

Phylogeographic Analyses and Paleodistribution Modeling Indicate Pleistocene In Situ Survival of *Hordeum* Species (Poaceae) in Southern Patagonia without Genetic or Spatial Restriction

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Although many phylogeographic studies have been conducted to analyze the impact of the ice age on species history of Northern Hemisphere mountain plants, such studies are nearly absent for plants of the Southern Hemisphere, particularly for lowland vegetation units. These species should have been primarily influenced by climate cooling and changes in precipitation regime instead of glaciers covering their distribution areas. It is thought that New World lowland species generally evaded climate changes by equatorial migration during Pleistocene cold cycles and recolonized their habitats at higher latitudes when climate warmed up again. In contrast to Eurasia, latitudinal orientation of the major mountain ranges in the Americas made these migrations easily possible.

In the huge steppe of the Patagonian plains and adjacent Andes of southern South America thrives a group of three sympatrically distributed diploid species of the barley genus *Hordeum*, which originated during the last 1.3 million years (My) from a common progenitor. To get insights into the speciation mode of the taxa and to test the hypothesis of longitudinal migration of steppe vegetation during the Pleistocene, we conducted population genetic and phylogeographic analyses based on sequences of the chloroplast *trnL-F* region from 922 individuals.

We found a high number of chloroplast haplotypes shared among species, which indicate speciation through vicariance events. Analysis of the distribution of genetic diversity within and among species inferred an origin of *Hordeum comosum* in the Central Argentine Andes, whereas *Hordeum patagonicum* and *Hordeum pubiflorum* originated in southern Patagonia. The extant occurrence of *H. comosum* in southern Patagonia and *H. pubiflorum* northward along the Argentine Andes was caused by reciprocal migration after the origin of the species.

Surprisingly, molecular data provided no evidence for range shifts toward the north during the last glacial maximum and recolonization of southerly habitats afterward, but indicated in situ survival of large populations of *Hordeum* species within their extant distribution ranges even in southernmost Patagonia and Tierra del Fuego. Ecoclimatic niche modeling used to reconstruct the potential paleodistribution areas of the species during the last glacial maximum shows that climate conditions were sufficient for the species to survive Pleistocene cold cycles in Patagonia without significant geographic restrictions. Molecular data together with ecological niche modeling indicate stable geographic distribution areas in two of the three species for at least the Holocene. As the *Hordeum* species are characteristic taxa of different steppe habitats, we speculate that the Patagonian steppe might be an old vegetation unit occurring for up to 4.5 My in southern South America.

Introduction

Pleistocene climate oscillations, involving several ice age cycles, heavily influenced the vegetation distribution on earth (Hewitt 1996; Comes and Kadereit 1998). They resulted in a depleted flora in most of temperate Eurasia due to east–west stretching migration barriers, preventing plants to retreat to more southerly habitats. Furthermore, most plant communities, currently present at higher latitudes in Eurasia, were pushed into southerly refugia and replaced by tundra vegetation. In contrast, north–south stretching mountain ranges in the Americas allowed latitudinal migration of vegetation units with changing climate conditions, resulting in lower species extinction in the New World. This difference is well documented in the fossil record, providing evidence for the occurrence of many extant New World plant taxa in pre-ice age Europe (Van der Burgh 1983). The heavily glaciated areas of the Northern Hemisphere resulted in distinct genetic patterning of populations depending on survival of species in or outside ice age refugia, their number and size, as well as tempo and mode of recolonization (Soltis et al. 1997; Abbott et al.

2000; Stehlik 2003; Kadereit et al. 2004; Koch et al. 2006; Alsos et al. 2007). Moreover, climate changes and therefore range shifts of plants during the last 2 million years (My) also left traces in the genetic composition of extant populations from formerly unglaciated areas (Kadereit et al. 2005; Soltis et al. 2006; Jakob et al. 2007). However, far less is known for plant species in the temperate regions on the Southern Hemisphere. Only the southern parts of South America and New Zealand are in geographic positions comparable with the glaciated areas on the Northern Hemisphere. Glaciers here were mostly restricted to mountain ranges (Hollin and Schilling 1981; Rabassa and Clapperton 1990; Hulton et al. 2002), as apart from Antarctica no large landmass at high latitudes promoted the formation of an extended ice shield. In any case, lower average temperatures and changed precipitation regimes would have influenced all vegetation at higher latitudes on the Southern Hemisphere. Although the huge Patagonian steppe, extending from the eastern slopes of the southern Andes to the Atlantic coast, is the major vegetation unit in this part of South America, the small number of existing phylogeographic studies from this area concern only tree species and/or species from the Andes (Pastorino and Gallo 2002; Marchelli and Gallo 2004, 2006; Muellner et al. 2005), whereas no steppe taxa were analyzed up to now.

In these steppe habitats of Patagonia and northern Tierra del Fuego, three closely related diploid *Hordeum* species occur (von B othmer et al. 1995), with *Hordeum*

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comosum being sister to *Hordeum patagonicum* and *Hordeum pubiflorum* (Pleines and Blattner 2008). They originated as part of a rapid radiation of the genus in South America and split within the last 1.3 My (Blattner 2004, 2006). That places their origin within the time of highest glacial maxima in Patagonia (Mercer 1976). The three species occur broadly sympatrically in southern Patagonia, south of 42°S. *Hordeum patagonicum* is a lowland species restricted to this area, whereas *H. comosum* and *H. pubiflorum* also stretch north along the Argentine Andes up to 19°S, reaching elevations of 4,000 m above sea level (von Bothmer et al. 1995). Although the species are often found within few meters distance, they are separated by different ecological preferences and show differences in flowering time (own field observations). *Hordeum comosum* grows in very dry steppe habitats and represents a key species of the climax vegetation unit of the Patagonian steppe. *Hordeum patagonicum* is a typical species at the species-poor margins of ponds and ablation hollows, whereas *H. pubiflorum* is characteristic for moister, nutrient, and species-richer grassland communities (Boelcke et al. 1985). In contrast to *H. comosum*, the latter two species are quite salt tolerant. As Pleistocene glaciations in South America were mostly restricted to the southern Andes, and the shallow shelf of the Patagonian Atlantic coast was partly exposed when sea level dropped during glacial maxima, plant species of the Patagonian steppe had plenty of room in the lowlands east of the Andes for latitudinal migration. Therefore, it is hypothesized that the Patagonian steppe communities occurred several degrees latitude to the north during the Pleistocene cold cycles (Heusser 1987) and shifted southward when the climate warmed up again.

The extant largely sympatric occurrence of the three species and their close relationship might allow a sympatric speciation scenario. Coyne and Orr (2004) defined four prerequisites that must be fulfilled for the assumption of sympatric speciation: 1) sympatric occurrence, 2) reproductive isolation, 3) sister relationship of the species, and 4) no indication of an allopatric phase during their history. As described above, the Patagonian *Hordeum* species meet all these prerequisites, although the last one is of course hard to prove. However, as plenty of unglaciated areas east of the Andes were available, during the last 1.3 My, there is no necessity to assume a priori allopatric speciation. Particularly the occurrence on salt-influenced soil, together with differences in water and nutrient availability, could promote sympatric speciation, as seeds dispersed into salty areas will generally develop slower and flower later, which should result in instant prezygotic reproductive isolation from their source population. This would circumvent the major problem of sympatric speciation, that is, the initial reduction of gene flow.

A previous study of chloroplast diversity in the entire genus *Hordeum* based on the *trnL-F* region revealed the allele distribution among all *Hordeum* species (Jakob and Blattner 2006), and thus set the stage for phylogeographic analyses (Avice 2000) in monophyletic groups within the genus. Particularly the Patagonian species share chloroplast haplotypes (HTs) due to long allele survival times and incomplete lineage sorting. These shared HTs might provide problems in phylogeographic analysis when only single species are analyzed without the knowledge of the HT dis-

tribution within a broader context (Jakob and Blattner 2006). Therefore, we decided to address the species together in a single study using nested clade analysis (NCA; Templeton et al. 1995), analyses of the frequencies of HTs and their geographic occurrence among and within the species (Avice 2000), and several population genetic parameters.

As our analyses resulted in unexpected phylogeographic patterns, that is, indicating ice age survival of *Hordeum* in the southernmost parts of Patagonia and recolonization of northern regions from there, we used extant and Pleistocene ecoclimatic distribution modeling to corroborate the results from chloroplast analysis (Peterson et al. 1999; Hugall et al. 2002; Yesson and Culham 2006; Carstens and Richards 2007). Ecological niche modeling predicts the potential distribution of species based on present occurrence points extrapolated to areas with similarly suitable ecological condition (Guisan and Thuiller 2005). Transferring the current climate envelope of a species onto climate models of the past gives an estimation of the species' potential paleodistribution, assuming stable ecological niches of species (Wiens and Graham 2005). This assumption is supported, at least over shorter evolutionary timescales, for some groups by studies of Peterson et al. (1999) and Martinez-Meyer and Peterson (2006) although fast niche shifts might occur in others (Broennimann et al. 2007; Pearman et al. 2008).

To disentangle the evolutionary history of the three *Hordeum* species and get insights into the history of the Patagonian steppe vegetation, here we address the following objectives. 1) What are the patterns of chloroplast diversity within and among the *Hordeum* species? 2) The three Patagonian species are sister taxa, occur broadly in sympatry today, and are ecologically differentiated. Thus, we want to explore whether the genetic and ecological data allow any conclusions about the geographic mode of speciation, that is, to discern between allopatric and sympatric speciation. 3) We want to analyze the distribution of these steppe species during the ice age, as it is assumed that in the Americas latitudinal migration of plant communities occurred to circumvent unsuitable climate conditions during Pleistocene cold cycles. Finally, 4) using palaeodistribution modeling, we have to take into account that niche shifts might have occurred in the studied taxa. Therefore, we want to see if we get indications either for shifts or stasis in the species' niches by comparing the results of phylogeographic analysis and modeling of paleodistributions.

Materials and Methods

Plant Material

We included 922 individuals of 3 diploid Patagonian *Hordeum* species (472 *H. comosum*, 164 *H. patagonicum*, and 286 *H. pubiflorum*) collected from 184 natural occurrence sites, supplemented by 48 lines from germplasm repositories. The sample covers the entire distribution areas of the species. It is dense and highly uniform for three of the four occurrence regions (Lake District, Inland Patagonia, Coastal Patagonia; see below), but is less dense for the Central Argentine Andes. All collection sites, numbers of individuals, their HTs, and collectors are listed in supplementary table S1 (tables and figures indicated by

“S” are available as supplemental online information or directly from the authors). Herbarium vouchers were deposited at GAT (IPK Gatersleben) and partly also at BA (Museo Argentino de Ciencias Naturales Buenos Aires).

Analyses of Chloroplast Sequences

DNA extraction, amplification, and sequencing of the *trnL-F* chloroplast region followed Jakob and Blattner (2006). Aligning of the chloroplast *trnL-F* sequences could be done manually by introducing gaps of 1- to 26-bp length at 20 positions. Length variation at three mononucleotide repeats (two T/A, one C/G) was excluded from the analyses due to uncertain homology of the sequence positions. Each insertion/deletion (indel) was considered as a single mutation event, and all indels were therefore coded as single positions in the final alignment. Identical sequences were grouped into HTs. TCS 1.13 (Clement et al. 2000) was used to calculate a statistical parsimony network reflecting the HT genealogy. We used gaps as a fifth state and calculated the 95% most parsimonious connection limit.

Phylogeographic Analysis

Phylogeographic patterns of each species were investigated primarily by considering the frequency and the geographic distribution of each HT as well as their distribution among and within the three species. Combining these results with the known relationship of the HTs, we wanted to detect possible reasons for the observed distribution patterns and the underlying historical processes.

We also applied NCA (Templeton et al. 1995; Templeton 1998, 2004) to investigate the phylogeographic patterns using the program GEODIS 2.0 (Posada et al. 2000) on the chloroplast HT network of each species. These HT networks were manually converted into a series of nested clades, following the rules given by Templeton et al. (1987). To determine if there is a significant match between genealogy and geography, statistically significant large or small values of D_C (clade distances) and D_N (nested clade distances) (Templeton et al. 1995) were interpreted using the inference key given by Templeton (2004). Although widely used, the results of NCA must be interpreted with caution because they are influenced by the number of sampled populations and the number of individuals per population (Petit and Grivet 2002; Templeton 2002). Moreover, NCA does not assess error levels in its inferences about historical processes or contemporary gene flow (Knowles and Maddison 2002). Thus, we lumped inferences in two categories (DeChaine and Martin 2005): 1) expansion events that included contiguous range expansion and long distance dispersal and 2) fragmentation events that could have occurred through past fragmentation or restricted gene flow, as defined by Templeton (1998). Then we compared whether similar historic events have occurred at the same clade level, that is, within the same time interval in different species.

Population Genetic Analyses, Test for Neutral Evolution and Population Expansion

As NCA has been several times heavily criticized (Knowles and Maddison 2002; Petit and Grivet 2002;

Knowles 2004; Panchal and Beaumont 2007; Petit 2008) we combined NCA with other analytical approaches to look for concordance among inferences. Thus, we divided the accessions among four geographical regions corresponding to areas with different glacial histories regarding duration and extent of glaciation (Auer 1956; Mercer 1976; Hollin and Schilling 1981). The northernmost region, the ‘Central Argentine Andes’ between 32°S and 38°S (single samples up to 19°S), is the region with the highest Andean Mountains but slightest extent and duration of glaciation (Hollin and Schilling 1981). This area also covers the foothills east of the Andes, as far as steppe vegetation is present. The second region is the “Lake District” around San Carlos de Bariloche, stretching along the Andes between 38°S and 44°S. Some glaciers in this area extended far to the east during the glacial maximum and nearly divided Patagonia into a northern and southern part (Auer 1956). The “Inner Patagonian” region stretches immediately along the Andes and their foothills between 70–73°W and 44–50°S and was highly glaciated either by local glaciers or the ice shield of the southern Andes (Auer 1956; Hollin and Schilling 1981). The “Coastal Patagonian” region between 65–70°W and 42–54°S was nearly never glaciated except for the southernmost parts of Tierra del Fuego and the southern Strait of Magellan during glacial maxima (Auer 1956; Mercer 1976).

To analyze population genetic parameters, the software package ARLEQUIN 2.0 (Schneider et al. 2000) was used. The gene diversity H (Nei 1987) was calculated to compare the genetic diversity of the three species within the four regions. Analysis of molecular variance (AMOVA) was used to test for genetic structure at various geographic scales: among geographic regions, among populations within regions, and within populations. To see whether the species have undergone a sudden population growth and if it is possible to assign this to a certain geographic region, we compared the mismatch distribution of pairwise differences among HTs with expectations of a sudden-expansion model (Rogers 1995) both for each of the three species in general and for each species within the four defined geographic regions. The goodness of fit between the observed and expected mismatch distribution was tested by a parametric bootstrap approach that uses the sum of squared deviations between observed and expected mismatch distribution as a test statistics, as implemented in ARLEQUIN. Statistical significance of population expansion was further assessed using Fu’s F_S -test of neutrality (Fu 1997). F_S was compared against a distribution generated from 10,000 random samples under the hypothesis of selective neutrality and population equilibrium. F_S tends to be negative under an excess of recent mutations and a significant negative value will be taken as evidence of population growth and/or selection. However, as the *trnL-F* intergenic spacer and the *trnL* intron region are noncoding regions, we have neglected selection as a reason for negative values of F_S .

Pleistocene and Present Ecological Predictive Models

To detect potential glacial refugia and their extent as well as to check and complement the phylogeographic

analyses, we modeled the present potential ecoclimatic niches of the species and projected them onto two climate models of the Pleistocene's last glacial maximum (LGM) about 21,000 years ago (Hugall et al. 2002; Martínez-Meyer et al. 2004; Peterson et al. 2004; Bonaccorso et al. 2006; Martínez-Meyer and Peterson 2006; Yesson and Culham 2006; Waltari et al. 2007, Peterson and Nyari 2008).

Ecological niches of *Hordeum* species were modeled in South America using two sources of primary data: 1) locality records for each species and 2) environmental data for the present and the LGM in the form of geographic information system (GIS) map layers. Occurrence information of species was used in the form of unique latitude–longitude combinations gathered from our own fieldwork, germplasm repositories, and online available herbarium information. In cases where no coordinates were given, point localities were geo-referenced using the global gazetteer version 2.1, available on www.fallingrain.com/world/ and the Britannica Atlas (Cleveland et al. 1994). For the modeling, we ended up with 137, 64, and 118 unique records for *H. comosum*, *H. patagonicum*, and *H. pubiflorum*, respectively (supplementary table S1, Supplementary Material online). These localities cover the entire distribution ranges of the taxa.

Environmental scenarios in the present and in the past were represented by a series of 19 variables summarizing aspects of climate (supplementary appendix S1, Supplementary Material online). Peterson and Nakazawa (2008) point out that the inclusion of all 19 bioclimatic variables will be prone to overfitting. However, this makes conclusion about distributional stability more conservative and thus more reliable. Present climates were drawn from the WorldClim database (Hijmans et al. 2005), and we used a new fine-resolution climatic data set for the LGM developed by R. Hijmans as follows: Two general circulation model outputs for the LGM, the Community Climate System Model (CCSM; <http://www.cesm.ucar.edu/> [Kiehl and Gent 2004]) and the Model for Interdisciplinary Research on Climate (MIROC, version 3.2; www.ccsr.u-tokyo.ac.jp/~hasumi/MIROC/), were scaled from their original resolution of 2.8–0.04° through interpolating the differences between past and present conditions using the spline function with the tension option implemented in ARCINFO (ESRI, Redlands, CA). Finally, the interpolated differences were added to the high-resolution current climate data sets from WorldClim and LGM bioclimatic maps created. Thus, final grids for the present were at a 0.01° pixel resolution and 0.04° for the LGM. This procedure has the advantage of producing data at a fine resolution and also of calibrating the simulated climate change data to the actual observed climate data. We used two LGM scenarios to reduce uncertainty due to particularities caused by the details of individual climate-model implementations and to detect consistencies in the resulted geographic patterns via a consensus approach.

Potential geographic distributions were predicted by modeling the ecological niches of the three *Hordeum* species using the Genetic Algorithm for Rule-set Production (GARP; Stockwell and Noble 1992; Stockwell and Peters 1999) implemented in DESKTOPGARP v. 1.1.6 ([http://](http://www.nhm.ku.edu/desktopgarp/)

www.nhm.ku.edu/desktopgarp/). GARP has been successfully used to predict present and past distributions for different taxonomic groups of plants and animals (Martínez-Meyer et al. 2004; Peterson et al. 2004; Bonaccorso et al. 2006; Martínez-Meyer and Peterson 2006). Because several models developed in GARP with the same data set present some variation due to all random processes involved, we developed 100 replicate models of the species' ecological niches in order to capture that variation and optimize model performance and selected a subset of the 10 best ones following Anderson et al. (2003). The 10 maps of these models were summed in a GIS to provide a best estimate of the potential geographic distribution of each species. Best subset niche models developed for each species in the present were then projected onto the climatic scenarios in the LGM, and maps describing the distributional potentialities were generated. These maps do not incorporate any explicit assumption on dispersal processes and assume a conservatism of species' niches in the distributional controlling variables (Peterson et al. 1999). Thus, maps should be interpreted as areas in the Pleistocene that held environmental conditions currently suitable for the species. Models for each of the three Patagonian *Hordeum* species were then used to see if they fit the interpretation of the molecular data, the phylogeographic results, the potentially underlying historic processes, and the present distribution patterns of species.

A necessary condition for projecting niche models to a different time period is that they are effectively predictive in the present time (Nogués-Bravo et al. 2008). Otherwise, a model that is not able to represent the distribution of the species in the same time period is clearly unreliable and should not be projected in a different climatic scenario. We tested model predictivity in two ways following Peterson and Shaw (2003). The first method consisted in calculating the median latitude and longitude for each species' locality data set and geographically splitting them into two sets: one set containing the SW–NE records and the other set containing the SE–NW ones. Then, models were built with set 1 following the procedure described above and occurrences of set 2 were overlain on the map produced and counted the number of points that were correctly predicted, and vice versa. The second method consisted in a simple 50–50 random division of the data sets, one for training and the other one for testing. Finally, a chi-square analysis was conducted to each test to evaluate whether model predictivity departed from random expectations.

Results

HT Sequences

Fifty-two chloroplast *trnL*-F HTs were identified within the three Patagonian diploids, 21 in *H. comosum* (in 472 individuals), 26 in *H. patagonicum* (164 individuals), and 21 in *H. pubiflorum* (286 individuals). The numbers of private (i.e., alleles restricted to a species) alleles for the species are 15, 14, and 11, respectively (table 1). The sequences were deposited in the European Molecular Biology Laboratory nucleotide database, and the accession numbers for each HT are given in supplementary table S1, Supplementary Material online. The HT distribution among the

three Patagonian *Hordeum* species as well as the HT distribution within the species among the four regions is given in table 1.

Statistical Parsimony Network

TCS calculated a 95% parsimony connection limit of 13 steps. Thirteen HTs occur at the interior, 39 at tip positions (fig. 1). Nine HTs inferred by TCS were not found in the analyzed individuals and occur as missing intermediates in the network. Ten positions in the TCS network revealed closed loops. These loops were each caused by single homoplastic alignment positions. Using the rules given by Templeton and Sing (1993) and Posada and Crandall (2001) together with the frequency of the HTs, their geographic positions, and their distribution among the species, nine of them could be resolved unambiguously.

Twelve HTs are shared among species, eight of them between two, and four among all three species. Although we cannot completely exclude ongoing gene flow, these shared HTs are nearly always the result of incomplete lineage sorting, thus, predating species separation (Jakob and Blattner 2006). This earlier conclusion was strengthened by the extended sample of this study, as we have found within 41 collection sites with sympatrically occurring species only 11 sites where shared HTs occur. In all cases, these were interiors (5× HT46, 1× each HT54, 57, 68, 85, 2× each HT47, 77), whereas shared tip HTs were never found at the same collection site.

All chloroplast HTs in the right part of the network (fig. 1a) are characterized by the absence of an A insertion within a poly-C repeat in the *trnL-F* intergenic spacer. These non-ACC HTs (as otherwise the A is followed by two Cs) occur in all three Patagonian diploid *Hordeum* species, although this part of the network is dominated by *H. comosum*. Only about one-third of the individuals of *H. patagonicum* and *H. pubiflorum* showed these non-ACC HTs. HTs on the left side of the network, starting with HT68, possess this A-insertion. These ACC HTs are restricted to *H. pubiflorum* and *H. patagonicum* (fig. 1a, table 1) and originated only after the common progenitor of these species split from the *H. comosum* lineage.

Apart from HT76 and 59, all tip HTs are private alleles for single species, whereas all interiors, except HT71, 78, and 118, are shared among the species. Actually, HT76 is not a real tip, as it has two descendants in taxa outside the species under study (data not shown). Generally, interior HTs are much more common (between 11 and 215 individuals/HT) than are tips (between one and nine individuals/HT, mostly only singletons).

Geographical Distribution and Frequencies of HTs

Generally, interior HTs are widely distributed, whereas the tips are locally restricted, mostly even to a single location. Only the tip HT126 has a larger distribution as it occurs from 35°S in the province of Mendoza up to 26°S in Tucuman, which approximates a distance of 1,120 km. There is, however, a conspicuous difference between the geographic distribution of the non-ACC and ACC interiors. The first ones (HT46, 47, 54, 57, 62, and 64) occur through-

out the entire distribution area of the three species (fig. 1b, table 1). The oldest Patagonian haplotype HT46, as inferred by Jakob and Blattner (2006), was found to be one of the common types in every geographic region. The ACC interiors (HT68, 75, 85, 71, 77, and 78) are restricted to the two Patagonian regions (figs. 1b and 2, table 1), only rarely occurring north of 44°S. Yet HT68, the oldest ACC HT, is very rare in the Lake District and completely missing in the adjacent Central Argentine Andean region, while all of its immediate descending tip alleles do not exceed 44°S at all. Thus, only the two Patagonian regions are complete regarding the presence of ACC interiors, whereas the northerly adjacent Lake District and the Central Argentine Andes are more or less depleted of these HTs (fig. 2). Furthermore, we observed a remarkable gap in the geographic distribution of ACC HTs. Between 40°S and 44°S, no individual of *H. pubiflorum* was collected at all bearing an ACC HT. There are, however, two exceptions from this general restriction pattern of the ACC HTs. The two highly derived interiors HT85 and, less pronounced, HT77 possess a disjunct distribution by being completely absent in the northern Patagonian provinces Chubut and Rio Negro, but showing a second distribution center in the province Neuquen, north of 40°S. Even the descendants of HT85 within *H. pubiflorum* (HT86, 114, and 126) reach up to 19°S.

Inferences from NCA

The nested designs for the species are given in figure 3. The non-ACC HT46 is the root of all Patagonian HTs (Jakob and Blattner 2006). Therefore, each clade containing HT46 was handled as an interior clade, although these clades were sometimes in an exterior position, at least at the level of the total cladogram. NCA reveals significant association of clades and their geographic distribution for all three species at all levels (tables 2a and 2b). It uncovered evidence for series of more recent and ancient fragmentation events and restricted gene flow across the distinct levels of the cladogram (tables 2a and 2b). Thus six, six, and five clades within *H. comosum*, *H. patagonicum*, and *H. pubiflorum*, respectively, have experienced fragmentation events, mostly due to restricted gene flow. The inference of restricted gene flow with isolation by distance is throughout all clade levels mostly caused by derived tip HTs, which are strictly locally distributed. However, restricted gene flow was also inferred on the level of the entire cladogram in *H. comosum*, concerning clade 2–3, which contains HT57 and its descendants.

A relatively recent fragmentation event was detected in *H. patagonicum* for clade 1–6, concerning HT73, which is geographically separated from the remaining HTs of this clade by the Strait of Magellan and occurs exclusively in Tierra del Fuego. No inferences at the level of the entire cladogram were possible for *H. patagonicum* due to the lack of significant D_C and D_N values. Both clades are geographically concordantly distributed.

Recent as well as ancient expansion events were detected within *H. patagonicum* and *H. pubiflorum* (two and four events, respectively), whereas no such event was inferred for *H. comosum* (tables 2a and 2b). For

Table 1
Distribution of HTs within and among the Species and Regions

Haplotype	<i>Hordeum comosum</i>					<i>Hordeum patagonicum</i>				
	Andes	Coastal	Inland	Lake	Sum	Andes	Coastal	Inland	Lake	Sum
HT046	13	1	36	73	123	11		3		14
HT047	3	24	20	35	82	5				5
HT054	3		1	1	5	6		2		8
HT057	4	22	46	135	207			8		8
HT058				6	6					
HT059		1			1	1				1
HT060	3				3					
HT062	3			5	8	3				3
HT064						5		2		7
HT065	3				3					
HT068						17		24		41
HT069										
HT070						1				1
HT071						13				13
HT072						1				1
HT073						6				6
HT074										
HT075						4		4		8
HT076								4		4
HT077						14		4		18
HT078						2				2
HT079								1		1
HT080										
HT082										
HT083								1		1
HT085								4		4
HT086										
HT088						1				1
HT091	3				3					
HT096										
HT098			1		1					
HT099			2		2					
HT100						1				1
HT101								4		4
HT106								4		4
HT107						1		4		5
HT108								2		2
HT109			2		2					
HT110						1				1
HT112				2	2					
HT113				2	2					
HT114										
HT115				5	5					
HT116										
HT117										
HT118				1	1					
HT119				5	5					
HT120				2	2					
HT121				8	8					
HT124										
HT125			1		1					
HT126										
No. individ.	35	48	109	280	472	93	71			164
No. HT	8	4	8	13	21	18	15			26
Percentage private alleles	38	0	50	62	71	44	40			54
No. ACC-HT	0	0	0	0	0	11	9			16
No. interiors	5	3	4	6	6	10	8			12
No. tips	3	1	4	7	15	8	7			14
Gene diversity H (SD)	0.83 (0.05)	0.55 (0.03)	0.68 (0.02)	0.68 (0.02)	0.71 (0.01)	0.90 (0.01)	0.86 (0.03)			0.90 (0.01)
Fu's F_S^a	-1.80	2.34	-0.52	-2.63	-8.33 **	-7.25**	-4.10			-13.28***

Number of individuals, number of HTs per species and region, percentage of private HTs per species and region, Nei's gene diversity H including standard deviation, Fu's F_S value and mean Π value (mean number of pairwise differences) are given.

^a Significance levels: *** $P \leq 0.001$, ** $0.001 < P < 0.01$.

**Table 1
Extended**

<i>Hordeum pubiflorum</i>					Regions				All species			
Andes	Coastal	Inland	Lake	Sum	Andes	Coastal	Inland	Lake	Number of			
									General Frequency of HT	Species/HT	Tip/Interior	ACC/Non-ACC
2	14	15	6	37	15	26	54	79	174	3	I	Non-ACC
	1	2	6	9	3	30	22	41	96	3	I	Non-ACC
4			3	7	7	6	3	4	20	3	I	Non-ACC
					4	22	54	135	215	2	I	Non-ACC
								6	6	1	T	Non-ACC
						2			2	2	T	Non-ACC
					3				3	1	T	Non-ACC
	1	4	3	8	3	4	4	8	19	3	I	N-ACC
	2	2	9	13		7	4	9	20	2	I	Non-ACC
					3				3	1	T	Non-ACC
	22	44	7	73		39	68	7	114	2	I	ACC
	2			2		2			2	1	T	ACC
						1			1	1	T	ACC
						13			13	1	I	ACC
						1			1	1	T	ACC
						6			6	1	T	ACC
	2			2		2			2	1	T	ACC
		3		3		4			11	2	I	ACC
		18		18					22	2	T	ACC
2	11		5	18	2	25	4	5	36	2	I	ACC
						2			2	1	I	ACC
							1		1	1	T	ACC
1				1	1				1	1	T	ACC
	2			2		2			2	1	T	ACC
								1	1	1	T	ACC
16	7	12	33	68	16	7	16	33	72	2	I	ACC
2				2	2				2	1	T	ACC
						1			1	1	T	ACC
					3				3	1	T	Non-ACC
		9		9					9	1	T	ACC
								9	1	1	T	Non-ACC
								2	2	1	T	Non-ACC
						1			1	1	T	Non-ACC
								4	4	1	T	Non-ACC
								4	4	1	T	ACC
						1		4	5	1	T	ACC
								2	2	1	T	Non-ACC
								2	2	1	T	Non-ACC
						1			1	1	T	ACC
								2	2	1	T	Non-ACC
								2	2	1	T	Non-ACC
2				2	2				2	1	T	ACC
								5	5	1	T	Non-ACC
			1	1				1	1	1	T	Non-ACC
			3	3				3	3	1	T	Non-ACC
								1	1	1	I	Non-ACC
								5	5	1	T	Non-ACC
								2	2	1	T	Non-ACC
								8	8	1	T	Non-ACC
		3	1	4				3	4	1	T	ACC
							1		1	1	T	Non-ACC
4				4	4				4	1	T	ACC
33	64	112	77	286	68	205	292	357	922			
8	10	10	11	21	14	23	23	20	52			
50	30	20	18	52	50	52	48	50				
6	6	6	4	14	6	15	11	4	25			
4	7	7	8	9	7	12	10	10	13			
4	3	3	3	12	7	11	13	10	39			
0.74 (0.07)	0.80 (0.03)	0.79 (0.03)	0.78 (0.04)	0.85 (0.01)	0.88 (0.02)	0.89 (0.01)	0.86 (0.01)	0.79 (0.02)				
-0.86	-2.34	-1.31	-1.27	-6.72**	-2.66	-8.86**	-7.42**	-4.94				

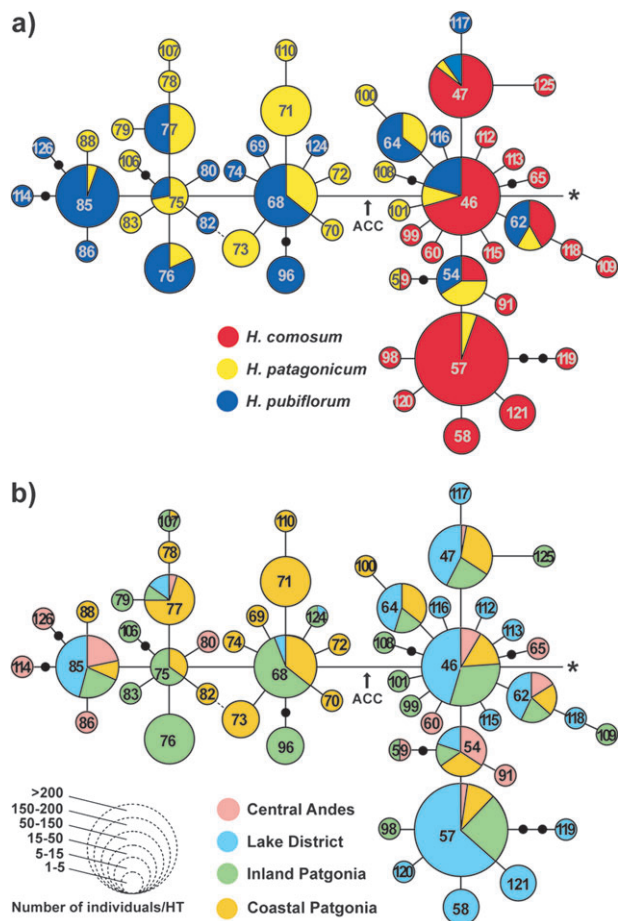


FIG. 1.—Chloroplast HT genealogy based on the *trnL-F* region for three Patagonian *Hordeum* species. An asterisk indicates the connection of the network to the HTs occurring in North American and Old World *Hordeum* species, thus being the root of the presented network. Black dots depict missing intermediate HTs not found in the analyzed individuals. Figure 1a shows the distribution of the HTs among the species, Figure 1b the geographical distribution within the four regions of Patagonia and adjacent Andes, as shown in Figure 2.

H. pubiflorum, these expansions generally took place from south to north, starting from distinct clade centers south of 42°S. They concern the non-ACC clade 1-1, comprising HT46 and its one-step descendants. In *H. pubiflorum*, HT46 is mainly restricted to the southernmost part of Patagonia in the province Santa Cruz, and its frequency decreases dramatically toward the north, whereas its one-step descending HTs (HT47, 54, 62, 64, and 116) occur mostly north of 44°S up to the province Mendoza (35°S). Also, ACC clades 1–5 and 1–6 revealed expansion events. These independent recent expansions are responsible for the disjoint appearance of HT85 and HT77 north of 40°S. Tip alleles of HT85, which are one step apart (HT86, 114, and 126), are private alleles (i.e., alleles exclusively restricted to a region) of the Central Argentine Andes. Also on the level of the entire cladogram, range expansion from south to north was inferred for *H. pubiflorum*, again indicating that it extended its range to the north.

For *H. patagonicum*, the more recent expansion (clade 1–1) also happened from south to north. However, in that species, the more ancient expansion event, inferred for

clade 3–2, took place from interior clade 2–4 in the Inner Patagonian region to the Atlantic coast in the east.

In general, the lower clade levels of NCA (first or second level) do not necessarily comprise only geographically restricted HTs as one would expect. Especially the shared interiors belong to the ancient stock of HTs in Patagonia and thus are geographically mostly widespread. However, sometimes they are a kind of dead end in certain species (e.g., HT47, 54, 62, and 64), as they have not experienced a radiation here and thus adopt highly derived positions in the HT network of single species. Therefore, in some cases, the geographic distribution of derived first-level clades is of similar magnitude like that of the deepest clade level (e.g., clade 1–8 in *H. comosum* or clade 1–6 in *H. pubiflorum*), and the HT genealogy of all three species corroborates the AMOVA findings of geographic structure (see below).

Population Genetics and Population Expansion

In general, Nei's gene diversity H was highest within *H. patagonicum*; the lowest value was found in *H. comosum*. That is in accord with the detected number of distinct HTs compared with the number of individuals analyzed for each species (table 1). There are, however, clear differences in the gene diversity H for the species within the four geographic regions. In *H. comosum*, the Central Argentine Andean region possesses the highest value. The lowest gene diversity was found in the Coastal Patagonian region.

For the other two species, the values for Nei's gene diversity do not differ significantly among the geographic regions. However, despite the comparably equal values of gene diversity for both regions in *H. patagonicum*, their HT compositions are quite different. Merely 7 out of 26 HTs are shared between both regions, all interiors with the exception of HT107. Thus, the number of private alleles for each region is quite high (table 1). Although HT68 is widely distributed, its descendants (HT70, 71, 72, 73, and 110) are restricted to the southern tip of the Patagonian coast south of 51°S. In general, all eight private alleles of the coastal Patagonian region occur south of 51°S, an area that is not predicted as climatically suitable for the species during the LGM (see below). Thus, we divided the *H. patagonicum* populations in Coastal Patagonian region into those south and north of 51°S and calculated Fu's F_S separately for each subregion. A significant deviation from neutrality was detected for the subpopulation south of 51°S (Fu's $F_S = -4.89$, $P < 0.01$), but not for the northern subpopulation, indicating expansion of the former.

The results of AMOVA for the three species are given in table 3. The percentage of variation explained at each hierarchical level was nearly identical among species, indicating that gene flow and dispersal barriers are similar in all of them. However, dispersal of rare tip alleles is not hampered by physical barriers but by insufficient time to spread through the entire population. Very low (*H. comosum*, *H. pubiflorum*) or even nonsignificant (*H. patagonicum*) genetic variation was detected among the defined geographic regions. This points either to no or weak regional differentiation in the three species and is mostly due to the wide geographic distribution of older interiors occurring

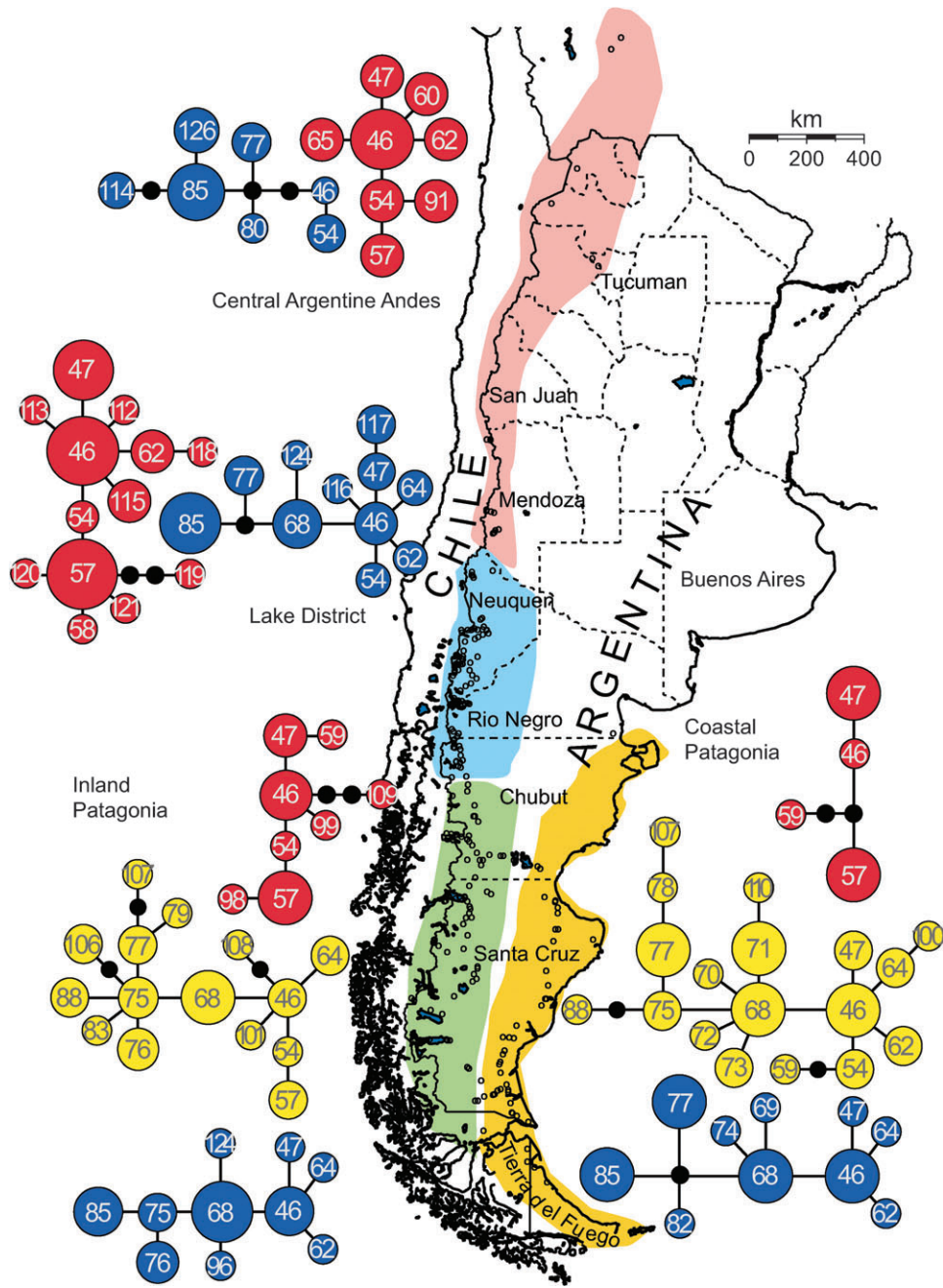


FIG. 2.—Map of southern South America indicating the four geographical regions (Argentine Andes = pink, Lake District = light blue, coastal Patagonia = orange, and inland Patagonia = green), collection sites (small black circles), and the regional chloroplast HT networks for each of the three *Hordeum* species in the four geographical regions. The chloroplast genealogies are given in red for *H. comosum*, in blue for *Hordeum pubiflorum*, and in yellow for *Hordeum patagonicum*. Circle sizes in the networks represent HT frequencies in the specific areas.

throughout the entire distribution ranges of the species. This also corroborates the absence of a significant association between the genealogy and geography on the highest clade level in *H. patagonicum* in NCA.

Mismatch distribution shows a smooth, unimodal wave in all species as predicted for a population that has undergone demographic expansion (Rogers and Harpending 1992). Additionally, Fu's F_S supports the demographic expansion for all species rejecting stasis at the 0.01 significance level (supplementary fig. S1, Supplementary Mate-

rial online, table 1). On a more regional scale, comparing the mismatch distributions of the species within the defined geographic areas, the curves also indicate a sudden population expansion. The F_S values were mostly negative as well, but for *H. patagonicum* in the coastal Patagonian region, this value was also significant ($P = 0.01$, see also above). In some cases, the rough outline of the wave rather points to stasis for the effective population size (e.g., *H. comosum* in the Coastal Patagonian region or *H. pubiflorum* in the Central Argentine Andes).

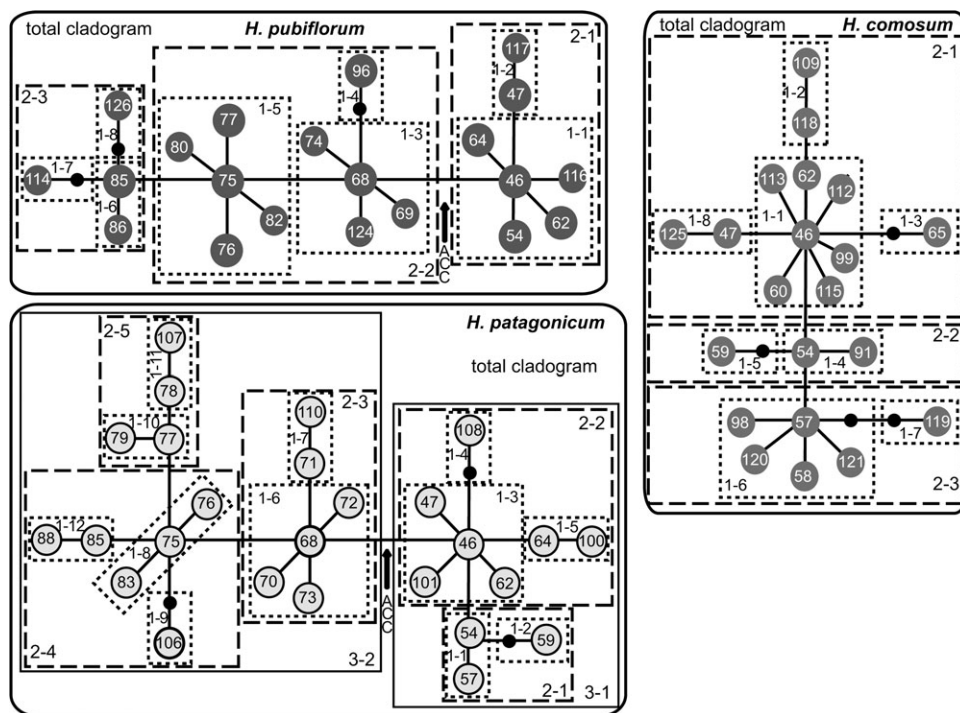


FIG. 3.—Nested clad designs of the chloroplast HT networks for three Patagonian *Hordeum* species.

Ecological Predictive Models and Potential Pleistocene Distribution

The present and Pleistocene ecological niche models and associated distributional predictions developed for each taxon are given in figure 4. Statistical tests indicated that predictions of independent data sets varied among species: For *H. comosum*, SE–NW models predicted 26.1% of SW–NE points ($\chi^2 = 50.24$), whereas SW–NE models predicted 100% of SE–NW points ($\chi^2 = 2,569.84$); for *H. patagonicum*, values were 40.6% ($\chi^2 = 1,821.04$) and 83.9% ($\chi^2 = 1,858.79$); and for *H. pubiflorum* were 61.7% ($\chi^2 = 195.79$) and 74.1% ($\chi^2 = 779.58$), respectively. Average predictivity of test points and χ^2 values of the 10 best subset models in the random tests for *H. comosum*, *H. patagonicum*, and *H. pubiflorum* were 94.4%, $\chi^2 = 8.22$; 92.4%, $\chi^2 = 3.72$; and 95.5%, $\chi^2 = 10.14$, respectively. In all cases, the *P* value was <0.001 .

The current distributional predictions (fig. 4, dark gray and black areas) are quite good representations of the taxa's extant geographical distribution (von Bothmer et al. 1995) although we see some differences between predicted and realized distribution areas toward the northeast, particularly for *H. pubiflorum*. All species are broadly sympatrically predicted in the cold, arid steppe area of southern Patagonia, with *H. comosum* and *H. pubiflorum* also stretching northward along the eastern Andes with warmer temperature and drier summer conditions.

The paleodistributional models for the LGM about 21,000 years ago (fig. 4, light gray and black areas) are given as a consensus from both Pleistocene climate models used. They do not differ substantially from the present day

distribution. According to the models, the species were co-distributed during the LGM and not substantially shifted to the north in comparison with present day models. Predicted suitable habitats for the species during the LGM were generally closer toward the Atlantic coast. Considering the four defined geographic regions, suitable ecoclimatic conditions during the Pleistocene prevailed within three of them: the Central Argentine Andes, the Lake District, and Coastal Patagonia. Paleodistribution models do not predict the species along the southernmost Andes and their foothills, which matches the Inner Patagonian region, and south of 50°S in the Coastal Patagonian region. That is consistent with expectations derived from the extent of glaciation (Mercer 1976; Hollin and Schilling 1981; Hulton et al. 2002). The models did not reveal fragmented, scattered, or isolated ice age refugia. Generally, between 40% and 50% of the areas currently occupied by the species were also suitable during the LGM (fig. 4, black areas), and for *H. comosum* and *H. pubiflorum*, the areas predicted as suitable during the LGM were of the same sizes or even slightly larger than at present, as the loss in inland Patagonia and the southern coastal region was counterbalanced by the exposed Patagonian shelf of the Atlantic coast. However, *H. patagonicum* seems to have been more restricted during the LGM, as its predicted current distribution area is about 20% larger than the potential Pleistocene distribution.

Discussion

Population Growth and Mode of Speciation

The chloroplast *trnL*-F genealogy of 922 individuals of 3 Patagonian diploid *Hordeum* species is concordant

Table 2a
Inferences of Historical Processes in the Patagonian *Hordeum* Species by NCA

Clade	Inferred Event
<i>Hordeum comosum</i>	
1-1	Restricted gene flow with isolation by distance for HT60
1-4	Restricted gene flow with isolation by distance for HT91
1-6	Restricted gene flow with isolation by distance for HT58 & 121
1-8	Restricted gene flow with isolation by distance for HT125
2-1	Inadequate sampling
2-3	Restricted gene flow with isolation by distance for HT119
Total	Restricted gene flow with isolation by distance for clade 2-3
<i>Hordeum patagonicum</i>	
1-1	Contiguous range expansion
1-3	Restricted gene flow with isolation by distance for HT62 & 101
1-6	Allopatric fragmentation for HT73
1-8	Restricted gene flow with isolation by distance for HT76 & 83
2-2	Restricted gene flow with isolation by distance for HT108
	Restricted gene flow with isolation by distance for clade 1-7 (HT71 und 110)
2-3	1-7 (HT71 und 110)
2-4	Restricted gene flow with isolation by distance for HT106
3-2	Contiguous range expansion from inner Patagonia (2-4) to the coast (2-5)
<i>Hordeum pubiflorum</i>	
1-1	Contiguous range expansion from S to N
1-2	Restricted gene flow with isolation by distance for HT117
1-3	Restricted gene flow with isolation by distance for HT74 & 124
1-5	Contiguous range expansion from S to N by 77
1-6	Contiguous range expansion from S to N by 85 & 86
	Restricted gene flow with isolation by distance for clade 1-2 (HT47 & 117)
2-1	1-2 (HT47 & 117)
2-2	Restricted gene flow with isolation by distance for HT96
2-3	Restricted gene flow with isolation by distance for HT114
Total	Contiguous range expansion from S to N

with a phylogenetic analysis by Pleines and Blattner (2008), who found *H. comosum* to be sister of *H. patagonicum* and *H. pubiflorum*. Although a high number of chloroplast HTs are shared among species, only so-called non-ACC alleles occur in all species. The ACC mutation obviously originated in the progenitor of *H. patagonicum* and *H. pubiflorum* after it split from the *H. comosum* lineage. All ACC HTs are therefore restricted to these species. Although interior HTs are mostly shared among species, the tip HTs are with two exceptions specific for the species in which they originated. Although hybridization occurs (own data), we never found tip HTs shared among species when they occur in mixed stands, indicating far-reaching reproductive isolation. Thus, extant hybridization plays a minor role regarding the distribution patterns of chloroplast HTs within diploid Patagonian *Hordeum* species (Jakob and Blattner 2006).

The occurrence of a certain number of shared alleles among species calls for a high number of individuals involved in a speciation event, as the persistence of ancient polymorphisms indicates the subdivision of relatively large populations during speciation. Thus, the chloroplast patterns found in Patagonian *Hordeum* species point to speciation through vicariance, where large populations became separated, but did not indicate population bottlenecks typical for speciation in peripheral isolates or due to founder events. Although prezygotic isolation together with ecological differences among the species might also allow a sympatric or parapatric speciation hypothesis, the chloroplast

Table 2b
Summary of NCA (E = Expansion, F = Fragmentation)

Clade Level	<i>Hordeum comosum</i>		<i>Hordeum patagonicum</i>		<i>Hordeum pubiflorum</i>	
	E	F	E	F	E	F
First	0	4	1	3	3	2
Second	0	1	0	3	0	3
Third	0	1	1	0	1	0
Fourth	—		Not significant		—	

data make this very unlikely. To arrive at the number of shared chloroplast alleles we observe today would involve either a high number of individuals shifting in parallel to a new ecological niche or prolonged gene flow between the diverging population and its progenitor lineage. However, high amount of gene flow would counteract the ecological diversification and therefore prevent speciation. Coyne and Orr (2004) analyzed which prerequisites must be fulfilled to assume sympatric speciation (see Introduction). Although these seem valid for Patagonian *Hordeum* and the species are in addition ecologically diverged, our data clearly show that at least during two periods in the past allopatric phases must have occurred resulting in lineage splitting. Therefore, the sympatric occurrence seen today originated obviously after speciation (see below).

Not only shared HTs but also the number and distribution of missing intermediate alleles in a genealogical network allow inference of population history (Jakob and Blattner 2006), as during population expansion the loss of alleles and therefore lineage sorting is low, whereas with shrinking population size or strong genetic bottlenecks allele extinction and therefore lineage sorting steeply increases. Comparable with branch length distributions in phylogenetic trees (Barracough and Vogler 2000; Barracough and Nee 2001), the number of missing intermediates reflects parameters of population history in gene genealogies when a representative sample of populations is analyzed. The chloroplast genealogy of the Patagonian species shows only a very low number of missing intermediates, and the main interiors are not only completely present but also quite frequent in the gene pool of the species (fig. 1a, table 1). This pattern is compatible with a constantly growing effective population size in all species, resulting in the preservation of nearly all newly arising chloroplast types (Avice 2000) and the maintenance of shared ancient polymorphisms. The analysis of mismatch distribution and Fu's F_S also point to the same direction. Taken together, the analyses of chloroplast data provide clear evidence for growing population size of the diploid Patagonian *Hordeum* species after this group split from the other South American taxa of the genus.

Phylogeography of the Species

Analysis of differences in the extant geographic distribution of genetic diversity is the major tool to reconstruct species histories during the Quaternary. It is assumed that regions with high levels of DNA variation have been inhabited for a long time or have served as glacial refugia for relatively large populations (Widmer and Lexer

Table 3
Test of Genetic Subdivision Using a Three-level AMOVA

Species	Source	df	ss	Variance	% ^a
<i>Hordeum comosum</i>	Among regions	3	7.752	0.018	4.88**
	Among populations within regions	89	76.162	0.13	34.83***
	Within regions	379	83.238	0.22	60.29***
	Total	471	167.153	0.36	
<i>Hordeum patagonicum</i>	Among regions	1	1.98	0.006	1.38
	Among populations within regions	45	44.793	0.23	48.95***
	Within regions	117	26.733	0.23	49.68***
	Total	163	73.506	0.46	
<i>Hordeum pubiflorum</i>	Among regions	3	10.692	0.03	6.87***
	Among populations within regions	72	73.306	0.23	52.70***
	Within regions	210	37.173	0.18	40.43***
	Total	285	121.171	0.44	

Regions were defined as Central Argentine Andes, Lake District, Inner Patagonia, and Coastal Patagonia. Values for the degrees of freedom (df), sum of squares (ss), the variance components (variance) and the percentage of variation explained at each hierarchical level are shown.

^a Significance levels: *** $P \leq 0.001$, ** $0.001 < P < 0.01$.

2001), whereas areas with low genetic variation are interpreted as recently colonized or harboring small relic populations. Therefore, many studies used these differences in the DNA variation of distinct geographic areas to identify putative Pleistocene refugia, recolonized areas, and migration corridors to reconstruct the evolutionary history of certain species (Soltis et al. 1997; Stehlik 2002; DeChaine and Martin 2005; Koch et al. 2006; Jakob et al. 2007).

For *H. comosum*, the highest gene diversity value was found in the Central Argentine Andes, whereas the lowest was detected in Coastal Patagonia. Although our sample is less dense in the Central Andean region, our data indicate a possible origin of *H. comosum* within the Central Argentine Andes and a relatively long in situ evolution in a large population. This is supported by the smooth, unimodal

curve progression of mismatch distribution (supplementary fig. S1, Supplementary Material online) and a negative value of Fu's F_S , although the latter is not significant. From there, the species extended its range southward into the Lake District. For this area, the chloroplast structure revealed an echo of possible former glacial refugia, predating the LGM. NCA analysis inferred restricted gene flow for the entire cladogram, concerning tip clade 2–3, which contains HT57 and its descendants. H57 is one of the most frequent ones within *H. comosum* in all geographic regions, but it has its highest frequency in the Lake District (table 1, fig. 2). Furthermore, all HTs derived from HT57, except singleton HT98, are private alleles of the Lake District. Thus, the D_C and D_N are significantly small, implying a pattern of restricted gene flow. We interpret this as an old

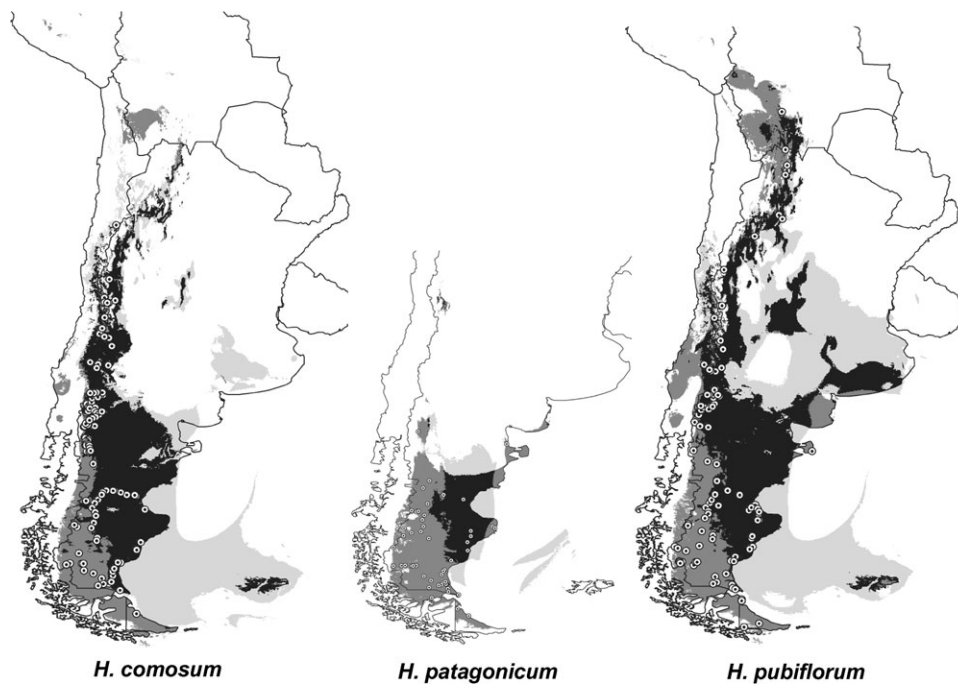


FIG. 4.—Present (dark gray and black) and past (light gray and black) distribution models of the three *Hordeum* species in southern South America calculated with GARP on the basis of extant occurrence points (white dots) of the species. The given potential distribution areas during the last glacial maximum (about 21,000 years ago) are consensus areas from two different Pleistocene climate models. Apart from inland Patagonia and Tierra del Fuego, the models infer the potential distribution of the *Hordeum* species in all areas where they occur today also during the last glacial maximum.

population bottleneck followed by fast population expansion (Posada and Crandall 2001), which is indicated by the dominance of HT57 and its star-like shape in the Lake District. Such a scenario is quite possible. During the highest glaciation in the early Pleistocene, about 1.2–0.8 Ma, on the eastern slope of the Andes glaciers extended from this region about 200 km into the lowlands and reached just south of 43°S the continental shelf on both sides of the Andes (Mercer 1976; Rabassa and Clapperton 1990). Moreover, in the area between 41°S and 42°S, small local glaciers existed, mostly not connected with the Andean ice shield (Rabassa and Clapperton 1990; Pastorino and Gallo 2002). All *Hordeum* collection sites within the Lake District are located in areas covered by ice at that time, whereas during the LGM, the Lake District itself and also the easterly adjacent areas provided suitable climate conditions for *H. comosum* (fig. 4). However, we cannot discern whether the other interior HTs are that rare compared with HT57 because they were restricted to small populations or recolonized the area afterward. The region-specific alleles derived from HT46, particular HT113 and 109 with their large distances between the collected individuals, argue against secondary recolonization. The colonization of both Patagonian regions by *H. comosum* happened quite recently. The gene diversity is comparably low, and the outline of mismatch distribution indicates no pronounced population growth in this area. Also, the regional chloroplast networks (fig. 2) indicate a recent colonization by *H. comosum*. Private chloroplast HTs occur in this species only in the northernmost part of Inland Patagonia close to the border of the Lake District, whereas the colonization of all southerly areas and particularly of Coastal Patagonia seems too young for the evolution of private tip HTs.

In general, compared with the other two species, *H. comosum* experienced only restricted gene flow according to NCA, that is, its most recent tips are still too young to have spread through the entire distribution area. Thus, the phylogeographic patterns within *H. comosum* point to a northern origin, possibly within the Central Argentine Andean region, and a two-step southward expansion, entering first the area of the Lake District where it was trapped for a longer time, then, quite recently, followed the colonization of the Patagonian regions.

Hordeum patagonicum and *H. pubiflorum* both are characterized by the possession of ACC HTs. In both species, the gene diversity of the different regions was rather similar and therefore not usable to infer the geographic origin of species. However, the geographic distribution and the frequencies of the ACC HTs, particularly of the older HT68 and 75, point to an origin of this mutation in Patagonia, possibly south of 44°S. Only the highly derived HT85 occurs at higher frequency north of 40°S, whereas the frequency of the oldest ACC HT68 decreases rapidly from south to north. Consequently, we assume the origin of *H. patagonicum* and *H. pubiflorum* in Patagonia.

Several expansion events from the south to the north were inferred for *H. pubiflorum* by NCA. Even the oldest Patagonian HT46, which certainly evolved in the north (where it is widely distributed in Central Argentine *H. flexuosum*; Jakob and Blattner 2006), moved with *H. pubiflorum* back from south to north as can be seen in the

expansion of clade 1–1. Furthermore, comparing the regional mismatch distributions between *H. comosum* and *H. pubiflorum*, the patterns change with opposite geographic directions. Although the curve progressions of mismatch distribution of the first species are rather smooth in the Central Argentine Andean region and ragged in the Patagonian regions, they are smooth in Patagonia and rough in the Lake District and the Central Argentine Andes for the latter. These findings together with the NCA and the distribution and frequency patterns of the ACC HTs support an origin of *H. pubiflorum* in Patagonia and a population expansion into the north. As the highly derived ACC interior HT85, which certainly also originated in Patagonia, has already a lot of descendants not only restricted to the Lake District but also reaching up to 19°S in Bolivia, this population expansion into the north by *H. pubiflorum* could not be a recent one and has to precede the LGM, if it was not highly accelerated by recent human activities.

The lowland species *H. patagonicum* shows the highest gene diversity H of all species analyzed, which might reflect extended substructuring of this species that is divided into five subspecies (von Bothmer et al. 1995). We did, however, find no correlation of the chloroplast distribution with these taxa and treated them here as a single unit. NCA inferred ancient range expansion from inland Patagonia toward the Atlantic coast within this species. In both regions, high values of genetic diversity exist, and mismatch distribution together with significantly negative F_S values indicate population growth particularly for coastal Patagonia. Within this area, all private alleles were found south of 51°S, indicating long-term persistence of the species within southernmost Patagonia and Tierra del Fuego. We found no signal in our data that would imply a colonization of this area from northern refugia or population expansion from coastal Patagonia toward the inland.

Present and Pleistocene Distribution Areas

Ecological niche modeling has been applied to a variety of research questions, including exploration of speciation mechanisms (Graham et al. 2004), species extinction (Swenson 2006), niche shifts (Broennimann et al. 2007; Jakob et al. 2007), and increasing the realism of historical models and thus improving phylogeographic inference (Carstens and Richards 2007). In the case of the southern Patagonian *Hordeum* species, molecular data led to the conclusion that these taxa were not pushed toward warmer northern areas during the last Pleistocene cold cycles but survived in situ within their extant distribution area. Moreover, we found no indication for reductions in population size for these species. As these results are counterintuitive, we were interested if climate conditions during glacial maxima were suitable in southern Patagonia for the species under study. Therefore, we modeled extant potential climatic distribution areas based on the occurrence points of the species and projected these models onto South American climate conditions during the LGM.

The models of the extant potential distribution areas are quite accurate representations of the species' realized distribution areas (fig. 4). For *H. pubiflorum*, we got however some differences toward the northeast, where the

models predict the species also in the southern pampas region of the province of Buenos Aires. This region provides more humid conditions than the Patagonian steppe and the vegetation consists of taller grasses, which might exclude *H. pubiflorum* from this area, although climate conditions generally fit the species.

Projecting extant climate envelopes to the conditions during LGM resulted in predictions of potentially suitable habitats during ice age cold cycles. The models showed that the extent of inhabitable areas during LGM was nearly identical to today for *H. comosum* and *H. pubiflorum* and predicted an approximate area reduction of 20% for *H. patagonicum*. These data are in accord with a proposed population growth during the last million years, as they show that at least climatic conditions during LGM did not limit the expansion of the *Hordeum* species in Patagonia. They also support our vicariance hypothesis of speciation, as large suitable areas are necessary for population subdivisions without marked reduction in genetic diversity. We deal, however, with two rather different time frames. Most probably the species were sympatrically distributed during the LGM, and since that time their relative distribution may have shifted but did not change dramatically. Yet, according to phylogenetic and population genetic analyses, the time of the origin of the species clearly predates the LGM, and the species were not sympatrically distributed when they originated. The paleodistributions were calculated for the LGM about 21,000 years ago, although we infer paleodistribution during the entire ice age, which might have been different from the LGM during some times in the Pleistocene (Dansgaard et al. 1993), particularly as it is known that the largest extent of glaciers occurred in Patagonia around 1.2 Ma (Mercer 1976). Therefore, our models show basically the possibility of occurrence of the species in the southern Patagonian steppe during cold cycles. They cannot exclude, however, that extended glaciers or more severe climate conditions might have subdivided the *Hordeum* populations earlier. This scenario would fit the split of *H. comosum* from the other species, which happened around the glacial maximum in Patagonia about 1.2 Ma.

Although the paleodistribution models of the *Hordeum* species generally support the hypothesis of their in situ survival of the ice age in southern Patagonia, we found inconsistencies between the molecular data and predicted areas in *H. patagonicum*. According to our climate models, inland Patagonia and Tierra del Fuego should not have harbored climatic suitable habitats during the LGM. Thus, the populations found today in these regions should be Holocene colonizers. The molecular data show however high genetic diversity and private alleles for *H. patagonicum* and *H. pubiflorum* in inland Patagonia, and also south of 51°S in *H. patagonicum*, thus suggesting long-term presence of *Hordeum* in both areas. This might indicate either niche shift toward an adaptation to warmer climate after the LGM or that the species could tolerate much colder climate conditions in the lowland, which are currently not present in southern South America, as high latitude habitats are missing due to the position of the species near the southern rim of the continent. The inconsistencies could also result from the paleodistribution models themselves. Although we have produced them at high resolution, they are per se rough es-

timations of the Pleistocene climate. Although the models do not predict suitable habitats south of 51°S and in inland Patagonia on a large spatial scale, such habitats might have existed on a local scale. Considering the Pleistocene history in this region, this seems possible. The ice cap in southern South America was mostly confined along the crest of the Andes (Hollin and Schilling 1981; Rabassa and Clapperton 1990; Hulton et al. 2002) but temporarily reached the edge of the continental shelf south of Chiloe Island (42°S) on the western slope of the Andes (Mercer 1976; Hollin and Schilling 1981; Hulton et al. 2002). On the Argentine side of the Andes, it was much more restricted, basically because of the westerly source of precipitation. Even during the highest glaciation about 1.2 Ma (Mercer 1976), glaciers were mostly only about few kilometers further east of the limit they reached at LGM (Rabassa and Clapperton 1990) and only locally reached the coast of the southern Atlantic. Also, the southernmost tip of South America was not permanently glaciated although the area around the Strait of Magellan was closed by ice during the highest glaciation (Mercer 1976) and subjected to a late glacier advance around 14,000 years ago (McCulloch et al. 2000). Thus, it seems conceivable that larger populations might have survived in climatically suitable pockets in inland Patagonia (see below).

Stability of Ecoclimatic Niches

Our analysis involved two independent approaches to reconstruct present and past species distributions. Phylogeography clearly indicated survival of large populations of *Hordeum* species within their extant distribution area at least for the last ice age cold cycle. These results were corroborated by the reconstruction of Pleistocene distribution models, indicating generally suitable climate conditions in this area for all analyzed species. Although these findings support the possibility of in situ survival of the *Hordeum* species in southern Patagonia, concordant results from both approaches allow also assuming niche stability in these species for at least the time since the LGM. As back- and forecasting of ecoclimatic niche envelopes rely on the stability of a species' niche, it is important to test this assumption with independent data sets. In our study, we did not infer potential ancient niches via integrating extant species niches along a phylogenetic tree (e.g., Graham et al. 2004), but inferred paleodistribution areas directly from population genetic diversity data. Thus, our molecular data generally confirm the results of paleodistribution modeling and support the importance of this tool in the reconstruction of species histories (Carstens and Richards 2007; Waltari et al. 2007). The incompatibilities we found for inland Patagonia can either be explained by population survival in climatically suitable pockets (Jackson et al. 2000), which also today occur in this area in sheltered valleys (e.g., the vicinity of Lago Buenos Aires/General Carrera), or the LGM models are not accurate for this area. As two *Hordeum* species seem to have inhabited inland Patagonia during LGM and the climate models are otherwise quite accurate and fit with the distribution of glaciated areas, we tend to assume survival in climatically favored parts within this region. In *H. patagonicum*, we found, however, high genetic diversity also in coastal Patagonia south of

51°S, where climatically favored shelters are harder to imagine. For this area, we see clear incongruity between the paleodistribution models and the phylogeographic results. Although smaller realized than potential distributions provide no problems, as competition with other species mostly prevent a taxon from filling the entire area with suitable climate conditions, larger realized paleodistribution than predicted can indicate range shift and therefore changes in the ecological niche. For this species, we can therefore either not exclude that a shift in the ecologic niche occurred after the LGM or that the species would otherwise be able to colonize today even more southerly habitats, if South America would provide areas at higher latitudes. In this case, we wonder, however, why it is restricted to lowland areas and not reaching higher altitudes in the Andes.

Conclusions

The *Hordeum* species studied here occur mainly in sympatry today, are sister taxa, show clear ecological differences and prezygotic reproductive isolation, which all together might indicate speciation in sympatry. However, high numbers of interior chloroplast HTs shared among the species indicate the subdivision of large populations prior to speciation, which is a hallmark of vicariance and therefore speciation in allopatry. For the division between *H. comosum* and the common progenitor of *H. patagonicum* and *H. pubiflorum*, the chloroplast data indicate a geographic pattern with *H. comosum* originating from a northern population, probably situated in the Central Argentine Andes, the latter species from a southern population. As the crown group age of the Patagonian *Hordeum* species coincides with the largest extent of glaciation in Patagonia and far-reaching separation of the southern and northern parts of the distribution area of the species, it suggests that this advance of glaciers about 1.2–0.8 Ma separated both subpopulations, ultimately arriving at two independent lineages. No such clear temporal or geographical pattern emerged for the later subdivision of *H. patagonicum* and *H. pubiflorum*. It is, however, clear that speciation did not occur in restricted ice age refugia, as niche modeling and molecular data indicate no major restriction of both species, neither geographically nor in population size. Only after the split of the latter species *H. comosum* and *H. pubiflorum* colonized their respective distribution areas, thus being largely codistributed today.

Our models indicate that the respective distribution ranges of all species did not diverge considerably from each other during LGM climate conditions. This, together with the congruent phylogeographic results, supports the concept of ecological niche stability in two of these *Hordeum* species, at least over time periods as far back as the Holocene. For *H. patagonicum*, we cannot exclude niche shift, as the molecular data predict for this species a larger LGM distribution than derived from paleodistribution modeling.

The Pleistocene scenario in Patagonia was very different from the Northern Hemisphere because large areas remained free of ice, and the climate conditions were less severe although temperatures decreased by 6–7°C (Markgraf et al. 1995; Moreno 1997; Hulton et al. 2002). Furthermore, the sea level was lowered during glacial cycles

(Hulton et al. 2002), and therefore the coastline of Patagonia was shifted to around four longitudinal degrees to the east (Auer 1956), which exposed additional habitats suitable to escape the glaciers and to survive. Therefore, one can easily imagine that today's Patagonian extra-Andean plant species were not necessarily pushed into small and scattered glacial refugia, which happened in large parts of the Northern Hemisphere. Thus, comparably large populations could have survived the Pleistocene east of the Andes. The surprising result of our study, however, was the occurrence of the *Hordeum* species within their extant distribution areas during LGM. The molecular data clearly indicate that they were not shifted toward the equator during Pleistocene cold stages, as assumed worldwide for most temperate species. Because the *Hordeum* species are characteristic taxa of a variety of steppe habitats, we hypothesize that not only *Hordeum*, but probably also large parts of the Patagonian steppe might have existed for a long time in situ in southern Patagonia, with no marked shifts toward north during cold climate cycles, at least not for the majority of species forming the extant steppe communities. This means that unlike steppes of the Northern Hemisphere, this vegetation unit might be quite old, possibly dating back 4.5 My when the increased rise of the southern Andes cut off westerly precipitation from the planes to their east. So Darwin was probably right about the age of the steppe when he wrote, reflecting on the harsh beauty of the Patagonian planes, "They bear the stamp of having lasted, as they are now, for ages, and there appears no limit to their duration through future time" (Darwin 1845, p. 501). Although the second part of his statement might no longer hold true, as the harsh but fragile steppe vegetation is now threatened due to severe overgrazing by introduced sheep and hares.

Supplementary Material

Supplementary table S1, figure S1, and appendix S1 are available at *Molecular Biology and Evolution* online (<http://www.mbe.oxfordjournals.org/>).

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