

Rapid Radiation in the Barley Genus *Hordeum* (Poaceae) During the Pleistocene in the Americas

Frank R. Blattner, Thekla Pleines, and Sabine S. Jakob

Abstract Evidence was found for a rapid radiation of the grass genus *Hordeum* in the Americas during the last 2 million years, accumulating 23 species in South and North America, while only 10 *Hordeum* species occur in other regions of the world. The differences in species richness are caused by distinct evolutionary mechanisms in the Americas and Eurasia, as recovered by the integration of phylogenetic and phylogeographic analyses with modeling of ecological niches. The Eurasian region is mainly characterized by a loss of biodiversity during the Pleistocene glaciations, while vivid speciation took place in the Americas during this time period. Thus, speciation in Eurasia was mainly affected by severe genetic bottlenecks probably due to small populations surviving in ice-age refugia, while such restrictions in New World species groups seem less pronounced. Particularly in southern Patagonia, speciation was due to multiple geographical subdivisions of relatively large populations during the last million years, without measurable reduction of genetic diversity or population sizes. This together with long-distance colonization of remote areas was the main cause of species diversity in the New World.

1 Introduction

Hordeum comprises about 33 species and belongs with other cereals, such as wheat and rye, and several important forage grasses, to the grass tribe Triticeae. *Hordeum* is characterized by three single-flowered spikelets (triplets) at each rachis node of the inflorescence, the lateral ones often sterile or only rudimentarily present. The economically most important species of the genus is barley, *H. vulgare*, which is used to feed livestock, as flour in human consumption, and malted for beer and

F.R. Blattner (✉), T. Pleines, and S.S. Jakob
Leibniz Institute of Plant Genetics and Crop Research (IPK), Corrensstraße 3, 06466 Gatersleben,
Germany
e-mail: blattner@ipk-gatersleben.de



Fig. 1 Examples of *Hordeum* species. From left to right: *H. marinum* from southwestern Europe, *H. brachyantherum* from western North America, flowering inflorescences of *H. patagonicum*, and *H. comosum* from southern South America

whisky production. *Hordeum* species are naturally distributed all over the northern hemisphere, in South Africa and in southern South America. With 16 native species, this latter region is also the main center of species diversity (von Bothmer et al. 1995). The species nearly always occur in open habitats (Fig. 1), often in steppe or meadow vegetation, along streams and ditches, many on salt-influenced soils, and nowadays also in disturbed habitats along streets and irrigation channels within their natural distribution areas. Some species like wall barley (*H. murinum*) and sea barley (*H. marinum* and *H. gussoneanum*) have been introduced nearly worldwide in temperate and dry regions, and occur now as weeds in disturbed and agricultural habitats. Several taxonomic treatments of *Hordeum* exist. The most extreme views are those of Dewey (1984) and Löve (1984) who divided the group into *Hordeum* s.str., consisting either of *H. vulgare* or *H. vulgare* and *H. bulbosum*, while all other species were included in the genus *Critesion*. This concept of generic delimitation was not generally agreed upon, and von Bothmer et al. (1995) in their last revision of the genus maintained the traditional circumscription as a single genus.

The infrageneric delimitation has also changed several times during the recent decades, involving criteria such as life cycle or awn length (Nevski 1941). von Bothmer and Jacobsen (1985) recognized four sections, *Hordeum*, *Anisolepis*, *Stenostachys*, and *Critesion*. These sections were maintained in the 1995 revision of the genus, although cytogenetic data did not entirely support this classification and the authors stated that it might be artificial (von Bothmer et al., 1995). Petersen and Seberg (2003) proposed a new sectional treatment of the genus based on phylogenetic analyses of single-copy nuclear and chloroplast DNA data. They delimited four different sections from von Bothmer et al. (1995): *Hordeum*, *Sibirica*, *Stenostachys*, and *Critesion*. Although based on a phylogenetic interpretation of DNA sequence data, this new classification also contradicted the earlier cytogenetic results, exemplified by the definition of so-called *genomes* in *Hordeum* and other Triticeae taxa. Within *Hordeum*, four genomes have been described,

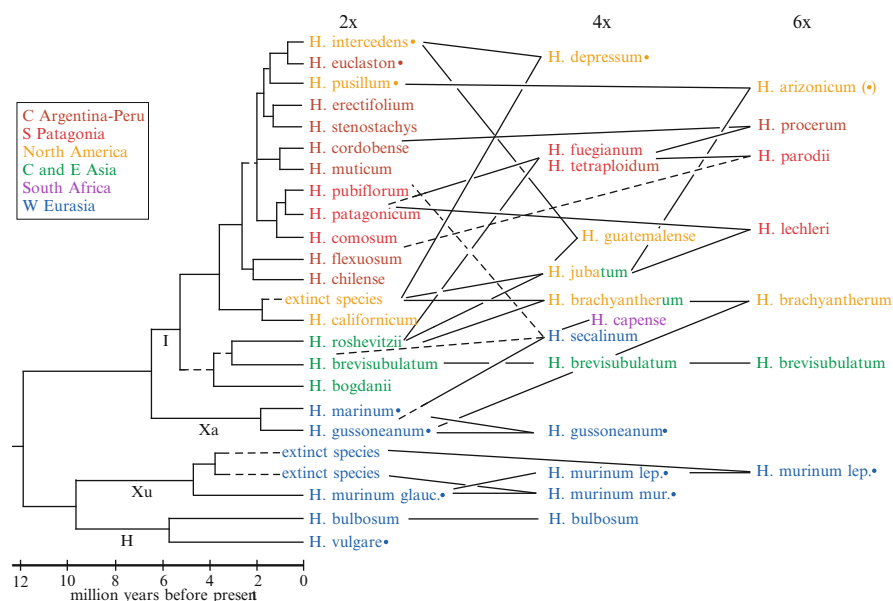


Fig. 2 Scheme representing our current understanding of phylogenetic relationships among *Hordeum* species (modified from Blattner 2009). It is derived from sequence analyses of nuclear rDNA ITS data for all species (Blattner 2004), ITS, DMC1 and EF-G for diploid taxa (Blattner 2006), AFLP fingerprints for the closely related diploid American species (Pleines and Blattner 2008), and for some species groups also chloroplast *trnL-F* sequences (Jakob and Blattner 2006). Diploids (2 \times) are drawn directly to the branches of the phylogenetic tree, while polyploids (4 \times , 6 \times) are mapped on the right. Lines connect the taxon names with their respective (partly putative) parental species. Dashed lines indicate topological uncertainties. Bullets behind taxon names mark annual taxa. Letters along the branches of the tree denominate the distribution of Triticeae genomes within the genus. While for the diploid species, estimations of clade ages were conducted, no such analyses were done for the polyploids. The existence of extinct species could be inferred from sequences, which are present in polyploid taxa, but do not occur in any extant diploid species

named **H**, **I**, **Xa**, and **Xu** (Linde-Laursen et al. 1992; Wang et al. 1996; Blattner, 2009). The distribution of the genomes among *Hordeum* species is given in Fig. 2. Although the concept of genomes is widely used in cultivated plants and their wild relatives, the systematic value of chromosomal similarity is debated (e.g., Petersen and Seberg 2003), as it is unclear how the amount of meiotic chromosome pairing in interspecific hybrids, that is used to define genomes, is connected to phylogenetic relationships.

Several attempts have been undertaken to clarify the phylogeny of *Hordeum* species, since barley is a major crop on the world scale and its wild relatives bear potential as genetic resources for crop improvement. However, most analyses suffered from often arbitrary taxon sampling or methodological shortcomings. Thus, surprisingly few phylogenetic studies with a complete or at least representative taxon sample have been conducted. Studies with higher species numbers or

including at least all diploid species have been published by Doebley et al. (1992), Komatsuda et al. (1999), Nishikawa et al. (2002), and Petersen and Seberg (2003, 2004). The results were contradictory, partly due to inconsistencies between chloroplast and nuclear phylogenies, and partly caused by differences in taxon sampling. Furthermore, all studies revealed poor resolution of the closely related New World taxa of the genus.

Speciation, the separation of a phylogenetically coherent group of individuals into two independently evolving lineages, is the major driving force of biodiversity. From a geographical perspective three major settings can result in speciation. These are: (1) speciation in allopatry, which means individuals of a species become geographically separated and evolve afterwards into two different lineages, as gene flow between sister populations is absent due to the geographical barrier; (2) parapatry, which means that a small peripheral population evolves into a different lineage, often involving restricted gene flow between the main population in the center and the peripheral isolate; and while these processes are probably involved in the majority of speciation events, the occurrence of (3) sympatric speciation is still under debate, as in this case no geographical barrier reduces gene flow between the differentiating lineages. As gene flow generally counteracts diversification and genetic recombination melts down diversifying allele combinations, strong selection against interbreeding has to maintain separated gene pools from a very early stage of diversification to arrive at separate species in a sympatric setting. Sympatric speciation can therefore be mainly postulated in situations such as those found in lakes or on small islands where geographic barriers are less likely (Coyne and Orr 2004). Otherwise, allopatric speciation involving geographical barriers is the preferred null hypothesis, particularly when taking into account the climate changes during the last 2 million years (my), when Pleistocene temperature oscillations resulted in range shifts and often forced species into isolated ice-age refugia. The major exception to the general rarity of sympatric speciation occurs nearly only in plants, where polyploidization, i.e., the doubling of chromosome numbers, is quite frequent while it seems comparatively rare in animals. Changes in chromosome number result in immediate reproductive isolation and therefore allow differentiation of two lineages in the same area without gene flow.

1.1 Framework of the Studies, Questions to Answer

Initially, the high number of *Hordeum* species in South America in comparison to other areas of the world allowed two hypotheses explaining this observation: (1) either the genus occurred for a longer time in South America and therefore had more time to evolve species diversity in this region, or (2) speciation rates increased in comparison to other areas after *Hordeum* arrived in South America. This second hypothesis was much more likely given that the majority of closely related genera of Triticeae occur in the northern hemisphere, particularly in western Asia, thus making a South American origin of the genus highly improbable. Also, the slight

morphological differences among several of the South American *Hordeum* species might indicate a relatively young age of this group. Thus, we assumed an ongoing rapid radiation of South American *Hordeum* taxa in comparison to species groups from other areas of the world. Putative causes for a rapid radiation could be (1) ecological differentiation of the taxa filling different open niches after an initial arrival in this area, or (2) several allopatric divisions of populations resulting in a multitude of geographically separated species. Clear ecological differences among species regarding soil and climate conditions together with often sympatric occurrence of species within a certain area could support an ecological speciation scenario in South America (Coyne and Orr 2004; Jakob et al. 2009). As seemingly increased speciation in South America could also be caused by higher extinction rates in areas of the northern hemisphere, we compared Old and New World *Hordeum* groups in a phylogenetic context to estimate speciation rates in different areas of the world. To understand possible reasons for shifts in speciation or extinction rates and to correlate them with climatic or geological changes it seemed also advisable to assign proximate ages to species groups within the genus. Moreover, the occurrence of *Hordeum* species on all continents apart from Australasia also demands the understanding of the historical biogeography, i.e., when and from where different regions of the earth were colonized.

The study of speciation processes first needs a sound phylogenetic framework. Therefore, several analysis approaches were conducted involving sequencing of nuclear loci and an analysis of amplified fragment length polymorphisms (AFLP; Vos et al. 1995) for the closely related species of the New World. In addition, analyses of chloroplast diversity were conducted to understand the nature of inconsistencies between nuclear and plastid phylogenies and to reconstruct the evolutionary history (phylogeography) of populations, species and species groups within the genus. To understand ecological differences among species, we also conducted cultivation experiments in greenhouses, and used modeling of the species' potential climatic niches to detect niche shifts among closely related taxa and probable Pleistocene refugia of species.

2 Materials and Employed Methods

In our molecular analyses of the evolution of species within the genus *Hordeum*, we generally tried to include a multitude of individuals per species in phylogenetic analyses to account for intraspecific variation. Thus, we transferred sampling schemes normally used in population genetic and phylgeographic analyses into phylogenetics. Wherever possible, we included material collected directly from wild populations. As we have up to now not been able to cover the entire distribution area of the genus with field trips, we complemented our *Hordeum* collection by materials from germplasm repositories (gene banks) and partly also herbaria. Seed material was germinated, grown in greenhouses, taxonomically determined, and ploidy levels were determined by flow cytometric analysis of genome sizes.

This was necessary, as *Hordeum* species are not always easily discernable, and materials from gene banks or botanic gardens often proved to be wrongly named. Moreover, in several species, cytotypes with different ploidy levels occur. Genome sizes are mostly reliable identifiers of species affiliation in *Hordeum* (Jakob et al. 2004), thus knowledge of the geographic origin of materials together with genome size provides in most cases enough information to verify species determinations.

To arrive at a robust species phylogeny, the ITS region of the nuclear ribosomal DNA was used to reconstruct phylogenetic relationships among all *Hordeum* species plus several outgroups from Triticeae, *Bromus*, and *Brachypodium* (Blattner 2004). As in most diploid *Hordeum* species two clusters of 45S rDNA located on two different chromosomes are present (Taketa et al. 1999, 2001) and most polyploid taxa originated from allopolyploidization, extensive sequencing of cloned ITS amplicons was used to determine the nature of the ITS regions present in individuals of most species. For all diploid and a few polyploid *Hordeum* species, ITS data were also combined with sequence data from two nuclear single copy genes (Blattner 2006): disrupted meiotic cDNA (DMC1; Petersen and Seberg 2004) and elongation factor gamma (EF-G; Komatsuda et al. 1999) which improved statistical support of basal branches in comparison to the analysis of only the ITS region. It did not, however, provide a better resolution of relationships among the American taxa. Therefore, we conducted in addition a phylogenetic analysis based on AFLP data (Vos et al. 1995) of the diploids from the Americas (Pleines and Blattner 2008) to define species groups particularly within the South American members of the genus. Nuclear rDNA ITS and the dataset combining three nuclear loci were also used to date branching points within the phylogeny of the genus. As calibration point, the split between barley and wheat about 13 my ago (mya) was used (Gaut 2002) in a penalized likelihood approach with r8s (Sanderson 2002) to estimate ages within *Hordeum*.

The historical biogeography of *Hordeum* was inferred from the phylogenetic tree derived from the combination of the nuclear loci, estimates of clade ages, and geographical distribution of the species. The age of the crown group of *Hordeum* (12 my) and the relevant subgroups (4–6 my) allowed for the assumption that the continents were in their present-day positions and that the relevant land bridges as Beringia and Central America were nearly or already in place at the time of origin of the taxa (Blattner 2006).

Chloroplasts, which are assumed to be maternally inherited in grasses, provide tools to analyze species histories. They are distributed by seeds only and have an effectively haploid genome and therefore a smaller effective population size than nuclear genes. This prevents problems with allelic recombination and results mostly in clearer geographically structured data in comparison to nuclear markers (Pleines et al. 2009). We used chloroplast data to analyze species or species groups to arrive at phylogeographic hypotheses of these taxa. To understand the general pattern of chloroplast allele distribution among species, we initially analyzed the chloroplast *trnL-trnF* region (*trnL-F*) in 875 individuals covering all taxa of *Hordeum* (Jakob and Blattner 2006). *TrnL-F* consists of two exons and the intron in the *trnL* gene, the intergenic spacer between this gene and *trnF*, and the *trnF* gene

itself. The transfer RNA (tRNA) genes are highly conserved among plants and are therefore suited as PCR primer binding sites, while intron and spacer sequences consist of variable DNA stretches, providing sequence differences useful in phylogenetic and population genetic analyses (Shaw et al. 2007).

For phylogenetic analyses of nuclear data, we used phenetic, cladistic, and model-based analysis algorithms implemented in PAUP* 4 (Swofford 2002) and MRBAYES 3.1 (Ronquist and Huelsenbeck 2003), which resulted in single or multiple trees. Statistical support of taxon groups was evaluated by bootstrap analyses (in neighbor-joining and parsimony analysis) and posterior probabilities from Bayesian inference. For chloroplast data, we also used a network approach (Posada and Crandall 2001), as data structure of the *trnL*-F region proved not to be tree-like. Thus, the reconstruction of an allele or haplotype genealogy provided a better representation of relationships among these haplotypes than all tree-based algorithms (Jakob and Blattner 2006). Haplotype genealogies were also used in studies of single species or species groups. In these cases, the number of included individuals was increased to get a good representation of species geographical distributions. In some of these more detailed studies, we included not only the *trnL*-F region but also sequenced highly variable parts of the chloroplast genome (Jakob et al. 2007; Jakob and Blattner 2010). These consisted either of AT-rich repetitive structures or mononucleotide repeat microsatellites, mostly poly-A/T stretches. Inclusion of microsatellites resulted in better-resolved relationships among chloroplast haplotypes within narrow taxonomic groups, but in more distantly related taxa, homoplasy was quite high. To overcome this problem, we invented a two-step procedure of network construction, where a backbone network was built on sequence variation at the slowly evolving parts of the analyzed loci, and sub-haplotypes of these backbone haplotypes were created taking into account variation at microsatellite loci (Bänfer et al. 2006; Jakob et al. 2007).

To understand the geographic settings of speciation processes, we used phylogeographic analyses. Phylogeography as a distinct discipline arose in the late 1980s and combines microevolutionary, i.e., population genetics, and macroevolutionary concepts, i.e., phylogenetics and systematics, with the distribution of genetic variation in space and time (Avise et al. 1987; Avise 2000). In contrast to population genetics, which explains allele distribution mainly by gene flow, phylogeography explicitly seeks to determine historical processes that shaped the extant distribution of genetic variation. Thus, the genetic variation within a species is organized into a genealogy and overlaid by the geographical distribution of the alleles of the marker region under study (Avise 1989). The analysis then interprets patterns of congruence or incongruence between the extant geographic distribution of alleles and their genealogical relationships on the background of different recent and historical processes influencing the structuring of genetic diversity within and among populations, i.e. geographic barriers, dispersal events, population size changes, and gene flow. As chloroplast alleles may persist through multiple speciation events, i.e. not reaching reciprocal monophyly for quite a long time, identical alleles might be found in several closely related taxa (Jakob and Blattner 2006). This can result in wrong interpretations of time axis and geographical patterns if only single

species are analyzed. To prevent such errors, we partly analyzed entire species groups together, if they are characterized by shared chloroplast haplotypes (Jakob et al. 2007, 2009).

To illustrate general differences in species evolution in *Hordeum* between Eurasia and the Americas, we here review three studies, dealing with (1) the *H. marinum* species group from the Mediterranean and adjacent regions, (2) the North American relatives of *H. californicum*, and (3) a group of southern Patagonian *Hordeum* species. In all cases, we conducted detailed phylogeographic analyses, partly in conjunction with modeling of climate niches of the species. Finally, we will discuss our current understanding of speciation processes in the genus and its general relevance for evolutionary biology.

3 Results and Discussion

3.1 Phylogeny of *Hordeum*

Based on several analysis approaches, our current understanding of species relationships within *Hordeum* is given in Fig. 2. In accordance with earlier cytogenetic work and in contrast to some other phylogenetic studies, the four genome groups in *Hordeum* proved monophyletic. The basal split in *Hordeum* was estimated to date back 12 my and separated the **H** and **Xu**-genome species from taxa belonging to the **I** and **Xa**-genome groups. Within the **H**, **Xa**, and **Xu**-genome groups only five extant species occur, while the majority of *Hordeum* species belong to the **I**-genome group (26 species). The latter segregated about 5 mya into two geographically defined lineages, of which one consists of all diploid Asian taxa, the other comprises all American species. Within this latter group, species are mostly not older than 1–2 my. While this separation is quite clear for diploid taxa, several allopolyploids exist combining **I** genomes from Asian and American taxa (e.g., tetraploid *H. brachyantherum*, *H. jubatum*, and *H. guatemalense*, or hexaploid *H. arizonicum* and *H. lechleri*). Allopolyploids involving two different genomes (**I** and **Xa**) are less frequent and restricted to tetraploid *H. secalinum* and its daughter taxon *H. capense*, as well as hexaploid *H. brachyantherum* that originated in historic times in a restricted area of California via hybridization of native tetraploid *H. brachyantherum* with introduced diploid *H. gussoneanum*. No naturally occurring taxa are known which combine the **H** or **Xu** genome or one of these with other Triticeae genomes, while the **I** genome can also be found in allopolyploid genera such as *Elymus* and *Elytrigia* (Dewey 1984, Mason-Gamer 2001).

The phylogenetic hypothesis (Fig. 2) shows that none of the up to now proposed infrageneric classifications of *Hordeum* (e.g., Nevski 1941; von Bothmer and Jacobsen 1985; Petersen and Seberg 2003) is sustained by the discovered monophyletic units, and also the split of the genus in *Hordeum* and *Critesion*, as proposed by Dewey (1984) and Löve (1984), would make *Critesion* paraphyletic. Thus, our

phylogenetic results support the concept of a single genus *Hordeum* (von Bothmer et al. 1995). To arrive at a closer match of phylogenetic relationships and infra-generic taxonomic units, a new classification of the genus was proposed (Blattner 2009) subdividing *Hordeum* in subgenus *Hordeum* with sections *Hordeum* (**H**-genome taxa) and *Trichostachys* (**Xu**-genome taxa), and subgenus *Hordeastrum* consisting of sections *Marina* (**Xa**-genome taxa), *Stenostachys* (**I**-genome taxa) and *Nodosa* (allopolyploids combining **I** and **Xa** genomes).

3.2 Biogeography

The dated phylogeny of the genus (Fig. 2) provides evidence that older lineages within extant *Hordeum* are all restricted to western Eurasia, while Central Asian and American clades are of younger age. The scenario (Fig. 3) for the historical biogeography of the genus (Blattner 2006) assumes, therefore, the origin of *Hordeum* somewhere in western Eurasia, maybe in Southwest Asia. It is not, however, possible to pinpoint the exact area of origin, as climate changes during the last 12 my may have resulted in range shifts of species. This area is in accord with the distribution of most other Triticeae genera, which also mainly occur in central Eurasia. From this ancient area, *Hordeum* migrated in a western direction into the Mediterranean basin and also eastwards into Central Asia. The lineage in this latter area belongs to the **I**-genome taxa and is most probably sister to all New World

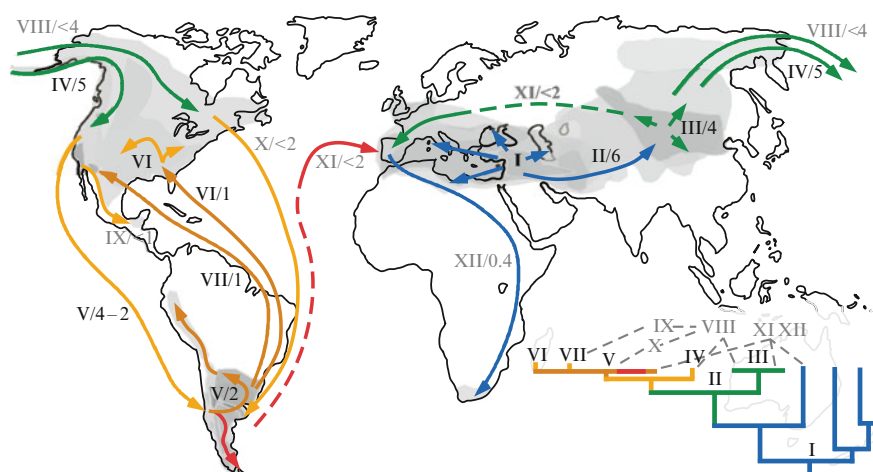


Fig. 3 Historical biogeography scenario of *Hordeum* plotted on the distribution map of extant *Hordeum* species. Shading of the areas reflects species numbers in the respective regions. Black roman numbers refer to distribution and colonization events in the diploid parts of the phylogenetic tree as shown in the insert, while the distribution events on the polyploid level were indicated in gray roman numbers. Numbers behind the slashes give approximate ages of colonization events in millions of years. Figure modified from Blattner (2006)

species of the genus. Therefore, a colonization of the Americas from Asia via Beringia is possible given the northern latitude climate during the relevant time interval about 5 mya was suitable for temperate taxa. From North America *Hordeum* reached South America between 4 and 2 mya, most probably via long-distance dispersal between western North America and today's Chile. Also, other long-distance dispersals were inferred. Thus, North America was colonized two times, independently from South America (resulting in *H. intercedens* and *H. pusillum*) and once again from East Asia (*H. jubatum*), South America one more time from North America (*H. lechleri*), Europe either from South America or Central Asia (*H. secalinum*), and South Africa from Europe (*H. capense*). Most of these dispersal routes coincide with migration routes of birds, which can often be found at ditches in *Hordeum* habitats (Blattner 2006). Thus, we guess that seeds glued to the legs or beaks of migratory birds by wet mud (Darwin 1859) were involved in long-distance colonization in *Hordeum*. Although we infer up to eight intercontinental long-distance dispersals involved in shaping the extant distribution area of *Hordeum*, species within a certain area are more closely related to each other in comparison to species occurring in other regions, apart from the two North American species nested within the South American species group. This means that colonizations of distant areas are still rare events in the genus and do not obliterate the general phylogenetic–geographic correlation.

3.3 Speciation Rates

Phylogenetic and biogeographic analyses of *Hordeum* revealed that the New World species and particularly the species in South America originated quite recently, mostly during the last 2 my. This confirmed our initial hypothesis of a young and rapid radiation on this continent leading to the high extant species number, as opposed to an ancient occurrence of the genus on that continent as assumed by Baum and Johnson (2003). In contrast to South America, we inferred in Eurasia only one speciation event at the diploid level during this time period (*H. gussoneanum*–*H. marinum*). A comparison of average net diversification rates for diploids from both areas resulted accordingly in $0.42 (\pm 0.11)$ species^{−my} in the Americas compared to $0.11 (\pm 0.03)$ species^{−my} in Eurasia (Jakob and Blattner 2006). This rate is quite high, particularly as it does not even include speciation at the polyploid level, and for the Americas is close to continental peak diversification rates (Baldwin and Sanderson 1998). The calculation of net diversification could not, however, finally answer our questions whether speciation rates are exceptionally high in South America or if speciation is occurring at a normal *Hordeum*-specific pace there, while Eurasia is characterized by high extinction rates resulting in much lower net diversification. To answer this question, we used an approach derived from the distribution of missing alleles in a chloroplast genealogy of the genus.

3.4 Chloroplast Genealogy of *Hordeum*

To identify reasons for inconclusive results of earlier chloroplast phylogenies in the grass genus *Hordeum* and to get insights in population demography, we established a genealogy of chloroplast haplotypes by sequencing the *trnL-F* region in 875 individuals, covering all 33 species of the genus (Jakob and Blattner 2006). Our analysis resulted in 88 chloroplast haplotypes. Their relationships are given in the genealogy shown in Fig. 4. The network reveals a clear geographical structure, with chloroplast lineages distributed mainly in the Old World or the New World all converging at the central haplotype HT1. In the New World, the major division is between North American and South American haplotypes, the latter subdivided into a northern (Chile, central Argentina, Uruguay, and northern Andes) and a southern group (southern Patagonia and Andes) with one chloroplast lineage exclusive to this southern group.

A pronounced difference between Eurasia and the Americas is the occurrence of missing intermediates (38 in the Old World vs 9 in the New World). A second conspicuous difference between these areas concerns the number of haplotypes per species and the number of shared haplotypes among different species. Most species in the Old World possess only a small number of haplotypes, in some cases even just one single haplotype was found. The coalescence of these haplotypes is shallow and they converge comparatively early into their last common ancestor. In the New World, we found up to 18 different chloroplast haplotypes within southern Patagonian species with quite deep coalescences. Furthermore, up to six species share

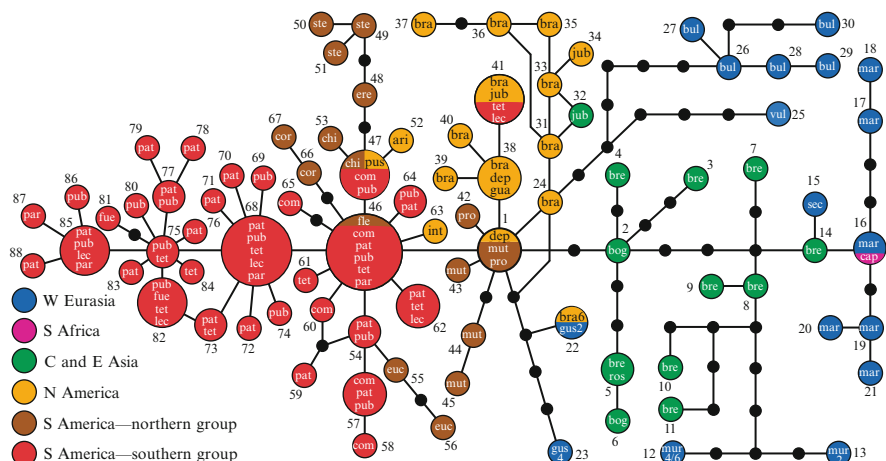


Fig. 4 Chloroplast haplotype genealogy of *Hordeum* based on a statistical parsimony network analysis of sequences of the *trnL-F* region from 875 individuals covering all *Hordeum* species. Species names are abbreviated by the first three characters of the species epithet. The network is characterized by a high number of missing intermediate haplotypes (black dots) in the Eurasian part, and many haplotypes shared among up to six species in the New World part. Figure modified from Jakob and Blattner (2006)

single haplotypes. In Eurasia, missing intermediates and mostly species-specific chloroplast types indicate far-reaching loss of genetic diversity, probably caused by repeated genetic bottlenecks for the populations in this area. Also, in the northern South American species group, species-specific chloroplast haplotype lineages indicate genetic bottlenecks, although probably less severe than in Eurasia (Jakob et al. 2010). The patterns found in southern Patagonia are consistent with long-term stable population sizes and large populations involved in speciation events. We explain these differences with quite different Pleistocene histories of South America and Eurasia, and also within southern South America. While east–west stretching mountain ranges and the Mediterranean Sea prevented Eurasian species from migration to southerly refugia, no such barriers were present in the Americas. Thus, species could shift with changing climate conditions to suitable habitats. This resulted in low species extinction rates in the New World, while extinction was quite high in Eurasia.

3.5 The Eurasian *Hordeum marinum* Species Complex

The *H. marinum* group consists of two annual grass species of western Eurasian saline meadows or marshes. The two taxa split in the Pleistocene about 2 mya (Blattner 2006). *Hordeum marinum* and the diploid cytotype of *H. gussoneanum* co-occur throughout the Mediterranean basin, while the tetraploid cytotype of *H. gussoneanum* overlaps with its diploid progenitor geographically only in the very far eastern Mediterranean, extending from there eastwards into Asia. Using chloroplast sequences of the *trnL-F* region and six chloroplast microsatellite loci, ecological predictive models based on climate data and the present geographical distribution of the two species, we analyzed differentiation processes in the *H. marinum* group (Jakob et al. 2007).

The chloroplast data indicated clear differences in the history of both species. Interestingly, both species possess highly distinct chloroplast lineages (Fig. 4), with *H. gussoneanum* revealing chloroplast haplotypes, which are otherwise extinct in Eurasia but have survived in the New World (Jakob and Blattner 2006). Phylogeographic and population genetic analyses clearly showed that the two diploid species originated allopatrically within different small and isolated ice-age refugia. For *H. marinum*, we found a subdivision between genetically quite variable populations from the Iberian Peninsula and more uniform populations from the remaining Mediterranean. As an explanation, we assume Pleistocene fragmentation of an earlier widespread population and survival in a southern Iberian and a southern Central Mediterranean refuge, where temperatures were also suitable for the species during Pleistocene cold cycles.

Chloroplast variation was completely absent within the cytotypes of *H. gussoneanum*, indicating a severe and recent genetic bottleneck. Due to this lack of chloroplast variation, only the combination of ecological habitat modeling with molecular data analyses allowed conclusions about the history of this taxon. The distribution areas of the two cytotypes of *H. gussoneanum* overlap today in parts of

Turkey, where the polyploid probably originated. Afterwards, it underwent a pronounced ecological shift, compared to its diploid progenitor, allowing it to colonize mountainous inland habitats between the Mediterranean basin and Afghanistan.

3.6 North American *H. californicum* Group

Analyses of the North American group of diploid *H. californicum* and three tetraploid taxa possessing related chloroplast haplotypes in the *Hordeum* chloroplast genealogy (Jakob and Blattner 2006) show that genetic diversity is high in comparison to *H. marinum*, particularly in the polyploid taxa. *Hordeum californicum* and *H. depressum* are both today mainly restricted to California and possess very similar haplotypes. *Hordeum brachyantherum* and *H. jubatum*, on the other hand, show a wider distribution range and also diverse chloroplast haplotypes. Many haplotypes are shared between these two taxa, even among individuals not occurring in the same area (Pleines and Blattner, unpublished). Reasons for the high haplotype diversity could either be multiple origins of the polyploids, each time introducing different maternal chloroplast types, or extended gene flow among polyploids and older diploid taxa, enriching chloroplast diversity in the polyploids. *Hordeum jubatum* probably survived the last ice-age in a northern refuge in Beringia, as can be deduced from high chloroplast diversity in Alaska. A different pattern was found in *H. brachyantherum* where we assume survival of widespread populations south of the North American ice shields in California and coastal regions of British Columbia and Newfoundland (Pleines and Blattner, unpublished).

In North America, the number of missing haplotypes is as equally low as in South America, indicating no severe genetic restrictions during the ice-age cold cycles. Contrary to the situation in Europe, *Hordeum* species in North America were probably geographically widespread during the Pleistocene. This may be because of fewer east–west oriented mountain ranges hindering migration, which is often used to explain higher extant biodiversity in North America in comparison to Europe (Thingsgaard 2001). This does not, however, hold for the only diploid of this species group, which today occurs in relatively small populations in California. This difference between diploid and polyploid taxa of this group can also indicate a wider ecological amplitude of the latter, resulting in fewer geographic restrictions from changing climate.

3.7 Southern Patagonian Species

In the huge steppe of the Patagonian plains and adjacent Andes of southern South America thrives a group of three sympatrically distributed diploid *Hordeum* species (*H. comosum*, *H. pubiflorum*, and *H. patagonicum*), which originated during the last 1.3 my from a common progenitor. For this group, we conducted population genetic

and phylogeographic analyses based on sequences of the chloroplast *trnL*-F region from 922 individuals (Jakob et al. 2009). We found a high number of older chloroplast haplotypes shared among these species. Furthermore, the interior haplotypes are geographically widespread whereas young tip haplotypes are mostly species specific and locally restricted (Fig. 4). Almost no missing haplotypes were detected, in great contrast to the Eurasian species. 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. The combination of many shared haplotypes together with the low number of missing intermediate haplotypes is furthermore 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.

Analysis of the distribution of genetic diversity within and among species inferred an origin of *H. comosum* in the central Argentine Andes, while *H. patagonicum* and *H. 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 neither evidence for Pleistocene genetic bottlenecks nor evidence for range shifts towards the north during the last glacial maximum and recolonization of southerly habitats afterwards, as is commonly assumed for lowland species of the southern hemisphere. Rather, our molecular data 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 Pleistocene distribution of the species about 21,000 years ago during the last glacial maximum shows that climate conditions were sufficient for the species to survive Pleistocene cold cycles in Patagonia itself without significant geographic restrictions. Molecular data together with ecological niche modeling indicate stable geographic distribution areas of the 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.

4 Conclusions

Earlier phylogenetic analyses in *Hordeum* encountered different and partly contradicting species relationships when analyzing chloroplast and nuclear loci. To explain these differences, we conducted a genealogical analysis of chloroplast alleles in 875 individuals covering all *Hordeum* species. This analysis revealed far-reaching incomplete lineage sorting in the New World taxa of the genus due to chloroplast alleles surviving several speciation events for up to 4 my. These shared alleles together with several distinct alleles occurring within single species makes the

outcome of species relationships almost arbitrary when only one or few individuals per species are included in phylogenetic analysis. The long allele survival times of haploid chloroplasts might imply that the time to reach reciprocal monophyly could even be higher in diploid nuclear alleles, rendering single copy loci also possibly problematic for phylogenetic analyses in *Hordeum*. Furthermore, in phylogeographic analyses, it seems advisable to include not only individuals from the species under study but also a representative sample from closely related taxa. From our experience with closely related species from genera of other plant families (*Allium*, *Crocus*, and *Hypericum*), we predict that the phenomenon of shared chloroplast alleles is not restricted to *Hordeum* but might be found in most other plant groups.

Our data support high speciation rates in the New World and particularly in South America, involving constantly growing effective population sizes in this area. On the other hand, *Hordeum* in Eurasia clearly suffered from far-reaching extinction, erasing populations and most probably also many species (Jakob and Blattner 2009), resulting in low extant species numbers and also relatively low chloroplast diversity. As far as this can be inferred from still existing genetic diversity, speciation mostly took place in small populations in geographically separated Pleistocene refugia. Other processes involved in speciation in *Hordeum* are multiple intercontinental long-distance dispersals, resulting in immediate reproductive isolation between the source and the newly founded population (Pleines and Blattner 2008). Even in southern South America where ecological speciation seemed possible to us, our analyses resulted up to now only in the inference of allopatric speciation through vicariance events. Thus, although many *Hordeum* species occur today in sympatry, sympatric speciation seems very rare in the genus. This supports the view that sympatric speciation might only occur in very exceptional cases, while normally, geographic barriers are involved in speciation events. In the future, we want to understand how these sympatrically occurring and still crossable *Hordeum* species prevent hybridization when occurring in close proximity, and analyze speciation processes in the polyploid taxa.

Acknowledgments We thank M. Arriaga and R. Gomez (Natural History Museum “Bernado Rivadavia, Buenos Aires) for kindly providing help in the organization of field work in Patagonia, P. Cichero (APN, Buenos Aires) for research permits in Argentine national parks, many friends, colleagues and gene banks for plant materials, and the Deutsche Forschungsgemeinschaft for financial support in the frame of SPP 1127.

References

- Avice JC (1989) Gene trees and organismal histories: a phylogenetic approach to population biology. *Evolution* 43:1129–1208
- Avice JC (2000) *Phylogeography: the history and formation of species*. Harvard University Press, Cambridge
- Avice JC, Arnold J, Ball RM, Bermingham E, Lamb T, Neigel JE, Reeb CA, Saunders NC (1987) Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. *Annu Rev Ecol Syst* 18:489–522

- Baldwin BG, Sanderson MJ (1998) Age and rate diversification of the Hawaiian silversword alliance (Compositae). *Proc Natl Acad Sci USA* 95:9402–9406
- Bänfer G, Moog U, Fiala B, Mohamed M, Weising K, Blattner FR (2006) A chloroplast genealogy of myrmecophytic *Macaranga* species (Euphorbiaceae) in Southeast Asia reveals hybridization, vicariance and long-distance dispersals. *Mol Ecol* 15:4409–4424
- Baum BR, Johnson DA (2003) The South African *Hordeum capense* is more closely related to some American *Hordeum* species than to the European *Hordeum secalinum*: a perspective based on the 5S rDNA units (Triticeae: Poaceae). *Can J Bot* 81:1–11
- Blattner FR (2004) Phylogenetic analysis of *Hordeum* (Poaceae) as inferred by nuclear rDNA ITS sequences. *Mol Phylogenet Evol* 33:289–299
- Blattner FR (2006) Multiple intercontinental dispersals shaped the distribution area of *Hordeum* (Poaceae). *New Phytol* 169:603–614
- Blattner FR (2009) Advances in phylogenetic analysis and new infrageneric classification of *Hordeum* (Poaceae). *Breed Sci* 59:471–480
- von Bothmer R, Jacobsen N (1985) Origin, taxonomy and related species. In: Rasmussen DC (ed) *Barley. Monographs in agronomy*, vol 26. American Society of Agronomy, Madison, WI, pp 19–56
- von Bothmer R, Jacobsen N, Baden C, Jørgensen RB, Linde-Laursen I (1995) An ecogeographical study of the genus *Hordeum*, 2nd edn, Systematic and ecogeographic studies on crop gene pools 7. International Plant Genetic Resources Institute, Rome
- Coyne JA, Orr HA (2004) *Speciation*. Sinauer Associates, Sunderland
- Darwin C (1859) *The origin of species by means of natural selection*, 6th edn. John Murray, London
- Dewey RD (1984) The genomic system of classification as a guide to intergeneric hybridization with the perennial Triticeae. In: Gustafson JP (ed) *Gene manipulation in plant improvement*. Plenum, New York, pp 209–279
- Doebley J, von Bothmer R, Larson S (1992) Chloroplast DNA variation and the phylogeny of *Hordeum* (Poaceae). *Am J Bot* 79:576–584
- Gaut BS (2002) Evolutionary dynamics of grass genomes. *New Phytol* 154:15–28
- Jakob SS, Meister A, Blattner FR (2004) The considerable genome size variation of *Hordeum* species (Poaceae) is linked to phylogeny, life form, ecology, and speciation rates. *Mol Biol Evol* 21:860–869
- Jakob SS, Blattner FR (2006) A chloroplast genealogy of *Hordeum* (Poaceae): long-term persisting haplotypes, incomplete lineage sorting, regional extinction, and the consequences for phylogenetic inference. *Mol Biol Evol* 23:1602–1612
- Jakob SS, Ihlow A, Blattner FR (2007) Combined ecological niche modelling and molecular phylogeography revealed the evolutionary history of *Hordeum marinum* (Poaceae) – niche differentiation, loss of genetic diversity, and speciation in Mediterranean Quaternary refugia. *Mol Ecol* 16:1713–1727
- Jakob SS, Blattner FR (2010) Two extinct diploid progenitors were involved in allopolyploid formation in the *Hordeum murinum* (Poaceae: Triticeae) taxon complex. *Mol Phylogenet Evol* 55:650–659
- Jakob SS, Martinez-Meyer E, Blattner FR (2009) Phylogeographic analyses and paleodistribution modeling indicates Pleistocene in situ survival of *Hordeum* species (Poaceae) in southern Patagonia without genetic or spatial restriction. *Mol Biol Evol* 26:907–923
- Jakob SS, Heibl C, Rödder D, Blattner FR (2010) Population demography influences climatic niche evolution: evidence from diploid South American *Hordeum* species (Poaceae). *Mol Ecol* 19:1423–1438
- Komatsuda T, Tanno K, Salomon B, Bryngelsson T, von Bothmer R (1999) Phylogeny in the genus *Hordeum* based on nucleotide sequences closely linked to the *vrs1* locus (row number of spikelets). *Genome* 42:973–981
- Linde-Laursen I, von Bothmer R, Jacobsen N (1992) Relationships in the genus *Hordeum*: Giemsa C-banded karyotypes. *Hereditas* 116:111–116

- Löve A (1984) Conspectus of the Triticeae. Feddes Repert 95:425–521
- Mason-Gamer RJ (2001) Origin of North American species of *Elymus* (Poaceae: Triticeae) allotetraploids based on granule-bound starch synthase gene sequences. Syst Bot 26:757–768
- Nevski SA (1941) Beiträge zur Kenntnis der wild wachsenden Gersten in Zusammenhang mit der Frage über den Ursprung von *Hordeum vulgare* L. und *H. distichon* L. (Versuch einer Monographie der Gattung *Hordeum*). Trudy Bot Inst Akad Nauk SSSR Ser 1(5):64–255
- Nishikawa T, Salomon B, Komatsuda T, von Bothmer R, Kadowaki K (2002) Molecular phylogeny of the genus *Hordeum* using three chloroplast DNA sequences. Genome 45:1157–1166
- Petersen G, Seberg O (2003) Phylogenetic analyses of the diploid species of *Hordeum* (Poaceae) and a revised classification of the genus. Syst Bot 28:293–306
- Petersen G, Seberg O (2004) On the origin of the tetraploid species *Hordeum capense* and *H. secalinum* (Poaceae). Syst Bot 29:862–873
- Pleines T, Blattner FR (2008) Phylogeographic implications of an AFLP phylogeny of the American diploid *Hordeum* species (Poaceae: Triticeae). Taxon 57:875–881
- Pleines T, Jakob SS, Blattner FR (2009) Application of non-coding DNA regions in intraspecific analyses. Plant Syst Evol 282:281–294
- Posada D, Crandall KA (2001) Intraspecific gene genealogies: trees grafting into networks. Trends Ecol Evol 16:37–45
- Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574
- Sanderson MJ (2002) Estimating absolute rates of molecular evolution and divergence times: a penalized likelihood approach. Mol Biol Evol 19:101–109
- Shaw J, Lickey EB, Schilling EE, Small RL (2007) Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: the tortoise and the hare III. Am J Bot 94:275–288
- Swofford DL (2002) PAUP*. Phylogenetic analysis using parsimony (* and other methods), version 4. Sinauer Associates, Sunderland
- Taketa S, Harrison GE, Heslop-Harrison JS (1999) Comparative physical mapping of the 5S and 18S–25S rDNA in nine wild *Hordeum* species and cytotypes. Theor Appl Genet 98:1–9
- Taketa S, Ando H, Takeda K, von Bothmer R (2001) Physical location of 5S and 18S–25S rDNA in Asian and American diploid *Hordeum* species with the I genome. Heredity 86:522–530
- Thinggaard K (2001) Population structure and genetic diversity of the amphiatlantic haploid peatmoss *Sphagnum affine* (Sphagnopsida). Heredity 87:485–496
- Vos P, Hogers R, Bleeker M, Reijns M, van de Lee T, Hornes M, Freuters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995) AFLP, a new technique for DNA fingerprinting. Nucleic Acids Res 23:4407–4414
- Wang RRC, von Bothmer R, Dvorak J, Fedak G, Linde-Laursen I, Muramatsu M (1996) Genome symbols in the Triticeae. In: Wang RRC, Jensen KB, Jaussi C (eds) Proceedings of the 2nd international Triticeae symposium. Utah State University, Logan, pp 29–34

Evolution in Action

Case studies in Adaptive Radiation, Speciation and the
Origin of Biodiversity

Glaubrecht, M. (Ed.)

2010, XXV, 586 p., Hardcover

ISBN: 978-3-642-12424-2