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# Phylogenetic analysis of the genus *Hordeum* using repetitive DNA sequences

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**Abstract** A set of six cloned barley (*Hordeum vulgare*) repetitive DNA sequences was used for the analysis of phylogenetic relationships among 31 species (46 taxa) of the genus *Hordeum*, using molecular hybridization techniques. *In situ* hybridization experiments showed dispersed organization of the sequences over all chromosomes of *H. vulgare* and the wild barley species *H. bulbosum*, *H. marinum* and *H. murinum*. Southern blot hybridization revealed different levels of polymorphism among barley species and the RFLP data were used to generate a phylogenetic tree for the genus *Hordeum*. Our data are in a good agreement with the classification system which suggests the division of the genus into four major groups, containing the genomes **I**, **X**, **Y**, and **H**. However, our investigation also supports previous molecular studies of barley species where the unique position of *H. bulbosum* has been pointed out. In our experiments, *H. bulbosum* generally had hybridization patterns different from those of *H. vulgare*, although both carry the **I** genome. Based on our results we present a hypothesis concerning the possible origin and phylogeny of the polyploid barley species *H. secalinum*, *H. depressum* and the *H. brachyantherum* complex.

**Key words** *Hordeum* · Phylogeny
Repetitive DNA sequences · RFLP · *In situ* hybridization

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## Introduction

Next to *Elymus*, *Hordeum* L. is the largest genus in the tribe Triticeae and comprises about 50 taxa (Bothmer et al. 1991). Modern classification of the genus is mainly based on morphometric data, the capacity of different species to produce fertile progeny in crosses, and the analysis of meiotic pairing of chromosomes in hybrids (cf. Bothmer et al. 1991). Löve (1982, 1984) suggested a division of *Hordeum* into two separate genera, namely *Hordeum* L. with the sole member *H. vulgare*, and *Critesion* Raf., including all other barley species. Dewey (1984) supported this division, but also included *H. bulbosum* in *Hordeum* as both *H. vulgare* and *H. bulbosum* carried the **I** genome. Later, Bothmer et al. (1986, 1987), based on the meiotic pairing behaviour of different interspecific combinations of *Hordeum* species, suggested the presence of four "basic genomes" designated **I**, **Y**, **X** and **H**. The wide circumscription of the genus *Hordeum* of Bothmer et al. (1991), including species with different basic genomes, has been generally accepted. Recently, molecular methods have been used to clarify phylogenetic relationships within the Triticeae. Repetitive DNA sequences such as rDNA, hordein genes and various non-coding sequences (Appels et al. 1989; Dvorák and Zhang 1992 a,b) as well as cDNA clones (Monte et al. 1993; Takumi et al. 1993) have been used. Repetitive DNA sequences from *H. vulgare* were used to analyze some *Hordeum* species (Vershinin et al. 1990; Shcherban and Vershinin 1992). Phylogenetic studies of barley species were also performed with the repetitive DNA sequence pSc119 from rye (Gupta et al. 1989), the ribosomal spacer DNA sequence pTa71 from wheat (Mol-

**Table 1** *Hordeum* species used, including accession numbers (or cultivars) from the collection of Department of Plant Breeding Research (Svalöv, Sweden), genomic constitution (Dewey 1984; von Bothmer and Jacobsen 1986), ploidy level and origin

| Species | Subspecies | Ploidy | Genome | Acc. code | Origin |
|---|---|---|---|---|---|
| **I-genome species:** | | | | | |
| *H. vulgare* L. | | | | | |
| | ssp. *vulgare* | 2x | I | cv Betzes | Obtained from Svalöf-Weibull AB |
| | ssp. *vulgare* | 2x | I | cv Sultan* | Obtained from Risø |
| | ssp. *vulgare* | 2x | I | cv Igri* | Obtained from Risø |
| | ssp. *spontaneum* (C. Koch) Thell. | 2x | I | H3142 | Cyprus, Proteras |
| | ssp. *agriocrithon* (Åberg) Scholz | 2x | I | H7019 | China, Tibet |
| *H. bulbosum* L. | | 2x | I | H3023 | Greece, Peloponnesos, Lakonia |
| *H. bulbosum* L. | | 2x | I | H391* | Greece, Ioannina |
| *H. bulbosum* L. | | 4x | II | H408 | Turkey, Yozgat |
| *H. bulbosum* L | | 4x | II | H2367* | Greece, Kos |
| **Y-genome species:** | | | | | |
| *H. murinum* L. | | | | | |
| | ssp. *murinum* | 4x | YY | H217 | Germany, Berlin, Dahlem |
| | ssp. *glaucum* (Steud.) Tzvel. | 2x | Y | H39 | Turkey, Izmir |
| | ssp. *leporinum* (Link) Arcanggeli | 4x | YY | H199 | Greece, Crete |
| | ssp. *leporinum* (Link) Arcanggeli | 6x | YYY | H796 | Iran, W Azarbaijan |
| **X-genome species**: | | | | | |
| *H. marinum* Huds. | | | | | |
| | ssp. *marinum* | 2x | X | H759 | Greece, Crete, Ag. Nikolaos |
| | ssp. *gussoneanum* (Parl.) Thell. | 2x | X | H126 | Greece, Amorgos, Cyclades |
| | ssp. *gussoneanum* (Parl.) Thell. | 4x | XX | H819 | Turkey, Vil. Erzurum |
| | ssp. *gussoneanum* (Parl.) Thell. | 4x | XX | H825* | Turkey, Vil. Bulo |
| **H-genome species:** | | | | | |
| H. *muticum* Presl. | | 2x | H | H6468 | Argentina, Jujuy, Senador Perez |
| H. *cordobense* Bothm. et al. | | 2x | H | H1711 | Argentina, Cordoba, Bialet Massé |
| H. *chilense* Roem. et Schult. | | 2x | H | H1816 | Chile, Coquimbo, Los Vilos |
| H. *stenostachys* Godr. | | 2x | H | H6437 | Argentina, La Rioja, El Potrerillo |
| H. *erectifolium* Bothm. et al. | | 2x | H | H1150 | Argentina, Buenos Aires, Bahia Blanca |
| H. *flexuosum* Steud. | | 2x | H | H1116 | Argentina, Buenos Aires, Juares |
| H. *euclaston* Steud. | | 2x | H | H2148 | Uruguay, Paysandu |
| H. *intercedens* Nevski | | 2x | H | H1941 | USA, California, Lakeview |
| H. *pusillum* Nutt. | | 2x | H | H1906 | USA, Colorado, Denver. |
| H. *jubatum* L. | | 4x | H | H1162 | Argentina, Buenos Aires, Guanini |
| H. *comosum* Presl. | | 2x | H | H1181 | Argentina, Rio Negro, S. C. Bariloche |
| H. *pubiflorum* Hook. f. | | 2x | H | H6360 | Argentina, Neuquen, Lago Tromen |
| H. *lechleri* (Steud.) Schenck | | 6x | HHH | H6344 | Argentina, Neuquen, Ao. Carrei |
| H. *procerum* Nevski | | 6x | HHH | H1781 | Argentina, San Juan, San José |
| H. *arizonicum* Covas | | 6x | HHH | H3253 | USA, Arizona, Picacho |
| H. *secalinum* Schreb. | | 4x | HH | H296 | Spain, 14 km E Huelva |
| H. *bogdanii* Wil. | | 2x | H | H8700 | China, Xinjiang, Altai co. |
| H. *roshevitzii* Bowd. | | 2x | H | H8787 | China, Xinjiang, Jeminay co. |
| H. *brevisubulatum* (Trin.) Link | | | | | |
| | ssp. *violaceum* (Boiss. et Hohen.) Tzvel. | 4x | HH | H306 | Turkey, Vil. Erzurum |
| | ssp. *nevskianum* (Bowd.) Tzvel. | 4x | HH | H10162 | Russia, Altai, Kuraj |
| | ssp. *turkestanicum* (Nevski) Tzvel. | 4x | HH | H9254* | China, Xinjiang |

| | | | | | |
|---|---|---|---|---|---|
| H. *brachyantherum* Nevski | ssp. *brachyantherum* | 4x | HH | H4216 | USA, Wyoming, Teton co. |
| | ssp. *brachyantherum* Nevski | 6x | HHH | H2001 | USA, California, Louis Obispo co. |
| | ssp. *californicum* (Covas et Stebb.) Bothm. et al. | 2x | H | H2401 | USA, California, San Diego co., |
| H. *depressum* (Scribn. et Smith) Rydb. | | 4x | HH | H2308 | USA, California, Kings co. |
| H. *guatemalense* Bothm. et al. | | 4x | HH | H2299 | Guatemala, Sierra de los Cuchumatanes |
| H. *capense* Thunb. | | 4x | HH | H334 | South Africa, Ventersted, Oviston |
| H. *parodii* Covas | | 6x | HHH | H6255 | Argentina, Chubut, Alto Rio Senguerr |
| H. *tetraploidum* Covas | | 4x | HH | H6364 | Argentina, Mendoza, Laguana Coipillaugen |
| H. *fuegianum* Bothm et. al. | | 4x | HH | H6156 | Argentina, Tierra del Fuego, Rio Grande |
| H. *patagonicum* (Haumann) Covas | | | | | |
| | ssp. *patagonicum* | 2x | H | H1520 | Argentina, Santa Cruz, Rio Deseado |
| | ssp. *mustersii* (Nicora) Bothmer et al. | 2x | H | H1358 | Argentina, Santa Cruz, Ea. Los Pozos |
| | ssp. *santacrucense* (Parodi et Nicora) Bothmer et al. | 2x | H | H6054 | Argentina, Santa Cruz, S. St. Julian |
| | ssp. *setifolium* (Parodi et Nicora) Bothmer et al. | 2x | H | H1352 | Argentina, Santa Cruz, NW Rio Gallegos |
| | ssp. *magellanicum* (Parodi et Nicora) Bothmer et al. | 2x | H | H6106 | Argentina, Tierra del Fuego |

* Indicates cytotypes which were used only for *in situ* hybridization

nar et al. 1989, 1992) and the tandemly-organized repetitive DNA sequence dpTa1 from wheat (Vershinin et al. 1994). However, limited numbers of species and/or probes have been used in each investigation.

In phylogenetic studies it is important to use a broad set of DNA sequences since more regions of the genome become involved and, consequently, more detailed information can be obtained. In this investigation, we have used six repetitive DNA sequences, comprising approximately 5–6% of the *H. vulgare* genome. Comparative RFLP mapping is playing an increasing role in phylogenetic studies but repetitive DNA sequences are still very informative since: (1) repetitive DNA represents 75–80% of the cereal genome (Flavell et al. 1977; Rimpau et al. 1980); (2) repetitive sequences change rapidly, whereas low-copy sequences are generally conserved (Jelinek and Schmid 1982); and (3) both tandemly-organized and dispersed repetitive DNA sequences are usually distributed throughout the whole genome. These facts are important in phylogenetic studies because different regions of the genome evolve at different speeds. Repetitive DNA sequence families also show low intraspecific variation which makes it possible to use only one or a few accessions per taxon or cytotype for analysis (Dvorák and Zhang 1992 b).

The aims of the investigation reported here were to study phylogenetic relationships in the genus *Hordeum* using barley repetitive DNA sequences as probes, to study differences between *Hordeum* species which belong to groups with different genomes and, finally, to elucidate relationships between diploid and polyploid barley species.

## Materials and methods

### Plant material

Forty-six cytotypes from the genus *Hordeum* (31 species) were studied (seed collection of Department of Plant Breeding Research, Svalöv). Species, accessions, their sources, genomic constitution and chromosome number are listed in Table 1.

### Probes

Six repetitive DNA sequences, pHv7036, pHv7161, pHv7191, pHv7223, pHv7241 and pHv7293, cloned from the *H. vulgare* genome were used as probes (see Vershinin et al. 1990). Plasmid DNA was labelled with digoxigenin as described by the supplier (DIG DNA Labeling and Detection Kit, Boehringer Mannheim).

### DNA isolation, blotting and hybridization

Total genomic DNA was extracted from green leaves according to Ausubel et al. (1987) and digested with restriction endonucleases. The DNA fragments were separated on 0.9% agarose gels and transferred to GeneScreen nylon membranes (Du Pont) by capillary blotting. After transfer, membranes were rinsed with 2×SSC and the DNA was cross-linked in a Stratalinker 2400 (Stratagene) using the "automatic crosslink" regime. Prehybridization (2 h) and hybridization (overnight) was carried out at 65 °C in 5×SSC, 0.5% blocking reagent (Boehringer Mannheim), 0.1% N-laurylsarcosine, and 0.02% SDS. DIG-labeled DNA (10–15 ng/ml) was included in the hybridization solution. After hybridization, membranes were consequent-

ly washed in 2× SSC; 2 × SSC, 0.1% SDS; 0.5 × SSC, 0.1% SDS and 0.1 × SSC, 0.1% SDS for 20 min each at room temperature. Chemiluminescent detection was carried out as described by Kreike et al. (1990).

*In situ* hybridization

Plant material for *in situ* hybridization included seedlings of *H. vulgare* cvs Sultan and Igri, *H. murinum* ssp. *murinum*, *H. marinum* ssp. *gussoneanum* (4x) and plants of *H. bulbosum* (2x and 4x) and *H. brevisubulatum* ssp. *turkestanicum* (4x). Root tips were pretreated, fixed and squashed after enzyme treatment according to Pedersen and Linde-Laursen (1994). All probes exept pHv7293 were labelled by nick-translation with biotin and *in situ* hybridization was performed essentially as described previously using the biotin-avidin-based fluorescence detection system (Pedersen and Linde-Laursen 1994). The slides were examined with a Zeiss Photomicroscope III and photographs were taken on Kodak Ektachrome P800/1600 professional film for colour slides.

## Results

*In situ* hybridization

The five probes hybridized more or less uniformly along the *H. vulgare* chromosome arms (Fig. 1). The strongest hybridization signal was obtained with pHv7191, followed by pHv7161, pHv7036, pHv7241 and pHv7223. Although all probes showed a dispersed organization, some differences in their chromosomal distribution were observed. The probes pHv7036 and pHv7223 hybridized more weakly in the regions proximal to the centromeres (Fig. 1 A and B), whereas the remaining probes hybridized rather uniformly along the chromosomes leaving only a narrow band free from yellow fluorescence at the site of the centromeres (Fig. 1 C and D).

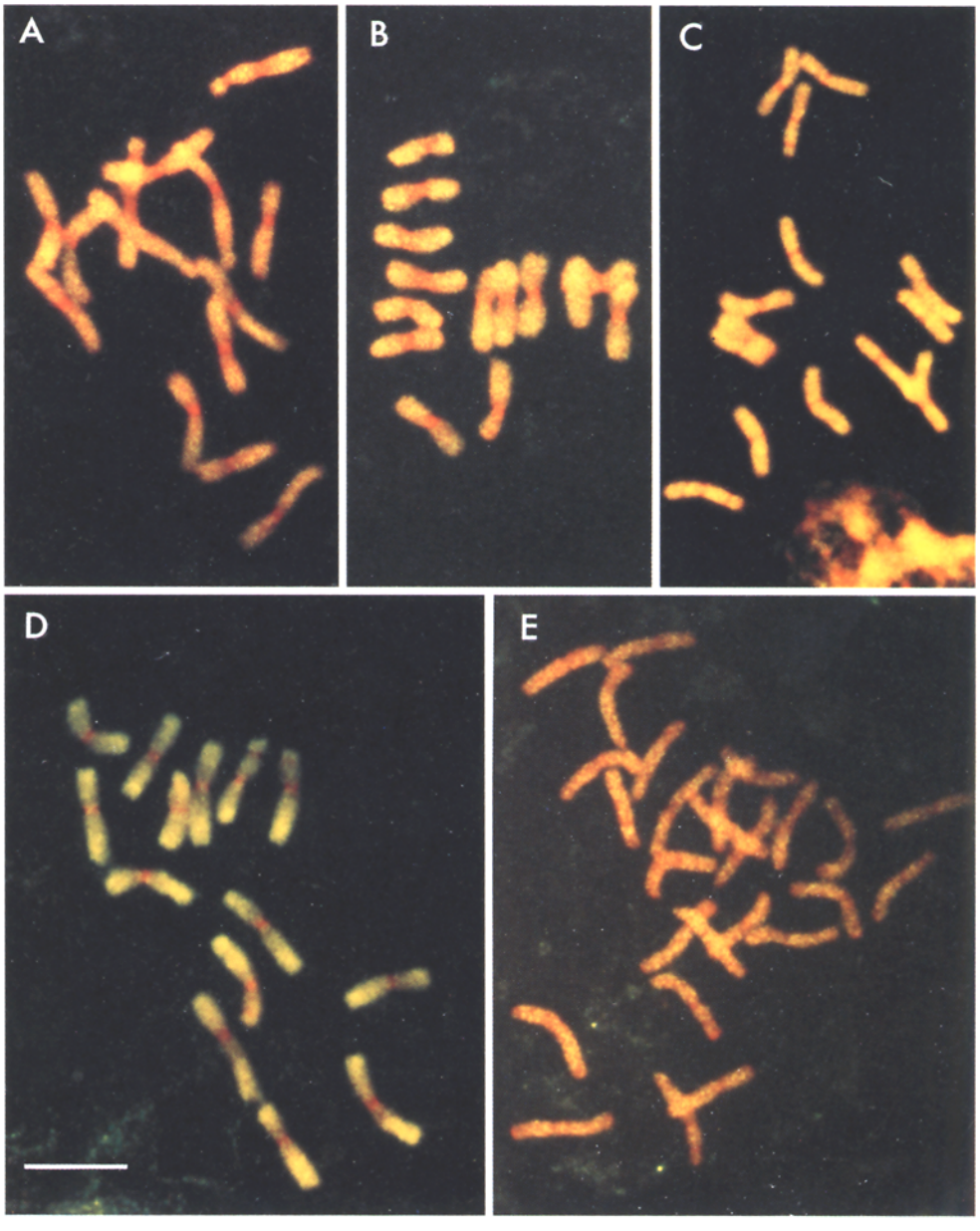


**Fig. 1** Chromosomes of barley (*Hordeum vulgare*) (**A–D**) and *H. brevisubulatum* ssp. *turkestanicum* (**E**) at somatic metaphase after *in situ* hybridization with the probes pHv7223 (**A**), pHv7036 (**B**), pHv7241 (**C**), pHv7191 (**D**) and pHv7161 (**E**). Bar = 10 μm

Among the wild barley species, the strongest hybridization signal was obtained with pHv7161, showing a dispersed organization in all species examined (Fig. 1 E). The hybridization signal was, however, significantly weaker in the wild species compared to cultivated barley.

## Phylogenetic analysis

For the analysis of intra- and inter-specific variation, six repetitive DNA sequences were hybridized to restricted DNA from 46 cytotypes of the genus *Hordeum* (31 species). A total of 14 probe/enzyme combinations were analyzed and 179 bands were scored. Only 17 bands were present in all analyzed taxa while the remaining were informative.

Each hybridizing band was treated as a unit character and all species were scored for the presence or absence of each band. Similarities in band patterns were then calculated for all pairwise combinations of species using the similarity index described by Nei and Li (1979). The calculated similarities were transformed to distances by subtracting the similarity index from unity. Finally, the estimated distance between taxa were compiled in a standard cluster analysis (UPGMA), using the SAS programme package, to reveal relationships among the investigated taxa.

When more than one probe/enzyme combination is used, a single evolutionary event can be scored several times so that the resulting phylogenetic tree would be incorrect (Dvorák and Zhang 1992 b). Different probes can give rise to various numbers of hybridizing bands and, consequently, some probes will have a higher input than others. To avoid these problems we created separate trees for each probe using several probe/enzyme combinations, which we later combined to generate an average tree (Fig. 2). By using this approach, each probe makes an equal contribution to the resulting tree. The use of separate trees also enabled us to determine the coherence of the results by comparing different trees. In addition, in order to further assess the robustness of the results, the data were analysed with the PHYLIP computer package (Felsenstein 1990) which permitted a formal bootstrap analysis.

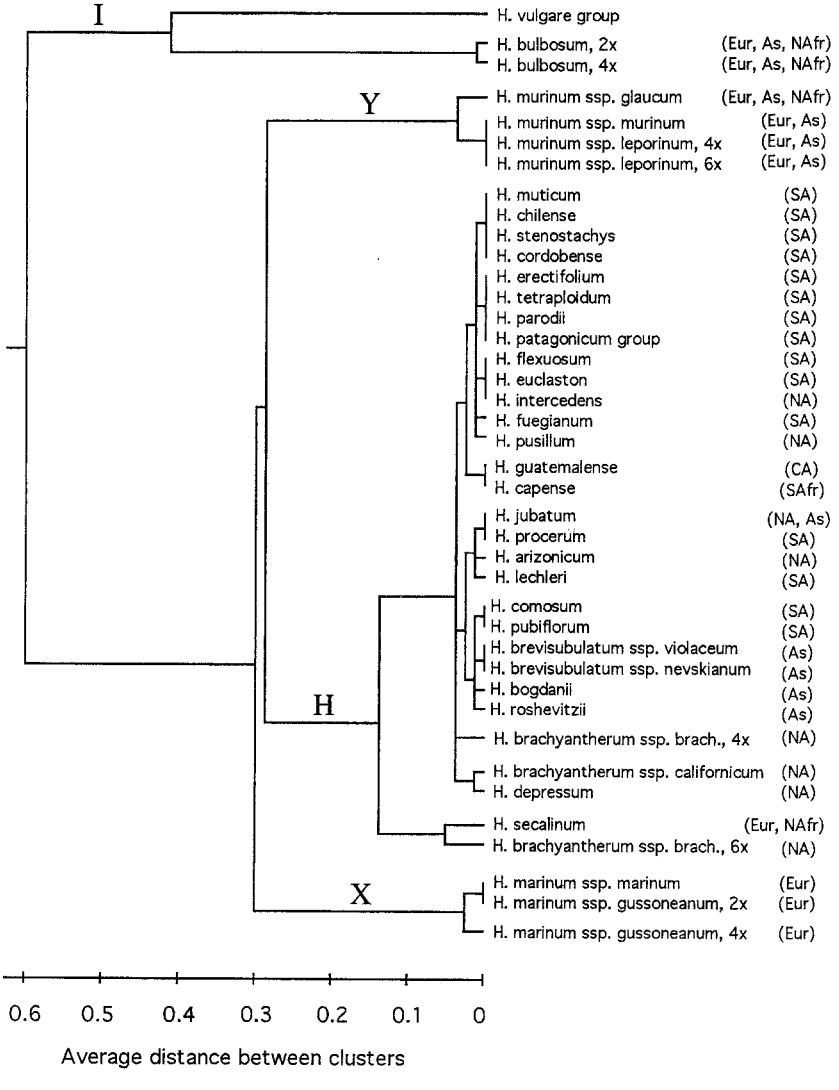


**Fig. 2** *Hordeum* genus dendrogram based on the UPGMA cluster analysis. The geographical distribution of the species is given in parentheses. *Eur*, Europe; *As*, Asia; *NAfr*, Northern Africa; *SA*, South America; *NA*, Northern America; *CA*, Central America; *SAfr*, South Africa

Differences in the intensity of the hybridization signal were often observed between species. This variation can be generated in various ways (Dvorák and Zhang 1992 b) and has not been considered in the present investigation.

## Interspecific polymorphism

High levels of polymorphism were observed between species with different genomes. The *H. murinum* group (**Y**), the *H. marinum* group (**X**) and a large group of wild barley species with the **H** genome could easily be distinguished. The trees based on each probe consistently separated the **H** genome, **X** genome and **Y** genome containing species into distinct clusters. The **I** genome species *H. vulgare* and *H. bulbosum* do not demonstrate a consistent clustering in the different probe trees generated. Five of the six probes used gave different patterns for *H. vulgare* and *H. bulbosum* (for pHv7161 see Fig. 3 A). Only one probe, pHv7223, produced a similar hybridization pattern in both species. This probe hybridized mainly to DNA from species belonging to the *H. vulgare* and *H. bulbosum* complex and showed very weak and mostly unclear patterns for other *Hordeum* species (Fig. 3 B).

Species containing the **H** genome generally show a low level of polymorphism. In spite of the fact that the branches within the group of **H** genome species do not show high levels of bootstrap confidence, our data are in agreement with previous classification systems and the geographical distribution of the species. The tree clustered together all South American diploids, except *H. comosum* and *H. pubiflorum*, two South American polyploids, *H. tetraploidum* and *H. parodii*, and two North American species, *H. intercedens* and *H. pusillum*. Another four American polyploids, *H. jubatum*, *H. procerum*, *H. arizonicum* and *H. lechleri*, form another group. Three Asian species *H. roshevitzii*, *H. bogdanii* and *H. brevisubulatum*, are also clustered together. In the **H** genome group, *H. secalinum*, *H. brachyantherum* and *H. depressum* are distinct from the rest of the species. *H. depressum* exhibits a hybridization pattern very similar to that of *H. brachyantherum* ssp. *californicum*. The hexaploid cytotype of *H. brachyantherum* and *H. secalinum* have bands which are typical for species of the *H. marinum* group when the clones pHv7161,

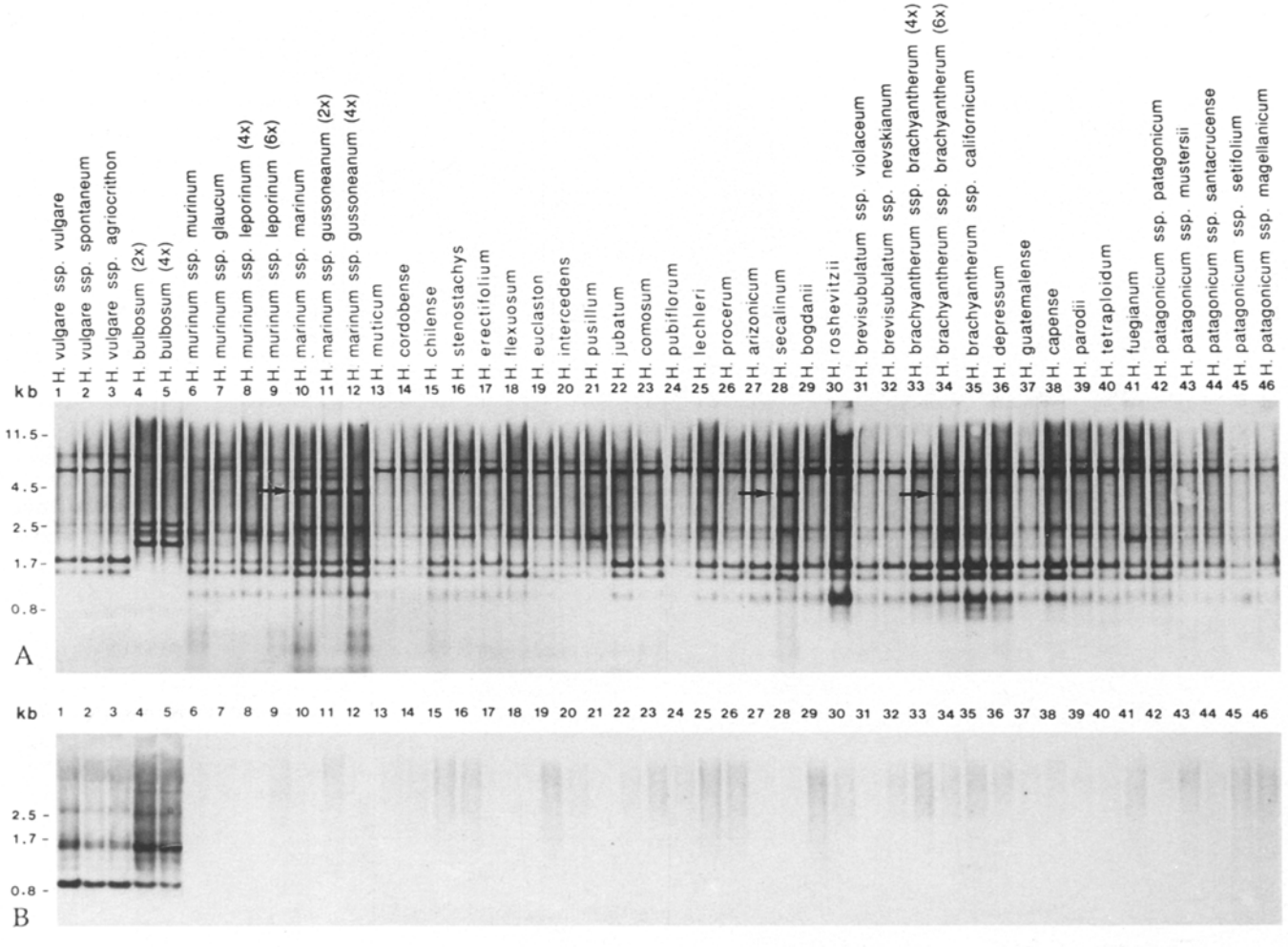


**Fig. 3** Hybridization of pHv7161 to *Bgl*II (**A**)- and pHv7223 to *Hin*dIII (**B**)- digested DNA from *Hordeum* species. *Arrows* indicate the hybridization bands which are characteristic for the *H. marinum* complex, *H. secalinum* and *H. brachyantherum* ssp. *brachyantherum* (6x)

pHv7036, pHv7191 and pHv7241 were used (for pHv7161 see Fig. 3 A). To confirm these intriguing results, other accessions of *H. secalinum*, *H. brachyantherum* ssp. *californicum*, *H. brachyantherum* ssp. *brachyantherum* (6x) and *H. depressum* were analyzed. Identical hybridization patterns were obtained (data not shown). Slight variation was also demonstrated for the two Asiatic species *H. roshevitzii* and *H. bogdanii* as well as for *H. guatemalense* and *H. capense* (Figs. 2 and 3 A).

### Intraspecific variation

Many species were represented by subspecies and/or cytotypes (see Table 1). In general, low levels of intraspecific variation were found (Figs. 2 and 3). No differences were observed for *H. murinum* ssp. *murinum*, ssp. *leporinum* (4x), and ssp. *leporinum* (6x), while *H. murinum* ssp. *glaucum* was clearly different. In the *H. marinum* complex, *H. marinum* ssp. *gussoneanum* (4x) was slightly different from ssp. *marinum* and ssp. *gussoneanum* (2x). A high level of polymorphism was seen in the three subspecies of the *H. brachyantherum* group, i.e. ssp. *brachyantherum* (4x and 6x), and ssp. *californicum*. Minor differences in the hybridization pattern were observed for diploid and tetraploid cytotypes of *H. bulbosum* while no variation was found in the *H. vulgare*, *H. brevisubulatum* and the *H. patagonicum* groups.

## Discussion

*In situ* hybridization with five of the six probes demonstrated a dispersed sequence organization in all investigated *Hordeum* species. This is in agreement with other highly repeated DNA sequences from barley, which also show a dispersed organization, e.g. the BIS 1 element (Moore et al. 1991), the Apa1 element (Lehfer 1992) and the Dialect-I species-specific repeat (Sonina et al. 1989). Only the $(GAA)_m(GAG)_n$ satellite sequence (Dennis et al. 1980) and the HvRT-family (Belostotsky and Ananiev 1990), which are both tandemly repeated, hybridize to distinct chromosomal positions (Röder et al. 1993; Pedersen and Linde-Laursen, 1994). The dispersed organization of the five repetitive DNA sequences used for *in situ* hybridization, and the fact that together they comprise about 5–6% of the *H. vulgare* genome, indicate that they are suitable candidates for phylogenetic studies.

The phylogenetic tree obtained in this study is consistent with previous data, particularly with the classification system which divides the genus *Hordeum* into four major groups with genomes **I**, **X**, **Y**, and **H** (Bothmer et al. 1986, 1987, 1991). A minor disagreement is evident in the relatively large difference between the *H. vulgare* complex and the diploid and tetraploid cytotypes of *H. bulbosum*. Based mainly on cytogenetic data, Dewey (1984) combined *H. vulgare* and *H. bulbosum* in one group with the genome designation **I**. This hypothesis was later supported by crossing experiments and studies of meiotic pairing in the $F_1$ hybrids (Bothmer et al. 1983, 1986, 1987), by C-banding studies (Linde-Laursen et al. 1990, 1992 a), isoenzyme analysis (Jørgensen 1986), cpDNA restriction analysis (Kataoka et al. 1987; Doebley et al. 1992), and by studies of hordein composition using monoclonal antibodies (Pelger and Bothmer 1992). However, Hsiao et al. (1986) and Linde-Laursen et al. (1990) showed that the karyotypes of these two species differ significantly in morphology. Recent phylogenetic studies, based on molecular hybridization techniques using the repetitive DNA sequence pSc119 (Gupta et al. 1989; Molnar et al. 1989; Xu et al. 1990), the ribosomal DNA sequence pTa71 (Molnar at al. 1992) and a repetitive *Bam*HI fragment (Shcherban and Vershinin 1992) as probes, have shown that these two species are probably not very closely related. Shcherban and Vershinin (1992) even proposed the separation of *H. bulbosum* into a fifth group with another genome designation. Molnar et al. (1989), who based their analysis on ribosomal DNA, suggested that *H. bulbosum* is more closely related to the *H. murinum* complex than to *H. vulgare*. In our experiments the hybridization patterns of *H. vulgare* and *H. bulbosum* are distinctly different for five of six probes. However, probe pHv7223 showed a similar hybridization pattern for both species. Moreover, this clone hybridized mainly to DNA from species belonging to the *H. vulgare* and *H. bulbosum* complex and did not hybridize distinctly to any of the other species (Fig. 3B). This observation suggests that *H. vulgare* and *H. bulbosum* may have a monophyletic origin. The separation from the other *Hordeum* species probably occurred at a relatively early stage and only later did *H. bulbosum* and *H. vulgare* diverge from their common ancestor. Our analysis suggests that *H. vulgare* and *H. bulbosum* are distinctly different and that their combination into one group with the same type of genome is open to discussion.

The *Hordeum murinum* complex is probably one of the best-studied groups in the genus *Hordeum*. However, there is no definite agreement concerning the taxonomy of this complex (Jacobsen and Bothmer 1994). Our data show that the *H. murinum* group is rather homogeneous and isolated within the genus (Figs. 2 and 3 A). This is consistent with previous reports based on C-banding patterns (Linde-Laursen et al. 1989, 1992 a), cpDNA variation (Doebley et al. 1992), and chromosome pairing in $F_1$ hybrids (Bothmer et al. 1988). Our study supports the suggestion that *H. murinum*, *H. leporinum* (4x and 6x), and *H. glaucum*, treated earlier as separate species, should be considered subspecies of *H. murinum* (Jørgensen 1986; Bothmer et al. 1987; Jacobsen and Bothmer 1994). *H. murinum* ssp. *glaucum* is the most deviant taxon within this group. Similar results were obtained with cpDNA analysis (Baum and Bailey 1991). Based on isoenzyme variation, Jaaska (1992) suggested that the only known diploid of the *H. murinum* group, i.e., ssp. *glaucum*, was not involved in the generation of the polyploids. Jørgensen (1986), also using isoenzyme analysis, considered the *H. murinum* complex a sister group of the *H. vulgare* and *H. bulbosum* complex. Pelger and Bothmer (1992) arrived at a similar conclusion

based on hordein variation. An analysis of rDNA showed similarities between ssp. *glaucum* and ssp. *murinum* and indicated that these two taxa were closer to *H. bulbosum* than to ssp. *leporinum* (Molnar et al. 1992). However, our data do not support this suggestion.

Studies of cpDNA (Kataoka et al. 1987; Baum and Bailey 1991; Doebley et al. 1992) and rDNA (Molnar et al. 1989) variation indicated a rather close relationship between *H. murinum* and *H. marinum*. Our investigation demonstrates no strong similarity between these two groups and, hence, does not support this hypothesis (Figs. 2 and 3 A). On the whole, the position of *H. marinum* and its relationship to other *Hordeum* species is rather unclear. Crosses and meiotic behaviour of chromosomes in hybrids revealed that the three *H. marinum* cytotypes are closely related (Bothmer et al. 1989). Linde-Laursen et al. (1989) showed that *H. marinum* ssp. *marinum* (2x) and ssp. *gussoneanum* (2x) have identical C-banding patterns which is in agreement with a classification of the taxa as subspecies. The analysis and physical localization of active and inactive rRNA gene loci by *in situ* hybridization (Linde-Laursen et al. 1992 b) further supported the autoploid origin of the tetraploid cytotype of *H. marinum*. However, analysis of isoenzyme variation (Jaaska and Jaaska 1986; Jørgensen 1986; Jaaska 1992) and cpDNA diversity (Baum and Bailey 1991; Doebley et al. 1992) suggested a more distant relationship between *H. marinum* ssp. *marinum* and *H. marinum* ssp. *gussoneanum* (syn. *H. geniculatum*). It has been proposed that the two taxa should be considered separate species. Both Baum and Bailey (1991) and Doebley et al. (1992) found *H. marinum* in the same clade as the *H. murinum* complex (the *H. leporinum* complex for Baum and Bailey). In our experiments the *H. marinum* complex was very homogeneous and gave specific hybridization patterns. A slight variation in the hybridization pattern was observed only for *H. marinum* ssp. *gussoneanum* (4x). This confirms the proposal that *H. marinum* ssp. *marinum* and *H. marinum* ssp. *gussoneanum* are subspecies which carry the **X** genome (Bothmer et al. 1987).

The large group of **H**-genome species is relatively homogeneous (Figs. 2 and 3 A). The most divergent species are *H. secalinum* and *H. brachyantherum* ssp. *brachyantherum* (6x). It has been suggested that *H. secalinum* is an alloploid species with one **H** genome and one genome of unknown origin (Bothmer et al. 1988; Jacobsen and Bothmer 1992; Linde-Laursen et al. 1992 a). There are some cytological (Bothmer et al. 1988), isoenzyme (Jørgensen 1986) and molecular (Shcherban and Vershinin 1992) data which imply a connection between *H. secalinum* and *H. marinum*. Doebley et al. (1992) based their study on cpDNA variation and found that tetraploid *H. marinum* ssp. *gussoneanum* deviates markedly from the diploid members of this species and is most closely related to the hexaploid form of *H. brachyantherum*, which is contradictory to other available information (Jaaska and Jaaska 1986; Jørgensen 1986; Bothmer et al. 1989). Our investigation may contribute to an understanding of these results and, presumably, the evolution of *H. secalinum* and hexaploid *H. brachyantherum*. We found that these two species most often have hybridization patterns which differ from the rest of the **H**-genome species, which in turn are very similar to patterns characteristic for the *H. marinum* complex (for pHv7161 see Fig. 3 A). This suggests that *H. marinum* might be involved in the evolution of *H. secalinum* and *H. brachyantherum* (6x). Geographically, both *H. marinum* and *H. secalinum* are of European origin and thus it is not unlikely that *H. marinum* has been involved in the evolution of *H. secalinum*. The hexaploid cytotype of *H. brachyantherum* occurs as a single population in California (Bothmer et al. 1993) and is possibly quite young in evolutionary terms. The participation of *H. marinum* in its evolution is not entirely impossible as *H. marinum* has been introduced to, and now exists as a weed, in this part of the world. Considering the results obtained by Doebley et al. (1992), *H. marinum* may have been the maternal ancestor of the hexaploid cytotype of *H. brachyantherum*. Based on morphological and cytological data it has been suggested that *H. secalinum* is close to the South African species *H. capense* (Bothmer and Jacobsen 1979; Linde-Laursen et al.1986; Bothmer et al. 1988, 1991). Our data do not support this proposition.

It has been suggested that the diploid and tetraploid cytotypes of the *H. brachyantherum* should be treated as separate species (Baum and Bailey 1990). The analysis of hordein variation did not support this proposal (Pelger and Bothmer 1992). However, our data demonstrate a high level of polymorphism for the *H. brachyantherum* group. All three cytotypes had clearly different hybridization patterns indicating that their taxonomic ranking might be questioned.

There have been several reports on the close relationship between two North American taxa, the diploid *H. brachyantherum* ssp. *californicum* and the tetraploid *H. depressum*, which are endemic to the western part of the United States. *H. depressum* has two very-closely-related genomes, and has been considered as a segmental alloploid by some authors (Covas 1949; Baum and Bailey 1991; Doebley et al. 1992; Linde-Laursen et al. 1992 a; Knutsson and Bothmer 1993) or even as an autoploid (Petersen 1992). Our results fully support the opinion that the genomes of *H. depressum* are very-closely related (Figs. 2 and 3 A). Moreover, in our experiments the hybridization pattern of *H. brachyantherum* ssp. *californicum* is much more similar to that of *H. depressum* than to those of the tetra- and hexa-ploid cytotypes of *H. brachyantherum*.

The set of repetitive DNA sequences used in our experiments is a useful tool for phylogenetic studies of the genus *Hordeum*. Our data are consistent with a classification system which divides the genus into four major groups representing the genomes **I**, **X**, **Y**, and **H** and with previous molecular studies in which the unique position of *H. bulbosum* has been demonstrated. Besides a confirmation of previous results on the origin and phylogeny of *H. depressum*, we have presented new evidence on the origin of *H. secalinum* and the *H. brachyantherum* group.

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