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An RFLP map of diploid *Hordeum bulbosum* L. and comparison with maps of barley (*H. vulgare* L.) and wheat (*Triticum aestivum* L.)

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Abstract This paper describes the first extensive genetic map of *Hordeum bulbosum*, the closest wild relative of cultivated barley. *H. bulbosum* is valuable for haploid production in barley breeding, and because of desirable agronomic characteristics, it also has potential for trait introgression into barley. A *H. bulbosum* map will assist introgression and provide a basis for the identification of QTLs for crossability with barley and other potentially useful genes. The present study used a population of 111 individuals from a PB1×PB11 cross to develop a genetic linkage map of diploid *H. bulbosum* (2n=2*x*=14) based on barley, wheat and other "anchor" cereal RFLP markers previously mapped in other species. Because of the cross-pollinating and highly polymorphic nature of *H. bulbosum*, up to four alleles showed segregation at any one locus, and five different segregation types were found. This enabled maps to be developed for the PB1 and PB11 parents, as well as a combined map. In total, 136 RFLP loci were mapped with a marker coverage of 621 cM. The markers were generally colinear with barley but *H. bulbosum* had less recombination in the centromeric regions and similar or more in the distal regions. Cytological studies on pollen mother cells at metaphase-I showed marked distal localization of chiasmata and a frequency consistent with the genetic map length. This study showed that *H. bulbosum* was highly polymorphic, making it suitable for trait analysis and supplementing maps of barley.

Keywords *Hordeum bulbosum* · *Hordeum vulgare* · RFLP · Genetic maps · Comparative mapping

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Introduction

A number of wild species in the genus *Hordeum* L. (tribe *Triticeae*, family *Poaceae*, *Gramineae*) are of potential importance for barley breeding. The species/taxa of *Hordeum* L. have the basic chromosome number of *x*=7, and with respect to cultivated barley the genus can be divided into a primary gene pool (*Hordeum vulgare* subsp. *vulgare* and *Hordeum vulgare* subsp. *spontaneum* (C. Koch Thell)), a secondary gene pool (*Hordeum bulbosum*) and a tertiary pool (all other *Hordeum* species). *H. bulbosum* L. occurs in diploid and tetraploid cytotypes, both of which show strong self-incompatibility. The diploid cytotype is found from Greece and Egypt westward (Brown 1992; von Bothmer et al. 1995).

H. bulbosum L. is the only obligatory cross-pollinating *Hordeum* species and has two major uses in barley breeding. The first is for doubled-haploid production by chromosome elimination (Simpson and Snape 1981). The second is as a source of novel genes for barley improvement by introgression. Traits of interest include winter hardiness and drought tolerance, anther extrusion and self-incompatibility; and resistance to pests and disease including powdery mildew, rusts, scab, barley yellow mosaic virus, net blotch, scald and Russian wheat aphid (Xu and Snape 1989; Kindler and Springer 1991; Xu et al. 1991; Xu and Kasha 1992; Walther et al. 2000). A number of recombinant *H. vulgare-H. bulbosum* lines have now been produced and stable gene introgression has been achieved, primarily with a view to introducing disease resistance (Pickering et al. 1994, 1997, 2000; Walther et al. 2000). Generally, however, the primary introgression is only the first step toward the utilization of these novel genes in agriculture. Normally, the transferred segment carrying the gene of interest also carries undesirable residual genes (linkage drag), which is a major limitation in introgression programs (Tanksley and Nelson 1996). Linkage drag can be reduced by selecting new reduced recombinants and this process is greatly helped by knowledge of the genetic map of the wild species. Thus, a *H. bulbosum* map will be useful for assist-

ing introgression and also as a basis for the identification of QTLs for crossability with barley for haploid production. The map is also useful in studying the homoeologous relationship of *H. bulbosum* with other *Triticeae* species. The aim of the present study was to develop a genetic linkage map of *H. bulbosum* and to carry out comparative genetic analysis within the Triticeae.

Materials and methods

Plant material and mapping population

A skeletal map of *H. bulbosum* was developed at the John Innes Centre, using a mapping population of 45 individuals derived from the cross of clones PB1×PB11 (Jaffé et al. 2000). These diploid cytotypes are very different in important characteristics. For example, PB1 is highly crossable with barley and produces a high frequency of haploid embryos for barley breeding. In contrast, PB11 has much lower crossability and produces a high frequency of hybrid embryos, making this clone suitable for alien gene introgression (Xu and Snape 1987, 1988). For detailed mapping work it is necessary to have a larger population than that originally developed, and the initial population was increased to 111 F_1 full-sib families by re-crossing the PB1 and PB11 parents. Basically, one spike from each parent was placed together in a crossing bag prior to anthesis, and once the seeds were well-developed they were collected separately from each of the parental spikes, noting the identification of the male and female parents.

Mapping procedures

Genomic DNA from the parents and from each F_1 recombinant clone was isolated from young leaves and digested with five restriction enzymes, *Bam*HI, *Dra*I, *Eco*RI, *Eco*RV and *Hin*dIII. Enzyme digestion, electrophoresis, Southern hybridization, probe labeling and the development of films was carried out using standard procedures, basically as described by Sharp et al. (1988).

A range of cDNA and genomic DNA clones from barley, wheat and oats, available in the Cereals Research Department, John Innes Centre, and including Cornell "anchor" probes (Van Deynze et al. 1998), were used (Table 1). Most probes had previously been mapped in barley (Langridge et al. 1995; Laurie et al. 1995; Qi et al. 1996) and/or other cereals. Because *H. bulbosum* is an obligate outbreeder up to four alleles can be segregating at each locus. This gave five segregation types that are listed, together with their relative frequencies, in Table 2. No clustering of segregation types was observed on any chromosome, showing that polymorphism levels were similar in all regions of the PB1 and PB11 genomes.

Segregation and linkage analysis

Pair-wise analysis, grouping of markers, and mapping, were performed with JoinMap Version 2 (Stam and van Ooijen 1995). This software can handle a wide variety of mapping population types, including outbreeding progenies involving markers with different segregation types, as with the cross here. Before linkage analysis, single-locus analysis (JMSLA module) was applied. Some loci with aberrant segregation ratios were discarded. The markers were assigned to linkage groups based on LOD scores of pairs of markers. Only in specific cases, and in particular genomic regions, was the combined map constructed by forcing the order using the expected fitted order of loci from the individual parental map. For the construction of the PB1 and PB11 maps, markers from the individual parents were separated using only the alleles from that particular parent to calculate recombination frequencies. In the case of *ab×ab* segregation types the linkage phase was unknown (Maliepaard et al. 1998) and such markers were only mapped on the combined map.

For the individual parental maps, each data set was analysed using the DH population type. For grouping, markers were assigned to linkage groups with LOD values of 3. However, when starting, LOD values from 1 to 8.5, at 0.5 increments, were used with the aim of detecting the stability of grouping. This also indicated which sets of markers formed tight linkage groups, which were doubtful, and which were definitely 'floating.' Markers within the groups were analyzed for pairwise linkages using JMREC

Table 1 Sources of RFLP probes

Anonymous unknown-function clones

Prefix	Source	Origin	Clone type
PSB	John Innes Centre	Barley	*Pst*I genomic fragments
PSR	John Innes Centre	Wheat	Anonymous cDNAs or *Pst*I genomic fragments
MWG	Graner et al. (1991)	Barley	*Pst*I genomic fragments
CMWG	Graner et al. (1991)	Barley	cDNAs
BCD	Heun et al. (1991) Van Deynze et al. (1998)	Barley	cDNAs
ABC	Kleinhofs et al. (1993)	Barley	cDNA
CDO	Heun et al. (1991) Van Deynze et al. (1998)	Oat	cDNAs
WG	Heun et al. (1991)	Wheat	*Pst*I Genomic fragments
Zen	Zeneca Seed Ltd.	Barley	*Pst*I Genomic fragments
RGR	Harushima et al. (1998)	Rice	Root cDNA
HvCO	S. Griffiths, John Innes Centre (pers comm)	Barley	Genomic fragment

Known-function cDNA clones

Clone	Name	Function
PSR8	*Cxp3*	Carboxypeptidase
PSR466	*Nar-1*	Nitrate reductase
PSR121	*Glb3*	1–3, 1–4-beta-glucanase
PSR13	*Gli-1*	Gliadin seed storage protein

(REC and LOD thresholds of 0.499 and 0.01, respectively). The linkage groups were ordered with the JMMAP module using the following parameters: JMMAP LOD threshold 0.01, REC threshold 0.49, jump threshold 5, tripled threshold 7, ripple value 3 and Kosambi's mapping function.

Meiotic studies

Meiotic studies were carried out on PB1 and PB11 plants, using anthers in which pollen mother cells (PMCs) were at metaphase-I. These were fixed in 3:1 100% ethanol and glacial acetic acid. As a deviation from conventional procedures, DAPI (4',6-diamidino-2-phenylindole, Sigma) was used to visualize pairing configuration at metaphase-I. Chromosome preparation and mounting was based on Schwarzacher and Heslop-Harrison (2000). The slides were examined for pairing configurations with an epifluorescence Nikon Microphot-SA microscope with a Microflex UFX-DX attachment and Nikon camera.

Results

Polymorphism levels and segregation patterns

A total of 160 probes were hybridised to genomic DNA on parental screening filters. Of these, 128 (80%) were polymorphic. After hybridization to the population filters, 103 gave clear hybridization patterns and a total of 131 loci were scored. Twenty nine probes from the Cornell anchor sets (Van Deynze et al. 1998) were used, of which 24 were successfully cross-hybridized, giving a total of 28 loci. Twenty one (16%) probes detected duplicated loci, five (4%) triplicated loci and one quadruplicated locus (0.8%). This gave a total of twenty seven with sequences presenting more than one copy in the *H. bulbosum* genome (see Fig. 2). This figure is similar to studies in other diploid cereals. The different segregation types are illustrated in Fig. 1 and their relative frequencies are given in Table 2.

Map construction and comparison with barley and Triticeae consensus maps

Separate RFLP maps were developed for PB1 and PB11. Because these plants were highly polymorphic, it was possible to combine the maps using common loci. As expected, the maps formed seven linkage groups (Fig. 2). The total map lengths were 365 cM, 615 cM and 616 cM for the PB1, PB11 and combined maps, respectively. The PB1 map had 75 loci while the PB11 had 84. On the combined map, certain loci such as *Xpsr167* $6H^b$ are given as contiguous duplicated loci in situations where they were mapped separately in PB1 and PB11 using different polymorphic alleles and had slightly different map locations. This is probably an artifact of the mapping software, rather than true gene duplication.

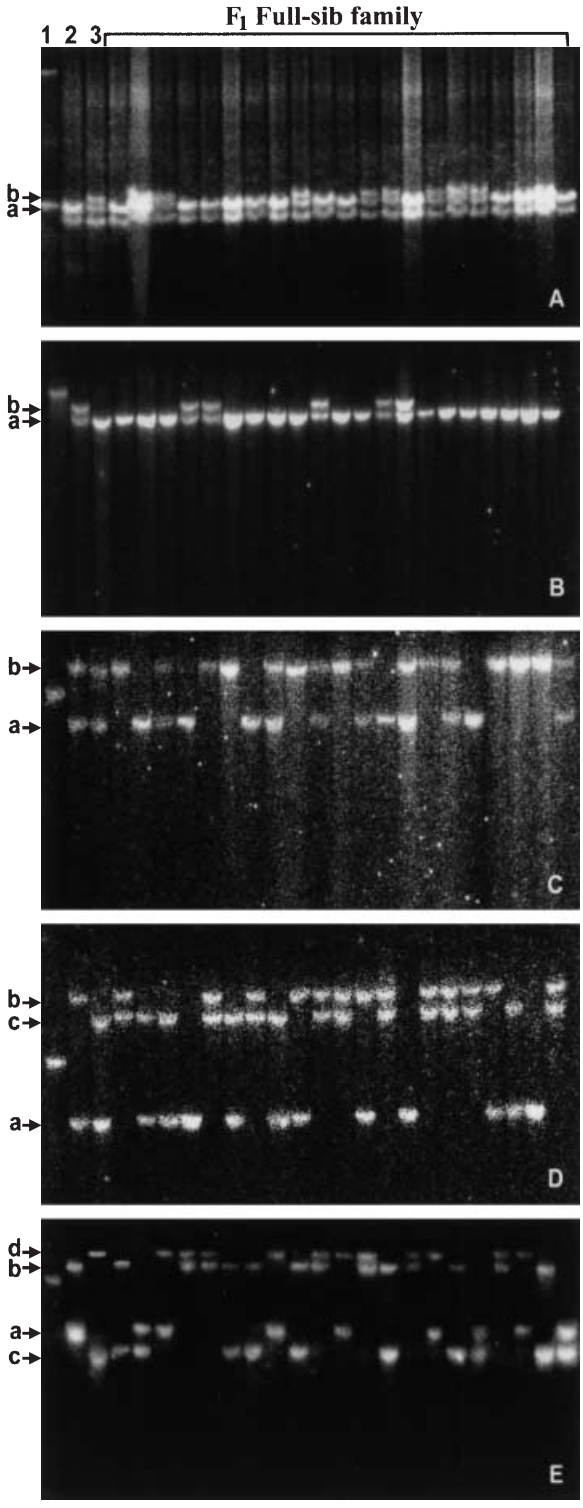

Fig. 1A–E RFLP banding patterns for different segregation types (inverted contrast). Total genomic DNA of *1* Barley variety Triumph, *2* PB1 parent, *3* PB11 parent. The *arrowhead letters a, b, c* and d indicate the alleles per locus. **A** *aa×ab* (two alleles), PB1 monomorphic. *Dra*I-digest hybridized with PSR108. **B** *ab×aa* (two alleles), PB11 monomorphic. *Eco*RI-digest hybridized with BCD450. **C** *ab×ab* (two alleles), both parents polymorphic. In this segregation type the linkage phase is unknown. *Eco*RI-digest hybridized with BCD135. **D** *ab×ac* (three alleles), both parents polymorphic. *Eco*RV-digest hybridized with PSB44. **E** *ab×cd* (four alleles, fully informative), both parents polymorphic. *Hin*dIII-digest hybridized with PSR8

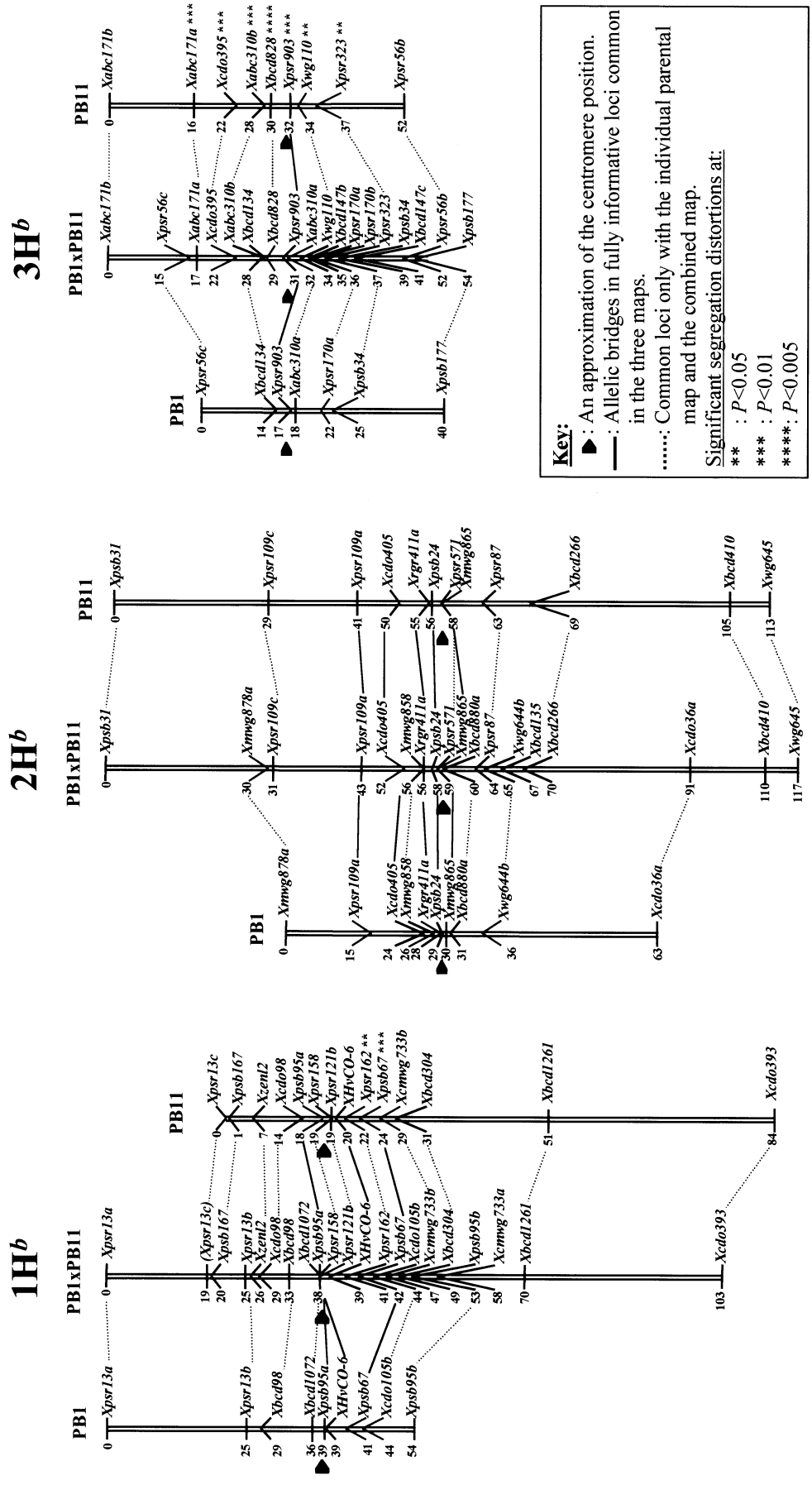

Fig. 2 Genetic linkage maps of diploid *H. bulbosum*: PB1, PB11 and combined maps are shown. Because of the overall colinearity of the *H. bulbosum* maps with those of barley and wheat (see text), centromere positions (▶) could be estimated from the position of markers previously assigned to short or long arms using aneuploid stocks. *Markers between parenthesis* in the combined maps of chromosome 1H^b and 6H^b indicate that these are probably the same locus

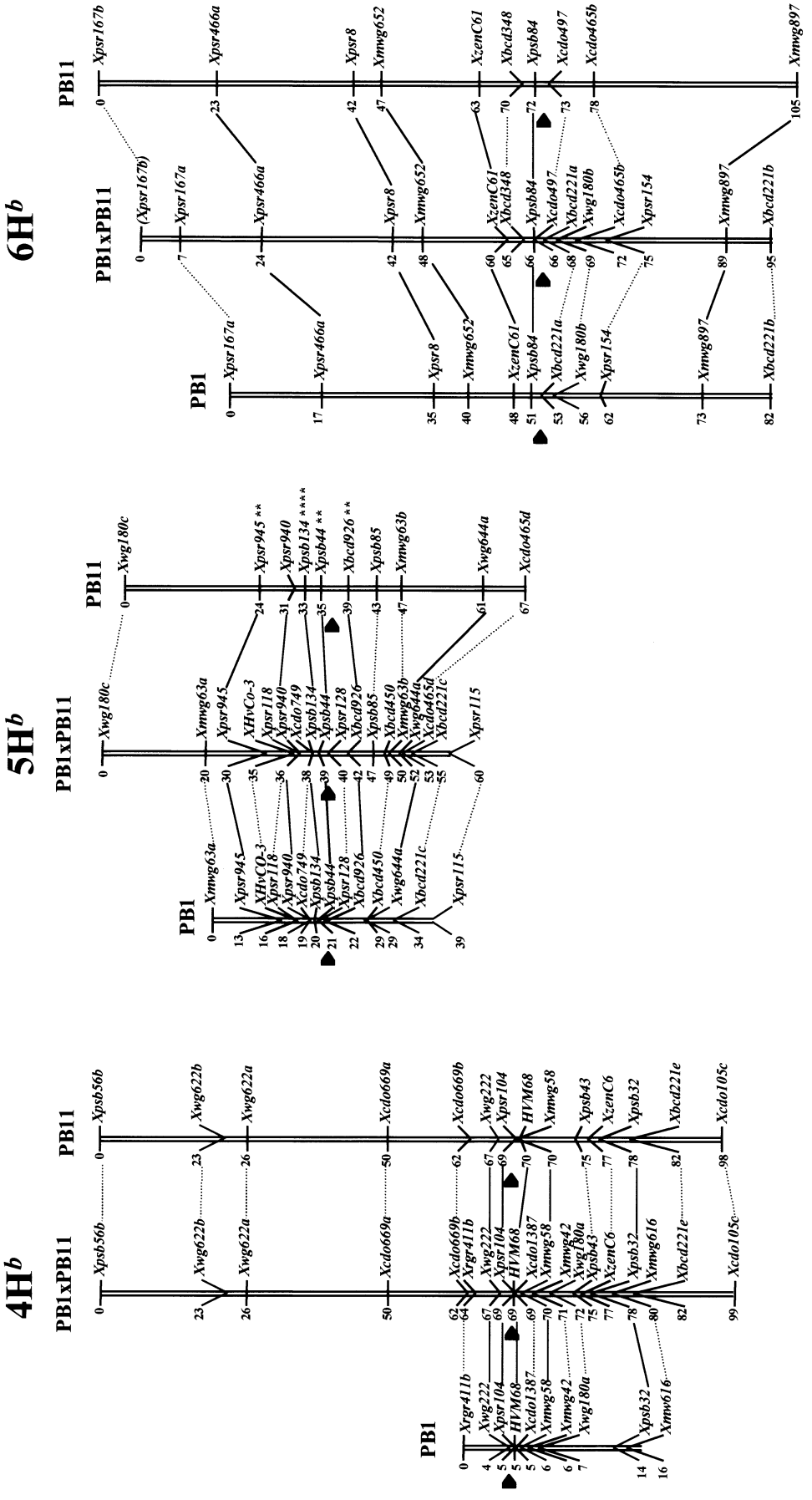

Fig. 2 (continued)

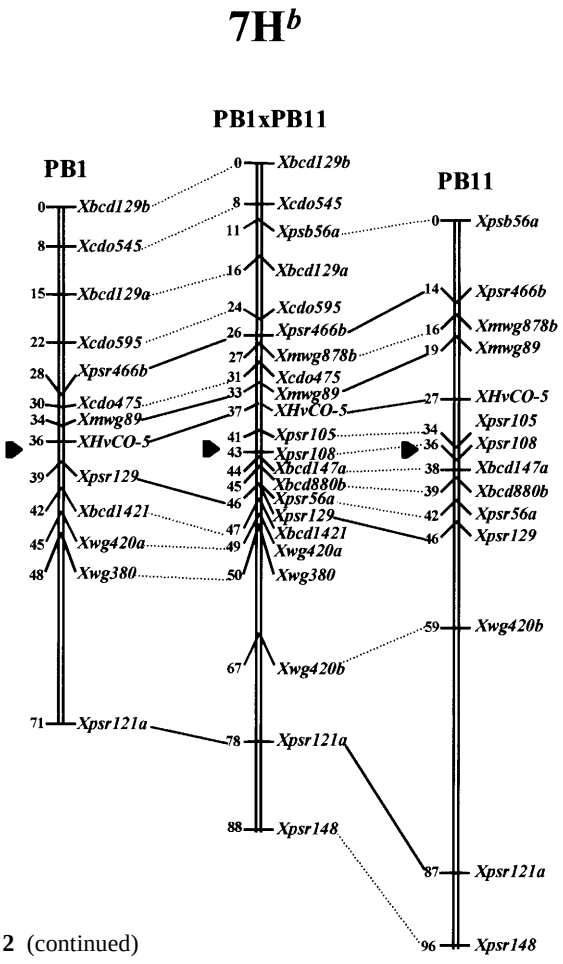

Fig. 2 (continued)

Chromosome 1H^b

For this chromosome the PB1 map contained nine loci and covered 54 cM. Two barley anchor marker cDNA loci, *Xbcd1072* and *Xbcd98*, were mapped in the proximal part of the short arm, similar to their positions in barley and the Triticeae consensus group-1 maps (Langridge et al. 1995; Van Deynze et al. 1995a). An oat anchor marker (*Xcdo105*) mapped on the long arm of this chromosome, in a similar position to barley (Kleinhofs et al. 1993; Qi et al. 1996), rice (Van Deynze et al. 1995a) and oat (Van Deynze et al. 1995b). The gliadin locus (*Gli-1*) was mapped on the short arm using the probe PSR13 (Wang et al. 1991, 1992). The PSR13 probe identified three loci, two of which were mapped in PB1 and the other in PB11. The *Xpsr13a* and *b* loci in PB1 are likely to be equivalents of the *Hor2* and *Hor1* loci of barley, respectively, while the single locus in PB11 probably correspond to *Hor1*, which was mapped close to *Xpsb67* in barley (Laurie et al. 1995).

The PB11 map contained 14 loci and covered 84 cM. The anchor loci *Xbcd1261* and *Xcdo393* were mapped on the distal region of the long arm, consistent with their position in barley (Heun et al. 1991) and the group-1 Triticeae consensus maps (Van Deynze et al. 1995a; Gill et al. 1996). In comparison to the latter, the two loci are separated by similar distances. Also, on the long arm, the barley cDNA locus *Xbcd304* and the wheat cDNA locus *Xcmwg733* seem to have an inverted position in the wheat and barley genomes. In *H. bulbosum*, MWG733 identified two loci, each one colinear to the respective loci in wheat and barley, which perhaps explains the inverted position in the homoeologous relationship between wheat and barley.

The combined map contained 21 loci, and had a total map coverage of 102 cM. It shows a clear maintained order from the PB1 and PB11 maps. The 1H^b maps were colinear with those maps of barley 1H and the consensus group-1 maps of the Triticeae. However the distance between common markers differed greatly, being shorter in all proximal regions and similar in the distal regions of the *H. bulbosum* maps.

Chromosome 2H^b

The PB1 map contained ten loci giving a marker coverage of 64 cM. Two oat anchor markers (*Xcdo405* and *Xcdo36a*) were mapped in this chromosome. *Xcdo405* has not been mapped in barley, but has been mapped on the Triticeae consensus group-2 map (Van Deynze et al. 1995a, b). Compared to its position in wheat it could be inverted with respect to *Xmwg858* but this apparent discrepancy could also be due to the reduced distance between genetic markers in *H. bulbosum*. Therefore, this was not considered to be convincing evidence of chromosome rearrangement. Two inter-chromosomally duplicated loci were identified. The rice probe RGR411 identified two loci, one mapping to 2H^b and one to 4H^b, consistent with its behaviour in barley (Laurie, unpublished data). The other inter-chromosomally duplicated locus was *Xwg644b*. WG644 was previously shown to detect a locus closely linked to vernalization loci on the long arm of the group-5 chromosomes (Laurie et al. 1995; Dubcovsky et al. 1998). In *H. bulbosum*, the 5H^b locus (*Xwg644a*, Fig. 2) was conserved and the 2H^b locus must be an additional copy of the sequence.

The PB11 map was constructed using 12 loci, with marker coverage of 113 cM. Markers on 2H^b were colinear with barley and other Triticeae maps (Nelson et al. 1995b), but genetic distances in the centromere region were reduced. For example, the *Xpsr571–Xmwg865* interval was approximately 25 cM in barley (Laurie et al. 1995) but only 1 cM in *H. bulbosum*. The combined map with 18 loci mapped was well-anchored in the presumed centromeric region. The barley anchor cDNA locus *Xbcd135*, mapped to wheat chromosome 2BL (Nelson et al. 1995b; Van Deynze et al. 1998), was only mapped in the combined map.

Chromosome 3H^b

The PB1 map had seven markers and covered 40 cM. The anchor loci *Xbcd134* and *Xpsr903* were mapped in

Table 2 Segregation types, total number of loci and genetic lengths of *H. bulbosum* PB1, PB11 and combined PB1×PB11 maps

Chromosome	Map	*aa×ab*	*ab × aa*	*ab×ab*	*ab×ac*	*ab×cd*	Total loci	Total map (cM)
1H^b	PB1	0	6	0	2	1	9	54
	PB11	11	0	0	2	1	14	84
	PB1×PB11	11	6	1	2	1	21	103
2H^b	PB1	0	5	0	5	0	10	63
	PB11	7	0	0	5	0	12	113
	PB1×PB11	7	5	1	5	0	18	117
3H^b	PB1	0	6	0	1	0	8	40
	PB11	8	0	0	1	0	10	52
	PB1×PB11	8	6	3	1	0	18	54
4H^b	PB1	0	5	0	4	1	10	16
	PB11	9	0	0	4	1	14	98
	PB1×PB11	9	5	0	4	1	19	99
5H^b	PB1	0	8	0	4	2	14	39
	PB11	4	0	0	4	2	10	67
	PB1×PB11	4	8	0	4	2	18	60
6H^b	PB1	0	5	0	4	2	11	82
	PB11	4	0	0	4	2	10	105
	PB1×PB11	4	5	0	4	2	15	95
7H^b	PB1	0	8	0	2	3	13	71
	PB11	9	0	0	2	3	14	96
	PB1×PB11	9	8	0	2	3	22	88
Total	PB1	0	43	0	22	9	75	365
	PB11	52	0	0	22	9	84	615
	PB1×PB11	52 (40%)	43 (33%)	5 (4%)	22 (17%)	9 (7%)	131	616

the proximal region of this chromosome, consistent with their positions on the barley consensus maps (Langridge et al. 1995) and the Triticeae consensus group-3 maps (Nelson et al. 1995c). On the *H. bulbosum* map, both loci were mapped very close to the centromere. On the long arm, the PSR170 probe identified two closely linked loci, which is similar to the result reported on rye 3R (Devos et al. 1992). In *H. bulbosum*, one locus (*Xpsr170a*) was mapped on the PB1 map. The second locus (*Xpsr170b*) could only be placed on the combined map because it had a *ab×ab* segregation type. *Xpsr170a*, *Xpsb32* and *Xpsb177* were colinear to loci mapped by Laurie et al. (1995).

The PB11 map contained nine loci and covered 52 cM. All the markers except the most-distal on the long and short arms showed distorted segregation. It is possible that this segregation distortion is due to the presence of crossability genes, because the deviation from the expected Mendelian segregation ratio was present only in the PB11 map. In homoeologous chromosomes of wheat and barley, QTLs for crossability have been mapped in common genomic regions (Fedak and Jui 1982; Taketa et al. 1998; Tixier et al. 1998). The distortion is unlikely to be due the *S* or *Z* self-incompatibility loci of *H. bulbosum* (Lundqvist 1962) because these loci have been mapped on chromosomes 1R and 2R of rye, respectively (Börner and Korzun 1998), and would therefore be predicted to be on 1H^b and 2H^b.

The probe ABC171 identified four loci in *H. bulbosum* of which only two were polymorphic and mapped on this chromosome. The distal locus (*Xabc171b*) had a similar position to a locus on the barley consensus map (Langridge et al. 1995) and the barley map of Graner et al. (1994). *Xcdo395*, mapped on the short arm of this chromosome, had a different position on the barley consensus maps (Langridge et al. 1995). It was at a similar position in all other Triticeae consensus maps (Nelson et al. 1995c). In *H. bulbosum*, this probe identified two loci; one of them was monomorphic. Thus, the different locus mapped by Langridge et al. (1995), could be an intra-chromosomal duplication.

The combined map had 18 loci and covered 54 cM. In *H. bulbosum*, the *Xbcd134* and *Xabc171b* loci were mapped on the proximal and distal regions in the short arm, respectively, consistent with their positions on the consensus maps, Langridge et al. (1995). On the long arm, three loci were mapped only in the combined map. Two of them were intra-chromosome duplicated anchor loci (*Xbcd147b* and *Xbcd147c*), which share positions with those mapped by Li et al. (1996).

For this chromosome, *H. bulbosum* shared marker order with the homoeologous group-3 chromosomes of wheat and barley, with similar map lengths to barley consensus maps on the short arm, but drastically reduced distances in the predicted centromere region and in the mapped regions of the long arm. Considering that the most-distal barley markers on the short and long arms

are *Xbcd171b* and *Xpsb177*, the *H. bulbosum* maps only covered about one-third of the chromosome relative to 3H and the Triticeae consensus group-3 maps (Nelson et al. 1995c).

Chromosome 4H^b

The PB1 map had ten loci but with a marker coverage of only 16 cM. The rice probe RGR411 identified the end locus on this map (*Xrgr411b*) which probably corresponds to a locus mapped near to the centromere on barley 4H (Laurie, unpublished). This indicates that this PB1 map covers mainly the long arm of chromosome 4H^b. All the loci that formed a cluster on this map have been mapped in the centromeric region of wheat, barley or oat (Kleinhofs et al. 1993; Gale et al. 1995; Langridge et al. 1995; Laurie et al. 1995; Dubcovsky et al. 1996; Qi et al. 1996). This clustering of markers is an indication of the low recombination frequency in *H. bulbosum* in this genomic region.

The PB11 map contained 14 loci and covered 98 cM. *Xpsb56b* was the most-distal locus on the short arm. Another copy of this probe was mapped on 7H^b, and both locations differed from that found in barley 6HL (Laurie et al. 1995). The locus on 4H^{b}S was linked to *Xwg622*, which has provided the most-distal marker on barley chromosome 4HS on the maps of Heun et al. (1991), Kleinhofs et al. (1993) and Laurie et al. (1995), on bread wheat and *Triticum tauschii* 4DS (Boyko et al. 1999) and *Triticum monococcum* 4A^{m}S (Dubcovsky et al. 1996). Because *Xpsb56b* was 23 cM distal to *Xwg622*, this suggests that the *H. bulbosum* map is relatively longer in the distal region of the short arm than in barley and wheat. The oat cDNA probe CDO669 identified three loci in the *H. bulbosum* genome, two of which mapped on this chromosome. In barley maps, only one locus has been mapped on this chromosome (Kleinhofs et al. 1993; Langridge et al. 1995; Qi et al. 1996). The combined map had 19 loci and covered 99 cM. Because *H. bulbosum* is completely colinear with barley for this chromosome, *H. bulbosum* clearly does not have the reciprocal 4AL/5AL and 4A^m/5A^m translocations of wheat (Gale et al. 1995; Dubcovsky et al. 1996, 1998).

Chromosome 5H^b

The PB1 map had 14 loci, covering 39 cM. *Xmwg63a*, the most distal locus on the short arm, corresponds to the *Xmwg63* locus in barley 5HS (Graner et al. 1991; Bezant et al. 1997). The loci *Xpsr945*, *Xpsr118* and *Xpsr940*, homoeologous with wheat, and the loci *Xpsb134* and *Xpsb44*, homoeologous with barley, were mapped on the proximal region of this chromosome. All have been mapped in a similar genomic region on barley chromosome 5HS and wheat group 5. However, *Xpsr118* was in a different position in *H. bulbosum*, and *Xpsb134* and *Xpsb44* are inverted with respect to barley (Gale et al. 1995; Laurie et al. 1995; Nelson et al. 1995a). The long arm of this chromosome was colinear with barley and wheat, confirming that *H. bulbosum* does not have the 5A/4AL reciprocal translocation of wheat (Gale et al. 1995; Laurie et al. 1995). Two anchor markers, *Xbcd926* and *Xbcd450*, were colinear with those reported on wheat 5AL (Nelson et al. 1995a) and 5BL (Gill et al. 1996). *Xbcd450* was completely linked to *Xwg644a* in the *H. bulbosum* genome, as has been found with *T. monococcum* 5A^m (Dubcovsky et al. 1998). Both probes were closely linked to the *Sgh2*/*Vrn-A1* locus determining vernalization response (Galiba et al. 1995; Laurie et al. 1995; Dubcovsky et al. 1998) and therefore provide useful markers for investigating the genetic basis of vernalization response in *H. bulbosum*.

The PB11 map contained ten loci covering 67 cM. All the loci surrounding the predicted centromere region showed segregation distortion. As in chromosome 3H^b, distortion was only found in PB11, the parent with low crossability with barley. The PB11 map, in common with the PB1 map, showed that *Xpsb134* and *Xpsb44* were inverted with respect to barley. The most distal locus mapped on the long arm was one of the inter-chromosomally duplicated loci, *Xcdo465d*. The cDNA oat probe CDO465 has previously been mapped on 5A^{m}L, close to *Xwg644* (Dubcovsky et al. 1996).

The combined map of this chromosome had 18 loci, with a marker coverage of 60 cM. Although there were six fully informative loci (*Xpsr945*, *Xpsr940*, *Xpsb134*, *Xpsb44*, *Xbcd926* and *Xwg644a*) the combined map was difficult to construct, probably because of the segregation distortion in the PB11 map. Therefore, the combined map was fixed to the fitted order of the PB1 map. The long arm was colinear in all maps and there was no need to fix the order for this part of the map.

Chromosome 6H^b

The PB1 map had 11 loci, covering 82 cM. The probe PSR167 identified the most-distal locus, *Xpsr167a*, which was mapped in a similar location in barley and wheat (Laurie et al. 1995; Marino et al. 1996). The known function genes *Xpsr466* (*Nar1*, nitrate reductase) and *Xpsr8* (*Cxp3*, carboxypeptidase) were also mapped in this distal region. Both have been reported in the barley and wheat maps (Kleinhofs et al. 1993; Langridge et al. 1995; Marino et al. 1996). In the proximal region of this map, loci were colinear with barley (Laurie et al. 1995), but the distance between them was drastically reduced in *H. bulbosum*, being only a sixth of that in barley. In the long arm of this chromosome, the wheat genomic probe MWG897 identified a fully informative locus which mapped on the three maps. This locus is one of the most distal on barley maps (Langridge et al. 1995; Qi et al. 1996).

The PB11 map had ten loci and covered 105 cM. The distal locus mapped on the short arm was *Xpsr167b*. Two *Xpsr167* loci were mapped in barley (Laurie et al. 1995) but one was much closer to the centromere. Thus, although two *Xpsr167* loci appear on the *H. bulbosum*

Table 3 Pairing configuration and mean chiasma frequency per nucleus in 50 metaphase-I pollen mother cells of *H. bulbosum* (PB1 and PB11 scores pooled)

Pairing configuration	No of nuclei	Ring bivalent		Rod bivalent one chiasmata	Total chiasmata
		three chiasmata	two chiasmata		
7 II	35	0	7	0	490
7 II	7	1	6	0	105
7 II	6	0	6	1	78
7 II	2	1	5	1	28
Totals	50				701
Mean chiasma frequency per nucleus					14

combined map these probably represent the same locus. All the other loci mapped on the short arm were fully informative and served to align the PB1 and PB11 maps. Two anchor loci (*Xbcd348* and *Xcdo497*) were mapped in the proximal region of this chromosome. Both loci have been reported in a similar position and order by Teulat et al. (1998). The combined map had 15 loci and a map length of 95 cM.

Chromosome 7H^b

The PB1 map had13 loci and covered 71 cM. The barley probe BCD129 gave a locus *Xbcd129b* that was the most distal on the short arm, and a locus *Xbcd129a* that was mapped 15-cM proximal to the terminal locus. In barley maps, the probe BCD129 has identified only one locus, which was in a similar position to *Xbcd129a* (Kleinhofs et al. 1993; Qi et al. 1996). Two anchor loci (*Xcdo545* and *Xcdo475*) were mapped on the short arm of this chromosome and were colinear with a barley consensus map (Langridge et al. 1995). PSR466 (nitrate reductase) detected two fully informative loci in *H. bulbosum* (6H^{b}S and 7H^{b}S). Duplication of this gene has not been reported in other Triticeae maps, but a 7AS/7DS/4AL copy has been identified in wheat using aneuploid lines. Other fully informative loci were *Xmwg89*, which mapped very close to *Xcdo475*, as in barley and wheat (Hohmann et al. 1995; Langridge et al. 1995; Qi et al. 1996), and *XHvCO-5*. *Xpsr129*, *Xwg420a* and *Xwg380* on the long arm were colinear with barley and consistent with wheat physical maps (Kleinhofs et al. 1993; Hohmann et al. 1995; Langridge et al. 1995; Laurie et al. 1995). The most distal locus that mapped on the long arm of this chromosome was *Xpsr121a* (*Glb3*), a locus highly conserved within the Triticeae (Gale et al. 1995; Laurie et al. 1995).

The PB11 map had 14 loci and covered 96 cM. The barley genomic probe PSB56 identified the most-distal locus on the short arm. This genomic probe was previously mapped on 6H in barley (Laurie et al. 1995) but, as it detected multiple loci in *H. bulbosum*, *Xpsb56a* is likely to be due to sequence duplication rather than to translocation. In the proximal region, two anchor loci were mapped (*Xbcd147a* and *Xbcd880b*). The former was mapped to the centromeric region in a saturated and high-resolution map of barley containing malting quality QTLs (Han et al. 1997). *Xpsr148* was the most-distal on the long arm and was linked to *Xpsr121*. In *H. bulbosum* these loci were co-

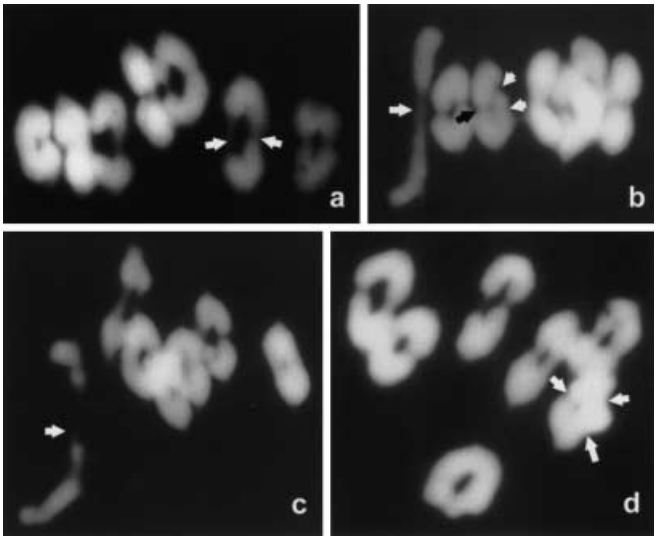

Fig. 3 Metaphase-I of meiosis in DAPI-stained PMCs of diploid *H. bulbosum*, clones PB1 and PB11, showing seven bivalents: (**a**) seven ring bivalents with two chiasmata per ring; (**b**) six ring bivalents with two or three chiasmata per ring and one rod bivalent with one chiasma; (**c**) six ring bivalents with two chiasmata per ring and one rod bivalent with one chiasma; (**d**) seven ring bivalents with two or three (*arrowheads*) chiasmata per ring

linear with barley and, therefore, inverted relative to wheat (Gale et al. 1995; Laurie et al. 1995). The combined map had 22 loci and was well-anchored by fully informative loci. Compared to barley and Triticeae consensus maps, this chromosome seems to be longer in the distal regions, similar in the whole short arm, and drastically reduced in the proximal regions and the long arm.

Chiasma frequency and distribution in *H. bulbosum* chromosomes

Cytological observations on chiasma frequency in *H. bulbosum* can give an approximation of its expected total map length. PMCs at metaphase-I from PB1 and PB11 plants were analyzed for pairing configurations. As both parents gave the same mean pairing configurations, a pooled result is presented. The chiasma counts from 50 nuclei with clear meiotic pairing configurations gave a mean of 14 chiasmata per nucleus (Table 3). All cells had seven bivalents (7II) and the means for ring (Fig. 3a) and rod bivalents (Fig. 3b and c) were 6.8 and 0.2 per nucle-

us, respectively. In only 18% of the nuclei was there a chromosome with a chiasma in a proximal region (Fig. 3b and d). Distal chiasma formation was very frequent and, assuming that the frequency of chiasma formation is similar in male and female meiosis, this gives an estimated total map length of about 700 cM. The observed map length of 616 cM therefore represents about 90% genome coverage, consistent with the observation that distal markers on the *H. bulbosum* maps generally corresponded well to distal markers on equivalent wheat and barley maps. The maps of some chromosome arms, such as 3H^{b}L and 5H^{b}L, will probably be extended if polymorphism for additional distal markers can be found.

Discussion

This paper provides the first extensive genetic map of the *H. bulbosum* genome. The PB1×PB11 cross proved highly polymorphic, with linkage maps that are colinear with barley except for the predicted centromere regions in chromosomes 1H^b, 5H^b and 7H^b, where some markers seem to be inverted. However, these regions also had a clustering of markers and/or segregation distortion, which may have affected the accuracy of mapping. No translocations were found that distinguished *H. bulbosum* from barley, which is consistent with previous phylogenetic studies showing that barley and *H. bulbosum* are closely related (Doebley et al. 1992). The high levels of polymorphism in *H. bulbosum*, and the high colinearity with barley, means that this map complements and adds to the genetic maps of cultivated barley in specific genomic regions not well-mapped because of low levels of polymorphism.

The *H. bulbosum* map represents between 53 and 56% of the length of the barley maps reported by Langridge et al. (1995) and Laurie et al. (1995), respectively. However, these differences were not evenly distributed over the maps. Map lengths in the distal regions of *H. bulbosum* chromosomes 2H^b, 4H^b, 6H^b and 7H^b were greater than those seen in barley, while distances in the putative centromere regions in *H. bulbosum* were greatly reduced. This indicates a more-extreme localization of chiasmata in *H. bulbosum*, consistent with the results of the cytological studies. This situation is clearly illustrated by comparison of *H. bulbosum* and barley group-2 maps (Figs. 4 and 5). These show that the recombination frequency in barley is higher in the centromeric region, while in the distal regions the recombination frequency in *H. bulbosum* is higher than barley. This situation is similar to that reported in the comparison of barley with *T. monoccocum* (Dubcovsky et al. 1996).

Comparisons of genetic linkage maps based on C-banding with those based on physical distance indicate that recombination is drastically reduced in centromere regions in cereals (Curtis and Lukaszewsky 1991; Kota et al. 1993; Gill et al. 1996; Künzel et al. 2000). However, in *H. bulbosum* this phenomenon may be even more extreme. *H. bulbosum* has heterochromatin only at

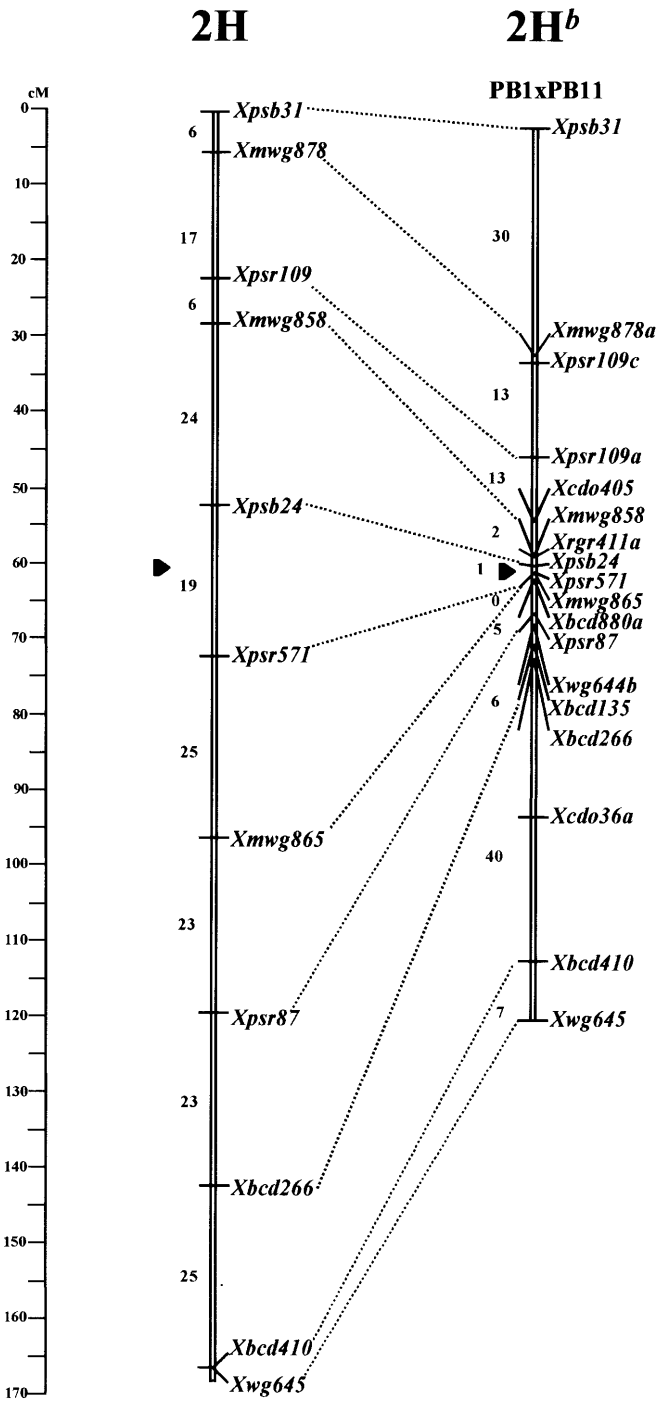

Fig. 4 Comparison of barley chromosome 2H (Laurie et al. 1995) with chromosome 2H^b of *H. bulbosum* (this paper)

the centromeres but, in comparison to barley, the heterochromatic blocks are not conspicuously larger (Linde-Laursen et al. 1992). Therefore, heterochromatin distribution and amount is unlikely to be the cause of the observed recombination differences. Strong distal localization of chiasmata is therefore more likely to be genetically controlled. This may have implications for introgression from *H. bulbosum* into barley if hybrids show similarly strong localization. To analyse this in more detail it

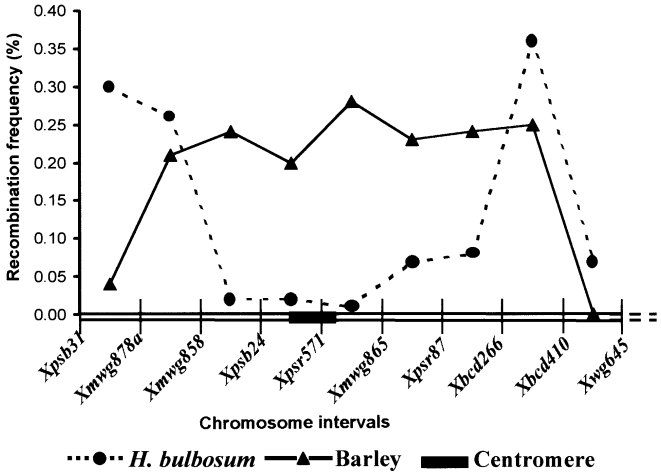

Fig. 5 Comparison of recombination frequency along chromosome $2H^b$ of *H. bulbosum* and 2H of barley

will be useful to identify additional probes for the most-distal chromosomal regions of *H. bulbosum*.

Recently, stable introgression from *H. bulbosum* into barley has been achieved (Pickering et al. 2000). However, it has not so far been possible to identify the *H. bulbosum* regions involved. The map shown in this paper provides markers to analyse introgression, thereby allowing detailed analysis of recombinants and translocation break points. Additionally, it is now possible to map loci controlling potentially agronomically valuable traits in *H. bulbosum*. Examples include the self-incompatibility genes and genes regulating haploid and hybrid formation in crosses with barley.

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References

Bezant J, Laurie D, Pratchett N, Chojecki J, Kearsey M (1997) Mapping QTLs controlling yield and yield components in a spring barley (*Hordeum vulgare* L.) cross using marker regression. Mol Breed 3:29–38

Börner A, Korzun V (1998) A consensus linkage map of rye (*Secale cereale* L.) including 374 RFLPs, 24 isozymes and 15 gene loci. Theor Appl Genet 97:1279–1288

Bothmer R von, Jacobsen N, Baden C, Jorgensen RB, Linde-Laursen I (1995) An ecographical study of genus *Hordeum*. 2nd edn. Systematic and ecogeographic studies on crop genepools 7. International Plant Genetic Resources Institute, Rome

Boyko EV, Gill KS, Mickelson–Young L, Nasuda S, Raupp WJ, Ziegle JN, Singh S, Hassawi DS, Fritz AK, Namuth D, Lapitan NLV, Gill BS (1999) A high-density genetic linkage map of *Aegilops tauschii*, the D-genome progenitor of bread wheat. Theor Appl Genet 99:16–26

Brown AHD (1992) Genetic variation and resources in cultivated barley and wild *Hordeum*. In: Muck L (ed) Barley genetics VI vol II. Proc. 6the Int Barley Genet Sym Munks gaard Int, Copenhagen, pp 669–682

Curtis CA, Lukaszewski AJ (1991) Genetic linkage between C-bands and storage protein genes in chromosome 1B of tetraploid wheat. Theor Appl Genet 81:245–252

Devos KM, Atkinson MD, Chinoy CN, Liu CJ, Gale MD (1992) RFLP-based genetic map of the homoeologous group-3 chromosomes of wheat and rye. Theor Appl Genet 83:931–939

Doebley J, von Bothmer R, Larson S (1992) Chloroplast DNA variation and the phylogeny of *Hordeum* (Poaceae). Am J Bot 79:576–584

Dubcovsky J, Luo M-C, Zhong GY, Bransteitter R, Desai A, Kilian A, Kleinhofs A, Dvorák J (1996) Genetic maps of diploid wheat, *Triticum monococcum* L., and its comparison with maps of *Hordeum vulgare* L. Genetics 143:983–999

Dubcovsky J, Lijavetzky D, Appendino L, Tranquilli G (1998) Comparative RFLP mapping of *Triticum monococcum* genes controlling vernalization requirement. Theor Appl Genet 97: 968–975

Fedak G, Jui PY (1982) Chromosome of Chinese Spring wheat carrying genes for crossability with Betzes barley. Can J Genet Cytol 24:227–233

Gale MD, Atkinson MD, Chinoy CN, Harcourt RL, Jia J, Li QY, Devos KM (1995) Genetics maps of hexaploid wheat. In: Li ZS, Xin ZY (eds) Proc 8th Int Wheat Genet Symp, Beijing China, 1993, pp 29–40

Galiba G, Quarrie SA, Sutka J, Morgounov A, Snape JW (1995) RFLP mapping of vernalization (*Vrn1*) and frost resistance (*Fr1*) on chromosome 5A of wheat. Theor Appl Genet 90:1174–1179

Gill KS, Gill BS, Endo TR, Taylor T (1996) Identification and high-density mapping of gene-rich regions in chromosome group 1 of wheat. Genetics 144:1883–1891

Graner A, Jahoor A, Schondelmaier J, Siedler H, Pillen K, Fischbeck G, Wenzel G, Herrmann RG (1991) Construction of an RFLP map of barley. Theor Appl Genet 83:250–256

Graner A, Bauer E, Kellermann A, Kirchner S, Muraya JK, Jahoor A, Wenzel G (1994) Progress RFLP map construction in winter barley. Barley Genet Newslett 23:53–59

Han F, Ullrich SE, Kleinfhofs A, Jones BL, Hayes PM, Wesenberg DM (1997) Fine-structure mapping of the barley chromosome-1 centromere region containing malting quality QTLs. Theor Appl Genet 95:903–910

Harushima Y, Yano M, Shomura A, Sato M, Shimano T, Kuboqui Y, Yamamoto T, Lin SY, Baltazar A, Parco A, Kajiya H, Huang N, Yamamoto K, Nagamura Y, Kurata N, Khush G, Sasaki T (1998) A high-density rice genetic linkage map with 2275 markers using a single F_2 population. Genetics 148:479–494

Heun M, Kennedy AE, Anderson JA, Lapitan NLV, Sorrells ME, Tanksley SD (1991) Construction of a restriction fragment length polymorphism map for barley (*Hordeum vulgare*). Genome 34:437–447

Hohmann U, Graner A, Endo TR, Gill BS, Herrmann RG (1995) Comparison of wheat physical maps with barley linkage maps for group-7 chromosomes. Theor Appl Genet 91:618–626

Jaffé B, Caligari PDS, Snape JW (2000) A skeletal linkage map of *Hordeum bulbosum* L. and comparative mapping with barley (*H. vulgare* L.). Euphytica 115:115–120

Kindler SD, Springer TL (1991) Resistance to Russian wheat aphid in wild *Hordeum* species. Crop Sci 31:94–97

Kleinhofs A, Kilian A, Saghai Maroof MA, Biyashev RM, Hayes P, Chen FQ, Lapitan N, Fenwich A, Blake TK, Kanazin V, Ananiev E, Dahleen L, Kudrna D, Bollinger J, Knapp SJ, Liu B, Sorrells M, Heun M, Franckowiak JD, Hoffman D, Skadsen R, Steffenson BJ (1993) A molecular, isozyme and morphological map of the barley (*Hordeum vulgare*) genome. Theor Appl Genet 86:705–712

Kota RS, Gill KS, Gill BS, Endo TR (1993) A cytogenetically based physical map of chromosome 1B in common wheat. Genome 36:548–554

Künzel G, Korzun L, Meister A (2000) Cytological integrated physical restriction fragment length ploymorphism maps for the barley genome based on translocation breakpoints. Genetics 154:397–412

Langridge P, Karakousis A, Collins N, Kretschmer J, Manning S (1995) A consensus linkage map of barley. Mol Breed 1:389–395

Laurie DA, Pratchett N, Bezant JH and Snape JW (1995). RFLP mapping of five major genes and eight quantitative trait loci controlling flowering time in a winter×spring barley (*Hordeum vulgare* L.) cross. Genome 38:575–585

Li CD, Langridge P, Lance RCM, Xu P, Fincher GB (1996) Seven members of the (1-3)-β-glucanase gene family in barley (*Hordeum vulgare*) are clustered on the long arm of chromosome 3 (3HL). Theor Appl Genet 92:791–796

Linde-Laursen IB, von Bothmer R, Jacobsen N (1992) Relationships in the genus *Hordeum*: Giemsa C-banded karyotypes. Hereditas 116:111–116

Lundqvist A (1962) Self-incompatibility in diploid *Hordeum bulbosum* L. Hereditas 48:138–152

Maliepaard C, Alston FH, van Arkel G, Brown LM, Chevreau E, Dunemann F, Evans KM, Gardiner S, Guilford P, van Heusden AW, Janse J, Laurens F, Lynn JR, Manganaris AG, den Nijs APM, Periam N, Rikkerink E, Roche P, Ryder C, Sansavini S, Schmidt H, Tartarini S, Verhaegh JJ, Vrielink-van Ginkel M, King GJ (1998) Aligning male and female linkage maps of apple (*Malus pumila* Mill.) using multi-allelic markers. Theor Appl Genet 97:60–73

Marino CL, Nelson JC, Lu YH, Sorrells ME, Leroy P, Tuleen NA, Lopes CR, Hart GE (1996) Molecular genetic maps of the group-6 chromosomes of hexaploid wheat (*Triticum aestivum* L. em. Thell.) Genome 39:359–366

Nelson JC, Sorrells ME, van Deynse AE, Lu YH, Atkinson MD, Bernard M, Leroy P, Faris JD, Anderson JA (1995a) Molecular mapping of wheat: major genes and rearrangements in homoeologous groups 4, 5 and 7. Genetics 141:721–723

Nelson JC, van Deynse AE, Autrique E, Sorrells ME, Lu YH, Merlino M, Atkinson M, Leroy P (1995b) Molecular mapping of wheat. Homoeologous group 2. Genome 38:516–524

Nelson JC, van Deynse AE, Autrique E, Sorrells ME, Lu YH, Negre S, Bernard M, Leroy P (1995c) Molecular mapping of wheat. Homoeologous group 3. Genome 38:525–533

Pickering, RA, Timmerman GM, Cromey MG, Melz G (1994) Characterization of progeny from backcrosses of triploid hybrid between *Hordeum vulgare* L. (2×) and *H. bulbosum* L. (4×) to *H. vulgare*. Theor Appl Genet 88:460–464

Pickering RA, Hill AM, Kynast RG (1997) Characterization by RFLP analysis and genomic in situ hybridization of a recombinant and a monosomic substitution plant derived from *Hordeum vulgare* L.×*Hordeum bulbosum* L. crosses. Genome 40:195–200

Pickering RA, Malyshev S, Kunzel G, Johnston PA, Korzun V, Menke M, Schubert I (2000) Locating introgressions of *Hordeum bulbosum* chromatin within the *H. vulgare* genome. Theor Appl Genet 100:27–31

Qi X, Stam P, Lindhout P (1996) Comparison and integration of four barley genetic maps. Genome 39:379–394

Schwarzacher T, Heslop-Harrison JS (2000) Practical in situ hybridization. BIOS Scientific Publishers, Oxford

Simpson E and Snape JW (1981) The use of doubled haploids in a winter barley programme. Proc 4th Int Barley Genet Symp, Edinburgh, 716–720

Sharp PJ, Kreis M, Shewry PR, Gale MD (1988) Location of beta-amylase sequences in wheat and its relatives. Theor Appl Genet 75:286–289

Stam P, van Ooijen JW (1995) JoinMap version 2.0. Software for the calculation of genetic linkage maps. CPRO-DLO, Wageningen

Taketa S, Takahashi H, Takeda K (1998) Genetic variation in barley crossability with wheat and its quantitative trait loci analysis. Euphytica 103:187–193

Tanksley SD, Nelson JC (1996) Advanced backcross QTL analysis: a method for the simultaneous discovery and transfer of valuable QTLs from unadapted germplasm into elite breeding lines. Theor Appl Genet 92:191–203

Teulat B, This D, Khairallah M, Borries C, Ragot C, Sourdille P, Leroy P, Monneveux P, Charrier A (1998) Several QTLs involved in osmotic-adjustment trait variation in barley (*Hordeum vulgare* L.). Theor Appl Genet 96:688–698

Tixier MH, Sourdille P, Charmet G, Gay G, Jaby C, Cadalen T, Bernard S, Nicolas P, Bernard M (1998) Detection of QTLs for crossability in wheat using a doubled-haploid population. Theor Appl Genet 97:1076–1082

Van Deynze AE, Dubcovsky J, Gill KS, Nelson JC, Sorrells ME, Dvorák J, Gill BS, Lagudah ES, McCouch SR, Appels R (1995a) Molecular-genetic maps for group-1 chromosomes of Triticeae species and their relation to chromosomes in rice and oat. Genome 38:45–59

Van Deynze AE, Nelson JC, Yglesias ES, Harrington SE, Braga DP, McCouch SR, Sorrells ME (1995b) Comparative mapping in grasses. Wheat relationships. Mol Gen Genet 248:744–754

Van Deynze AE, Sorrells ME, Park WD, Ayres NM, Fu H, Cartinhour SW, Paul E, McCouch SR, (1998) Anchor probes for comparative mapping of grass genera. Theor Appl Genet 97:356–369

Walther U, Rapke H, Proeseler G, Szigat G (2000) *Hordeum bulbosum*– a new source of disease resistance-transfer of resistance to leaf rust and mosaic viruses from *H. bulbosum* into winter barley. Plant Breed 119:215–218

Wang ML, Atkinson MD, Chinoy CN, Devos KM, Harcourt RL, Liu CJ, Rogers WJ, Gale MD (1991) RFLP-based genetic map of rye (*Secale cereale* L.) chromosome 1R. Theor Appl Genet 82:174–178

Wang ML, Atkinson MD, Chinoy CN, Devos KM, Gale MD (1992) Comparative RFLP-based genetic maps of barley chromosome 5(1H) and rye chromosome 1R. Theor Appl Genet 84:339–334

Xu J, Kasha KJ (1992) Transfer of a dominant gene for powdery mildew resistance and DNA from *Hordeum bulbosum* into a cultivar (*H. vulgare*). Theor Appl Genet 771–777

Xu J, Snape JW (1987) Crossabilities of barley varieties with diploid and tetraploid clones of *Hordeum bulbosum*. Barley Genet Newslett 17:40–42

Xu J, Snape JW (1988) The cytology of hybrids between *Hordeum vulgare* and *H. bulbosum*. Genome 30:486–494

Xu J, Snape JW (1989) The resistance of *Hordeum bulbosum* and its hybrids with *H. vulgare* to common fungal pathogens. Euphytica 41:273–276

Xu J, Procunier JD, Kasha KJ (1991) Germplasm transfer from *Hordeum bulbosum* to *H. vulgare*. In: Muck L (ed). Barley genetics VI. Proc 6th Int Barley Genet Sym, Munks gaard Int, Copenhagen, pp 97–98