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Comparative physical mapping of the 5S and 18S-25S rDNA in nine wild *Hordeum* species and cytotypes

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Absract The physical locations of the 5S and 18S-25S rDNA sequences were examined in nine wild *Hordeum* species and cytotypes by double-target in situ hybridization using digoxigenin-labelled 5S rDNA and biotin-labelled 18S-25S rDNA as probes. *H. vulgare* ssp. *spontaneum* (2n = 2*x* = 14; I-genome) had a similar composition of 5S and 18S-25S rDNA to cultivated barley (*H. vulgare* ssp. *vulgare*, I-genome), with two major 18S-25S rDNA sites and minor sites on four of the other five chromosomes; three chromosomes had 5S rDNA sites. The closely related *H. bulbosum* (2*x*; also I-genome) showed only one pair of 5S rDNA sites and one pair of 18S-25S rDNA sites on different chromosomes. Four wild diploid species, *H. marinum* (X-genome), *H. glaucum* and *H. murinum* (Y-genomes) and *H. chilense* (H-genome), differed in the number (2–3 pairs), location, and relative order of 5S and the one or two major 18S-25S rDNA sites, but no minor 18S-25S rDNA sites were observed. *H. murinum* 4*x* had three chromosome pairs carrying 5S rDNA, while the diploid had only a single pair. Two other tetraploid species, *H. brachyantherum* 4*x* and *H. brevisubulatum* 4*x* (both considered to have H-type genomes), had minor 18S-25S rDNA sites, as well as the major sites. Unusual double 5S rDNA sites – two sites on one chromosome arm separated by a short distance – were found in the American H-genome species, *H. chilense* and *H. brachyantherum* 4*x*. The results indicate that the species *H. brachyantherum* 4*x* and *H. brevisubulatum* 4*x* have a complex evolutionary history, probably involving the multiplication of minor rDNA sites (as in *H. vulgare sensu lato*), or the incorporation of both I and H types of genome. The rDNA markers are useful for an investigation of chromosome evolution and phylogeny.

Key words In situ hybridization · *Hordeum* · Karyotype evolution · rDNA · Barley

Introduction

The genus *Hordeum* is divided into 32 species (in total 45 taxa), and 51 cytotypes exist at three ploidy levels (diploid, tetraploid or hexaploid) with a basic chromosome number of *x* = 7 (Bothmer et al. 1995). Studies on chromosome pairing in hybrids suggested that at least four different genomes, H, I, X and Y, are present in the diploid species of this genus (Bothmer et al. 1986)[1]. In the polyploid species, the nature of polyploidy (alloploidy or autoploidy) has long been uncertain, partially due to the control of chromosome pairing, presumably genetically regulated as in hexaploid wheat. To study karyotype evolution of this important genus, C-banding was applied to all cytotypes (Linde-Laursen

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[1] At the 7th International Barley Genetics Symposium, 1996, it was agreed to designate the barley chromosomes by their homoeologous groups followed by the letter H (Linde-Laursen et al. 1997), as already widely used in the barley genetics community. The Triticeae conference has recommended the use of I for *H. vulgare* and close relatives, and H for many wild species (Wang et al. 1996). In the present paper, because we are discussing the whole genus, we follow the Triticeae recommendation with the exception that individual barley chromosomes are referred to as 1H etc.; superscripts are used for other species

et al. 1980, 1986 a, b, 1989 a, b, 1990 a, b; Linde-Laursen and Bothmer 1984; Konishi and Linde-Laursen 1988; Linde-Laursen 1990) giving evidence for the unique position of the species with the I-genome, *H. vulgare* and *H. bulbosum*, which have predominantly proximal bands. C-banding analyses clarified the relationships between some species, but the nature of polyploidy and some relationships remain uncertain because of intraspecific polymorphisms and interspecific similarities.

Nucleolus organizer regions (NORs) are recognized as secondary constrictions in satellited (SAT) chromosomes. The 18S-5.8S-25S rRNA genes and intergenic spacer regions (18S-25S rDNA) exist as tandem repeats at the NORs and at other chromosomal sites where they may not be associated with an NOR. Recent in situ hybridization experiments using the 18S-25S rDNA as a probe have revealed the presence of minor 18S-25S rDNA sites in addition to the major 18S-25S rDNA sites with NOR-forming activity in various cereals (Mukai et al. 1991; Leitch and Heslop-Harrison 1992; Jiang and Gill 1994; Pedersen and Linde-Laursen 1994) which are of importance in phylogenetic studies. The origin of minor 18S-25S rDNA sites is not well understood; silver-staining or an analysis of the meiotic association of nucleoli cannot detect their expression. The 5S rRNA genes make up an independent multilocus, multigene family and are also detected by in situ hybridization (Mukai et al. 1990; Leitch and Heslop-Harrison 1993; Fukui et al. 1994; Cuadrado et al. 1995). The 18S-25S and 5S rDNA sites are useful chromosome landmarks and provide valuable evidence about genome evolution at both molecular (Molnar et al. 1989) and chromosomal (Castilho and Heslop-Harrison 1995) levels. Furthermore, the location and polymorphisms in the sites are valuable markers for the integration of genetic and physical chromosome maps. The data can be integrated with extensive data from the examination of molecular polymorphisms of the genes and intergenic spacers in both 18S-25S and 5S rDNA (e.g. Kellogg and Appels 1995) sequences.

Physical mapping of 18S-25S and 5S rDNA sites by in situ hybridization in cultivated barley (*H. vulgare* ssp. *vulgare*) (Leitch and Heslop-Harrison 1992, 1993; Fukui et al. 1994; Pedersen and Linde-Laursen 1994) showed an unexpectedly high number of both 18S-25S and 5S rDNA sites. A few wild *Hordeum* species have been studied: *H. marinum* (Linde-Laursen et al. 1992 b) and *H. chilense* (Cabrera et al. 1995) were analyzed for 18S-25S rDNA sites, and recently Bustos et al. (1996) have examined the locations of both rDNAs in five wild *Hordeum* species. Based on the value of the studies of Linde-Laursen et al. (1992 a, 1995) using the SAT chromosomes alone as evolutionary markers, we aimed here to analyze the number and location of both 5S and 18S-25S rDNA in nine wild *Hordeum* species and cytotypes by double-target fluorescent in situ hybridization. This molecular cytogenetic study, covering different geographical origins, ploidy levels, and inbreeding and outbreeding species, provides new information on the karyotypes, genome organization and phylogeny of the genus *Hordeum*, and about the evolution of both classes of rDNA sites.

Materials and methods

Plant material and chromosome preparation

Nine wild *Hordeum* species and cytotypes, along with an accession of cultivated barley, were used in this study (Table 1). Here, we refer to cultivated barley as barley, and *H. vulgare* ssp. *spontaneum* as *H. spontaneum*; together they are referred to as *H. vulgare sensu lato*. Seeds were germinated on moist filter paper for about 2 days and then kept at 4°C for 24 h followed by 25°C for 24 h to synchronize cell divisions. The excised root tips were treated in ice water for 24 h and fixed in ethanol:acetic acid (3:1). Chromosomes were prepared by squashing enzyme-treated root-tips as described by Schwarzacher et al. (1994).

Table 1 List of the materials used in the present study

Genome	Species	Ploidy	Accession	Figure
I	*H. vulgare* L. ssp. *vulgare*[a]	2*x*	Kinai 5[b]	Not shown
	H. vulgare ssp. *spontaneum* (C. Koch) Thell[c]	2*x*	John Innes collection line R1	1
	H. bulbosum L.	2*x*	6R52 × J1R132	2
H	*H. chilense* Roemer & Schultes	2*x*	John Innes line 1	3
	H. brachyantherum Nevski	4*x*	John Innes line 2	8
	H. brevisubulatum (Trinius) Link	4*x*	John Innes line 1	9
X	*H. marinum* Hudson ssp. *marinum*	2*x*	John Innes line 1 (H515[d])	4
Y	*H. glaucum* Steud. (syn: *H. murinum* ssp. *glaucum* (Steude) Tzvelev)	2*x*	John Innes line 2	5
	H. murinum L.	2*x*	John Innes line 71	6
	H. murinum L.	4*x*	John Innes line 8257C	7

[a] Referred to as barley in this paper
[b] Japanese Breeding line
[c] Referred to as *H. spontaneum* in this paper; together barley and *H. spontaneum* are referred to as *H. vulgare sensu lato*
[d] Collected in Algeciras, Spain, by Dr. N. Jacobsen

DNA probes

Two DNA clones, pTa794 and pTa71, were used as probes. Clone pTa794 contains a 410-bp *Bam*HI fragment of the 5S rDNA, harboring a 120-bp coding sequence for the 5S rRNA gene and the intergenic spacers isolated from common wheat, *Triticum aestivum* L. (Gerlach and Dyer 1980). Clone pTa71, the 18S-25S rDNA, is a 9-kb *Eco*RI fragment from common wheat (Gerlach and Bedbrook 1979), containing the coding sequences for the 18S, 5.8S, and 25S rRNA genes and the intergenic spacer sequences.

In situ hybridization

Chromosome preparations were treated with 500 μg/ml of pepsin in a 0.01 M HCl solution for 10 min at 37°C, washed for 5 min in 2 × SSC (0.3 M NaCl and 0.03 M sodium citrate) and then treated with 100 μg/ml of RNase in 2 × SSC at 37°C for 1 h. After washes in 2 × SSC, the slides were stabilized in a 4% solution of freshly depolymerized paraformaldehyde in water at room temperature for 10 min, washed in 2 × SSC, dehydrated in an ethanol series and air dried.

The 5S rDNA clone was labelled by the polymerase chain reaction (PCR) with digoxigenin-11-dUTP (Boeringer Mannheim, Germany) and the 18S-25S rDNA clone was labelled with biotin-16-dUTP (Boeringer Mannheim) by nick translation. The probe mixture consisted of 2.5–5 ng/μl of both labelled probes, 50% (v/v) formamide, 10% (w/v) dextran sulphate, 0.1% (w/v) SDS (sodium dodecyl sulphate) in 2 × SSC and 3 ng/μl of sonicated salmon-sperm DNA (hybridization stringency of 76%). The hybridization mixture was denatured at 70°C for 10 min and chilled on ice for 5 min. Then 30 μl of the probe mixture were loaded onto each slide and a plastic coverslip was applied. The chromosomes, together with the probe, were denatured for 10 min at 80°C by a modified programmable temperature controller (Heslop-Harrison et al. 1991). The temperature was gradually decreased to 37°C.

The hybridization was carried out overnight at 37°C in a humid chamber. After hybridization, the slides were washed in 2 × SSC at 42°C for 3 min and then given a stringent wash in 20% (v/v) formamide in 0.1 × SSC at 42°C for 10 min. This wash removes probe sequences with less than 85% homology to the chromosomal target sequences. In practice, this level of homology enables the wheat-origin 5S, 18S, 5.8S and 25S rRNA genes to remain stably hybridized. The slides were then washed for 2 × 5 min each in 2 × SSC at 42°C and in 2 × SSC at room temperature.

Sites of hybridization of digoxigenin-labelled 5S rDNA and biotin-labelled 18S-25S rDNA were detected with sheep anti-digoxigenin-FITC (fluorescein isothiocyanate) and streptavidin-Cy3, respectively. Slides were transferred into detection buffer (4 × SSC, 0.2% Tween 20) for 5 min, treated with 5% (w/v) bovine serum albumin (BSA) in detection buffer for 5 min, and then incubated in 10–20 μg/ml of anti-digoxigenin-FITC and 5–10 μg/ml of streptavidin-Cy3 in detection buffer containing 5% (w/v) BSA at 37°C for 1 h. Preparations were counterstained with 6 μg/ml of 4,6-diamidino-2-phenylindole (DAPI) in McIlvaines citrate buffer, pH 7.0, and then mounted in antifade solution (AF1, Citifluor). Slides were examined with a Leitz epifluorescence microscope with appropriate filter sets. Photographs were taken on Fujicolor Super HG 400 color print film. Negatives were scanned to PhotoCD and printed from Adobe Photoshop using only cropping and processing functions that affect all pixels in the image equally. The length of the chromosomes with hybridization sites and the distance of the site from the centromere were measured to calculate the fraction length. At least ten chromosomes were measured, except in some species where only six chromosomes were measured. Standard deviations of fraction lengths were 0.02–0.10. In the outbreeding species *H. bulbosum* and *H. brevisubulatum*, measurements were taken from metaphases of a single plant.

Results

Figures 1–9 show in situ hybridization results with the 5S and 18S-25S rDNA probes. Figure 10 summarizes the distribution of rDNA sites along the chromosomes of the I-, H-, X-, and Y-genome species shown in Table 1.

H. spontaneum

Two major pairs and four minor pairs of 18S-25S rDNA sites were detected (Fig. 1), as reported in barley (Leitch and Heslop-Harrison 1992; Pedersen and Linde-Laursen 1994). In *H. spontaneum*, three pairs of 5S rDNA sites, in contrast with four pairs in barley (Leitch and Heslop-Harrison 1993; Fukui et al. 1994 and present series of experiments), were observed. Chromosome identification on the basis of combinations of 5S and 18S-25S rDNA sites indicated that in the *H. spontaneum* accession a 5S rDNA site on the long arm of chromosome 4H was absent compared to barley (Fig. 10).

Wild diploid species

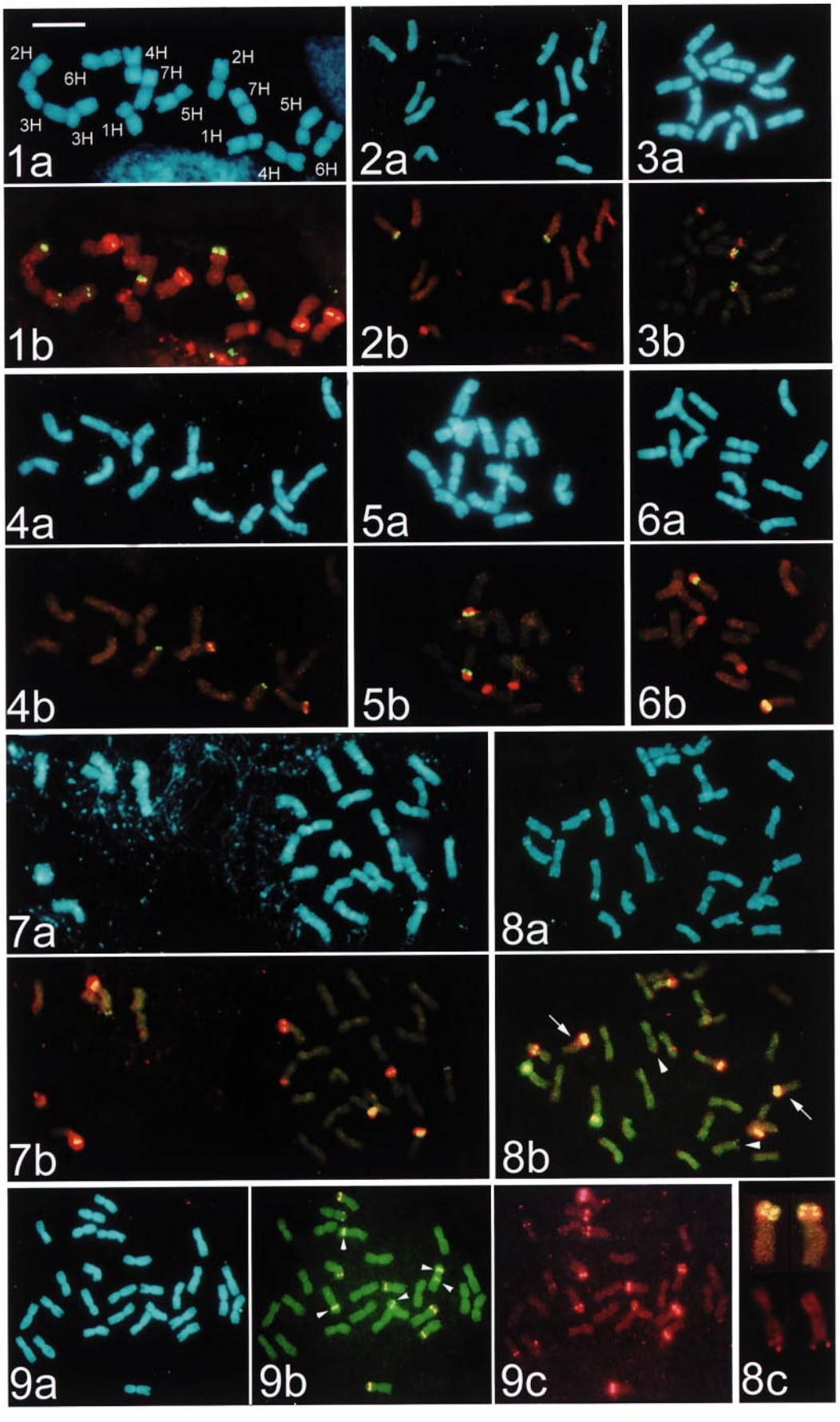
In the wild diploid species (excluding *H. spontaneum*) all 18S-25S rDNA sites were located in secondary constrictions of SAT chromosomes (Figs. 2–6, 10) and no minor 18S-25S rDNA sites were observed.

H. bulbosum 2*x* had a pair of 5S rDNA sites and a pair of major 18S-25S rDNA sites on different chromosomes (Fig. 2). Since the SAT chromosomes with the 18S-25S rDNA sites differed in length, their idiograms were prepared separately.

H. chilense 2*x* had 5S rDNA sites on one chromosome pair, and two pairs of major 18S-25S rDNA sites (Fig. 3). The SAT chromosomes ($5H^{ch}$ and $6H^{ch}$) with a major 18S-25S rDNA site were identified according to Cabrera et al. (1995). 5S rDNA sites were detected as unusual double sites in the distal region of the short arm of chromosome $5H^{ch}$ with two 5S signals separated by a short distance on one chromosome arm. It was impossible to resolve the relative order of the proximal 5S rDNA site and the 18S-25S rDNA site.

In *H. marinum* 2*x*, two pairs of 5S rDNA sites and a pair of major 18S-25S rDNA sites were observed (Fig. 4). The short arm of the submetacentric SAT chromosome pair carried a 5S rDNA site distal to the 18S-25S rDNA site. The other 5S rDNA site was located near the end of the long arm of a metacentric chromosome pair.

H. glaucum 2*x* (Fig. 5; sometimes recognized as a subspecies of *H. murinum*, Table 1) and *H. murinum* 2*x* (Fig. 6) each had a pair of 5S rDNA sites and two pairs of major 18S-25S rDNA sites. The 5S site and one of the 18S-25S rDNA sites were both located in the


2H
4H
2H
6H
7H
7H
5H
5H
3H
3H
1H
1H
4H
6H
1a
2a
3a
1b
2b
3b
4a
5a
6a
4b
5b
6b
7a
8a
7b
8b
9a
9b
9c
8c

short arm of the submetacentric SAT chromosomes pair, with the 5S rDNA proximal. The other major 18S-25S rDNA site was located in the long arm of the metacentric SAT chromosome pair. The arm ratios and fraction lengths of the chromosomes with 5S and 18S-25S rDNA sites were similar between these species.

Wild tetraploid species

H. murinum 4*x* had three pairs of 5S rDNA sites and four (three major and one minor) pairs of 18S-25S rDNA sites (Fig. 7). The pattern of 18S-25S rDNA-carrying chromosomes of *H. murinum* 4*x* did not represent an exact doubling of *H. murinum* 2*x* as an additional 5S site was subterminal on a metacentric chromosome pair and the 18S-25S rDNA sites varied in intensity (Fig. 10). In the tetraploid species, secondary constrictions were hard to observe with DAPI staining, presumably because of the variable expression of the rDNA sites; therefore, SAT chromosomes were difficult to identify.

H. brachyantherum 4*x* had two pairs of chromosomes carrying 5S rDNA sites and six chromosome pairs with 18S-25S rDNA sites (Fig. 8). Two pairs of submetacentric chromosomes had both 5S and 18S-25S rDNA sites with close proximity, but one of the pairs had a double 5S rDNA site with the two 5S signals separated by a short distance. Two pairs of metacentric chromosomes carried an 18S-25S rDNA site at a similar fraction length, but they differed in signal intensities. Two morphologically distinct chromosome pairs having 18S-25S rDNA sites on either both arms or one arm were also found.

H. brevisubulatum 4*x*, a self-incompatible species, had a heterogeneous composition of 5S and 18S-25S rDNA. In a single plant, a total of five 5S rDNA sites and 18 (eight major and ten minor) 18S-25S rDNA sites were observed. Six pairs of chromosomes (designated as A–F) were identified as putative homologues based on chromosome length, arm ratio, distribution and intensity of signals. A polymorphic 5S rDNA site was observed in the long arm of chromosome pair A. In the short arm of chromosome pair A, the 5S rDNA site was slightly distal to the major 18S-25S rDNA site, but in chromosome pair B the 5S and major 18S-25S rDNA sites existed in close proximity. Two morphologically distinct chromosomes with an 18S-25S rDNA site (designation G1 and H1) were detected and their homologous partner could not be determined.

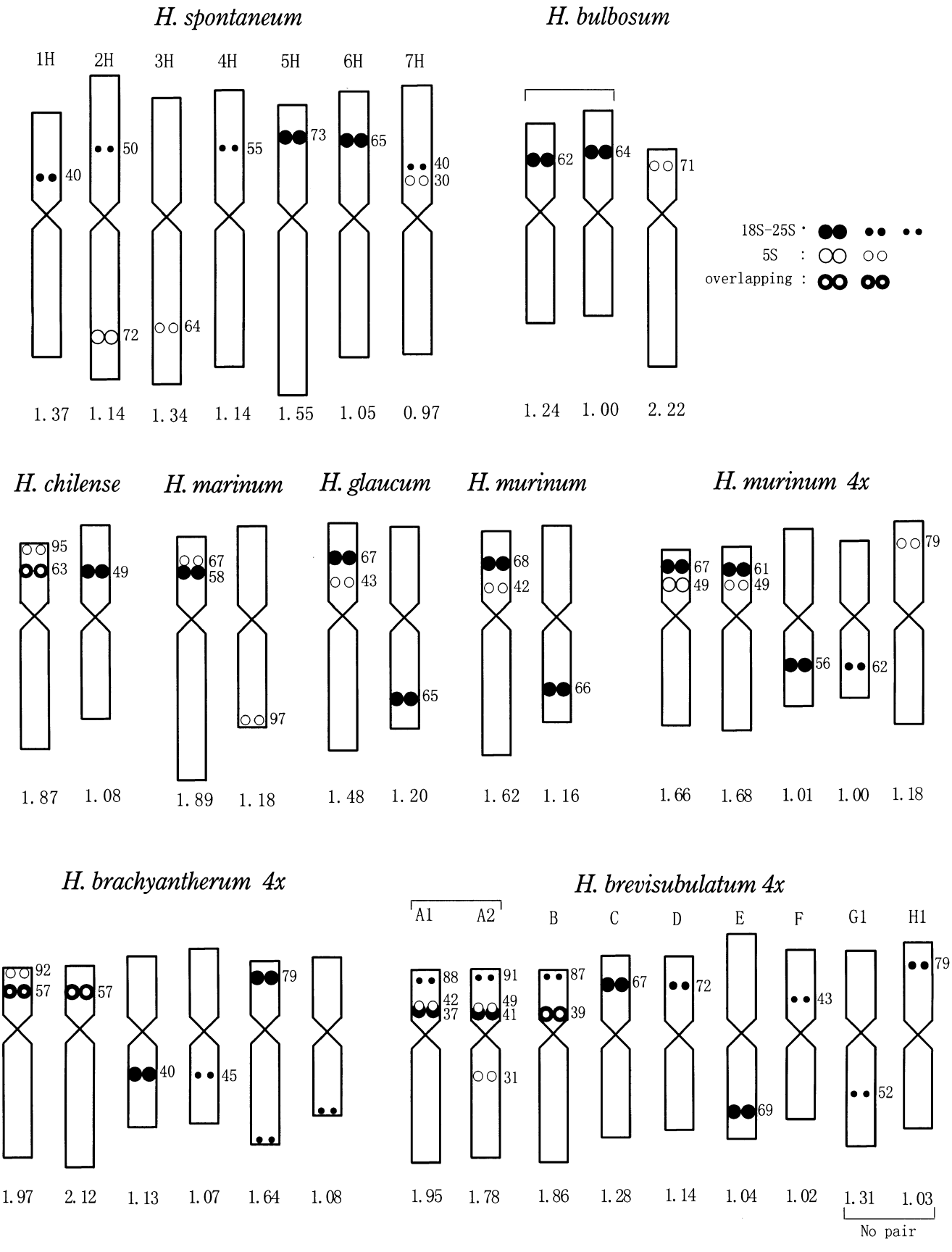
Figs. 1–9 In situ hybridization to root-tip metaphase cells from nine *Hordeum* taxa. *Blue DAPI staining* (parts **a**) shows the chromosomal DNA. *Red in situ hybridization signal* shows 18S-25S rDNA sites and (Figs. **1–8 b**) has been overlayed with a *green signal* showing 5S rDNA sites (parts **b**). Where the signals overlap, the colour is *yellow*. **1–6** are diploid, 2n = 2*x* = 14, cytotypes, while **7–9** show tetraploid, 2n = 4*x* = 28, cytotypes. The scale bar in **1 a** represents 10 μm in **1–6**; 12 μm in **7, 8 a, 8 b and 9**; 5.5 μm in **8 c**. (**1**) *H. spontaneum* has similar rDNA sites to barley with two major and four minor pairs of 18S-25S rDNA sites and three pairs of 5S rDNA sites. The other wild diploid species have fewer sites. (**2**) *H. bulbosum* has a single pair of sites of both rDNAs on different chromosomes. There is polymorphism between the chromosome pair carrying the 18S-25S rDNA in this outbreeding species. (**3**) *H. chilense* has two 18S-25S rDNA sites on chromosomes $5H^{ch}$ and $6H^{ch}$ and an unusual double 5S site on chromosome $5H^{ch}$. (**4**) *H. marinum* has two pairs of 5S rDNA sites, one distal to the single 18S-25S site. (**5**) *H. glaucum* and (**6**) *H. murinum* 2*x* both have two pairs of 18S-25S and one pair of 5S sites. (**7**) *H. murinum* 4*x* shows three major pairs of 5S and 18S-25S rDNA sites, with an extra minor pair of 18S-25S sites. Compared with the diploid cytotype (Fig. **6**), the additional 5S sites were found on a non-18S-25S rDNA-carrying chromosome pair. (**8**) *H. brachyantherum* 4*x* has seven pairs of 18S-25S rDNA sites and two pairs of 5S sites. **c** shows enlarged chromosomes from **b** with different processing to show (upper) the double 5S rDNA sites (*arrows* in **b**) and (lower) minor 18S-25S sites (*arrowheads* in **b**). (**9**) *H. brevisubulatum* 4*x*, an outbreeding species, with 18 polymorphic 18S-25S sites (on 14 chromosomes) and five 5S rDNA sites (*arrowheads*, on four chromosomes)

Discussion

18S-25S and 5S rDNA sites

The present in situ hybridization experiments revealed that the *H. spontaneum* accession has a similar composition of 5S and 18S-25S rDNA sites to barley, except that a 5S rDNA site on the long arm of chromosome 4H was not observed (Fig. 1). Because four pairs of 5S rDNA sites were detected in barley in the same series of experiments (data not shown), the sensitivity achieved under the conditions employed here was similar to that employed for the studies in barley (Leitch and Heslop-Harrison 1992; Pedersen and Linde-Laursen 1994), and hence comparisons are worthwhile. We expect that the 5S rDNA site on chromosome 4H of this accession is deleted or else has very few copies of the 5S sequence compared to that of the barley cultivars so far investigated. The presence of weak 5S signals on chromosome 4H in another *H. spontaneum* accession (Taketa et al., unpublished) gives evidence for variation in its copy number among different accessions of *H. spontaneum*.

In the other five wild diploid species including *H. bulbosum*, also with the I-genome (Fig. 2), the present in situ hybridization experiments detected only major 18S-25S rDNA sites at the secondary constrictions of SAT chromosomes and no minor 18S-25S rDNA sites were observed. This strongly contrasts with the results of *H. vulgare sensu lato* where four of the five non-SAT chromosomes have minor 18S-25S rDNA sites. All three wild tetraploid species, like barley itself, had both major and minor 18S-25S rDNA sites. The total numbers of 18S-25S rDNA sites detected by the present in situ hybridization experiments exceed the maximum numbers of nucleoli detected by silver staining for the


H. spontaneum
1H 2H 3H 4H 5H 6H 7H
40
50
72
64
55
73
65
40
30
1.37 1.14 1.34 1.14 1.55 1.05 0.97
H. bulbosum
62
64
71
1.24 1.00 2.22
18S-25S :
5S :
overlapping :
H. chilense
95
63
49
1.87 1.08
H. marinum
67
58
97
1.89 1.18
H. glaucum
67
43
65
1.48 1.20
H. murinum
68
42
66
1.62 1.16
H. murinum 4x
67
49
61
49
56
62
79
1.66 1.68 1.01 1.00 1.18
H. brachyantherum 4x
92
57
57
40
45
79
1.97 2.12 1.13 1.07 1.64 1.08
H. brevisubulatum 4x
A1 A2 B C D E F G1 H1
88
42
37
91
49
41
31
87
39
67
72
69
43
52
79
1.95 1.78 1.86 1.28 1.14 1.04 1.02 1.31 1.03
No pair

respective tetraploid species (Rajhathy and Morrison 1962; Linde-Laursen et al. 1986 a, 1989 b, 1995). Suppression or inactivity of the rRNA genes probably explains such discrepancies, although differences between accessions and the presence of incomplete, diverged rDNA sequences may also be involved.

Polymorphism for 5S rDNA sites was observed in a plant of *H. brevisubulatum* 4*x* (Fig. 9). The result from *H. murinum* 4*x* (Fig. 7) was the same as that of Bustos et al. (1996), indicating that the sensitivity of the techniques employed was similar; intraspecific polymorphisms were not detected. In *H. marinum*, Bustos et al. (1996) reported that subspecies *marinum* and *gussoneanum* have a third 5S rDNA site on different chromosomes in addition to the two 5S rDNA sites detected in the present study. These results, together with that from *H. spontaneum*, suggest the existence of intraspecific polymorphism for 5S rDNA sites.

In *H. chilense* (Fig. 3) and *H. brachyantherum* 4*x* (Fig. 8), novel double 5S rDNA sites were found. All 5S rDNA sites so far reported in wheat (Mukai et al. 1990) and barley (Leitch and Heslop-Harrison 1993; Fukui et al. 1994) showed a single hybridization site on each chromatid. Such double 5S rDNA sites have not been reported in the Triticeae and may be characteristic of American H-genome species. The double sites were visible on the micrograph of *H. chilense* shown by Bustos et al. (1996) but were not discussed by them. No double site was observed in *H. brevisubulatum* 4*x*, an Asian H-genome species.

Relative order of 5S and 18S-25S rDNA sites

The relative order of 5S and 18S-25S rDNA sites provides additional information on chromosome organization and evolution, as pointed out by Castilho and Heslop-Harrison (1995). The two possible relative orders are both observed in wild *Hordeum* species; in *H. marinum* (X-genome, Fig. 4) the 5S rDNA site is distal to 18S-25S rDNA, while in *H. glaucum* and *H. murinum* (Y-genome, Figs 5, 6) the order is reversed, supporting the distinction of the genomes as X and Y. In *H. chilense*, *H. brachyantherum* 4*x* and *H. brevisubulatum* 4*x* (H-genome species) 5S and 18S-25S rDNA sites were too close to unequivocally resolve their relative order, except for chromosome pair A of *H. brevisubulatum* 4*x*, in which 5S rDNA was distal to the major 18S-25S rDNA. Future studies employing prometaphase chromosomes or pachytene nuclei may resolve their relative orders. More complex combinations of 5S and 18S-25S rDNA sites were also found in the H-genome species. Knowledge of the relative order and distribution of 5S and 18S-25S rDNA sites, will help clarify the homoeology and genome assignment of individual wild barley chromosomes and might assist their use in crossing and substitution programmes aiming to assess the agronomic value of individual chromosomes.

Fig. 10 Idiograms of the morphology of the pairs of chromosomes carrying 5S and 18S-25S rDNA investigated here. *Numbers* under and besides the chromosomes are arm ratios and the percent arm length from the centromere, respectively. Single chromosome types are shown from *H. bulbosum* and *H. brevisubulatum* where chromosome pairs were heteromorphic

Phylogeny and karyotype evolution in the genus *Hordeum*

Studies on chromosome pairing indicate that *H. vulgare* and *H. bulbosum* have the same genome (Kasha and Sadasivaiah 1971; Bothmer et al. 1986). However, these two species markedly differed in the numbers and location of 5S and major and minor 18S-25S rDNA sites (Fig. 1). Such large differences indicate that massive reorganization of 5S and 18S-25S rDNA sites might have occurred after the differentiation of these species. The study of rDNA sequence restriction maps in *Hordeum* by Molnar et al. (1989) showed good agreement with conventional taxonomic groupings, except that *H. bulbosum* was separated from the other I-genome species. It is unclear why *H. vulgare sensu lato* has so many sites, and whether this is by chance alone or gives the species an advantage under agricultural selection conditions.

On the basis of the present rDNA data, none of the three tetraploid species can be considered as a simple autotetraploid. The chromosomes and rDNA sites found in *H. murinum* 4*x* (Fig. 7) differed from a doubling of the chromosomes in the diploid species (Figs. 6, 10), although the differences were small so that an autotetraploid origin cannot be ruled out. The chromosomes and rDNA sites in *H. brachyantherum* 4*x* could not be interpreted as arising from sets of four homologous chromosomes. This supports an alloploid origin of *H. brachyantherum* 4*x*, a self-pollinating species, as also suggested from meiotic and karyotype analyses (Rajhathy and Morrison 1959, 1962), isoenzyme pattern (Jorgensen 1986) and C-banding pattern (Linde-Laursen et al. 1986 a). The *H. brevisubulatum* 4*x* accession analysed in this study has a complex pattern of 5S and 18S-25S rDNA sites and shows no clear evidence for autotetraploidy with four sets of homologous genomes, as also indicated from C-banding analysis (Linde-Laursen et al. 1980). This sharply contrast with the results from meiotic studies which suggest its autoploid origin (Dewey 1979; Landström et al. 1984). Because heterozygosity for karyotype and rDNA is likely to be frequent in *H. brevisubulatum*, an outbreeding species, further work is needed to elucidate the nature of polyploidy in this species. The data on 5S and 18S-25S rDNA sites must be used to complement other

data; RFLP and physical mapping studies of wheat and barley showed that rDNA sites can be deleted or change their positions within a chromosome arm without disturbing the linkage groups (Castilho and Heslop-Harrison 1995; Dubcovsky and Dvorak 1995; Dubcovsky et al. 1996). In situ hybridization experiments using other repetitive sequences as probes would complement the results from rDNA sites. A dispersed repetitive sequence pHch950 cloned from *H. chilense* (Hueros et al. 1993) was successfully used to demonstrate an alloploid origin of *H. depressum* 4*x* (Ferrer et al. 1995), *H. murinum* 4*x* and *H. secalinum* 4*x* (Bustos et al. 1996).

The present results demonstrate that the physical mapping of major and minor 18S-25S and 5S rDNA sites within the genus *Hordeum* is providing valuable information about karyotype evolution and relationships for the species. Clearly, the analysis of rDNA sites in further species and additional accessions of the same species will be worthwhile to gain a more complete picture of variation in the genus, and enable a modelling of rDNA evolution, of particular significance for an understanding of genome evolution in the Triticeae as a whole.

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