The genome sizes of *Hordeum* species show considerable variation

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Abstract: *Hordeum*, distributed worldwide in temperate zones, is the second largest genus in the tribe Triticeae and includes diploid, tetraploid, and hexaploid species. We determined, by DAPI staining and flow cytometry, the nuclear DNA content for 35 accessions of the genus *Hordeum*, from a total of 19 species, including specimens of 2 cultivars and 2 landraces of *Hordeum vulgare* ssp. *vulgare* as well as samples of 12 *Hordeum vulgare* ssp. *spontaneum* populations. Genome sizes ranged from 5.69 to 9.41 pg for the G_1 nuclei of the diploids, and from 13.13 to 18.36 pg for those of the tetraploids. This constitutes a 1.7-fold variation for the diploids, contrasting with a 4% variation previously reported. For *H. vulgare* ssp. *vulgare* (barley), the accessions examined differed by 18%. These variations in genome size cannot be correlated with meiotic pairing groups (I, H, X, Y) or with proposed phylogenetic relationships within the genus. Genome size variation between barley accessions cannot be related to status as cultivated or wild, or to climatic or geological gradients. We suggest these data may indicate rapid but sporadic changes in genome size within the genus.

Key words: barley, *Hordeum*, Triticeae, genome size, flow cytometry.

Résumé : Le genre *Hordeum*, largement distribué dans les zones tempérées partout dans le monde, est le deuxième en importance parmi la tribu Triticeae et comprend des espèces diploïdes, tétraploïdes et hexaploïdes. Le contenu en ADN nucléaire a été déterminé par coloration au DAPI et cytométrie en flux chez 35 accessions du genre *Hordeum*. Ces accessions représentaient 19 espèces dont des spécimens de deux cultivars et de deux variétés locales du *Hordeum vulgare* ssp. *vulgare* ainsi que des échantillons de 12 populations du *Hordeum vulgare* ssp. *spontaneum*. La taille du génome variait de 5,69 à 9,41 pg dans les noyaux en G_1 chez les diploïdes et de 13,13 à 18,36 pg chez les tétraploïdes. Ceci indique une variation pouvant atteindre un facteur de 1,7 tandis que des travaux antérieurs n'avaient rapportés qu'une variation de 4%. Chez les accessions du *H. vulgare* ssp. *vulgare* (orge), une variation de 18% a été observée. Ces variations quant à la taille du génome ne peuvent être corrélées ni avec les groupes d'appariement méiotique (I, H, X, Y) ni avec les relations phylogénétiques proposées chez ce genre. La taille du génome n'est pas liée avec l'état de plante sauvage ou de plante cultivée pas plus qu'avec des gradients climatiques ou géologiques. Les auteurs suggèrent que ces données pourraient indiquer des changements rapides mais sporadiques de la taille du génome à l'intérieur de ce genre.

Mots clés : orge, *Hordeum*, Triticeae, taille du génome, cytométrie en flux.

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Introduction

The *Hordeum* genus, with some 50 species, is the second largest genus in the tribe Triticeae of the family Poaceae. It includes the important crop barley (*Hordeum vulgare* L.) and is widely distributed in the temperate zones of both hemispheres from sea level to more than 4500 m (von Bothmer et al. 1995). While *H. vulgare* (Löve 1984), or *H. vulgare* and *Hordeum bulbosum* (Dewey 1984), have sometimes been treated separately, all species are generally included in a single genus. Nevertheless, combinational analyses of chromosomal pairing during meiosis in *Hordeum* hybrids separate the genus into four genomic groups, I, Y, X, and H (von Bothmer et al. 1986; Jacobsen and von Bothmer 1992). Both genome diversification and meiotic pairing behavior in hybrids between species have been linked to repetitive DNA (Hoang-Tang et al. 1990; Irick 1994). Indeed, a cladogram produced from repetitive DNA analyses in *Hordeum* (Svitashev et al. 1994) largely supports the concept of four basic genomes. This and several preceding studies have employed the repetitive sequence pHv7161 and flanking sequences in analyzing *Hordeum* genomic relationships (Vershinin et al. 1990, 1992; Svitashev et al. 1994). These sequences are in fact part (bases 357–1461 of accession Z17327) of the long terminal repeat (LTR) of retrotransposon *BARE*-1 (Manninen and Schulman 1993).

We have been interested in the role of *BARE*-1 in the dynamics of genome organization and evolution in *Hordeum*. Sequences hybridizing to *BARE*-1 probes are

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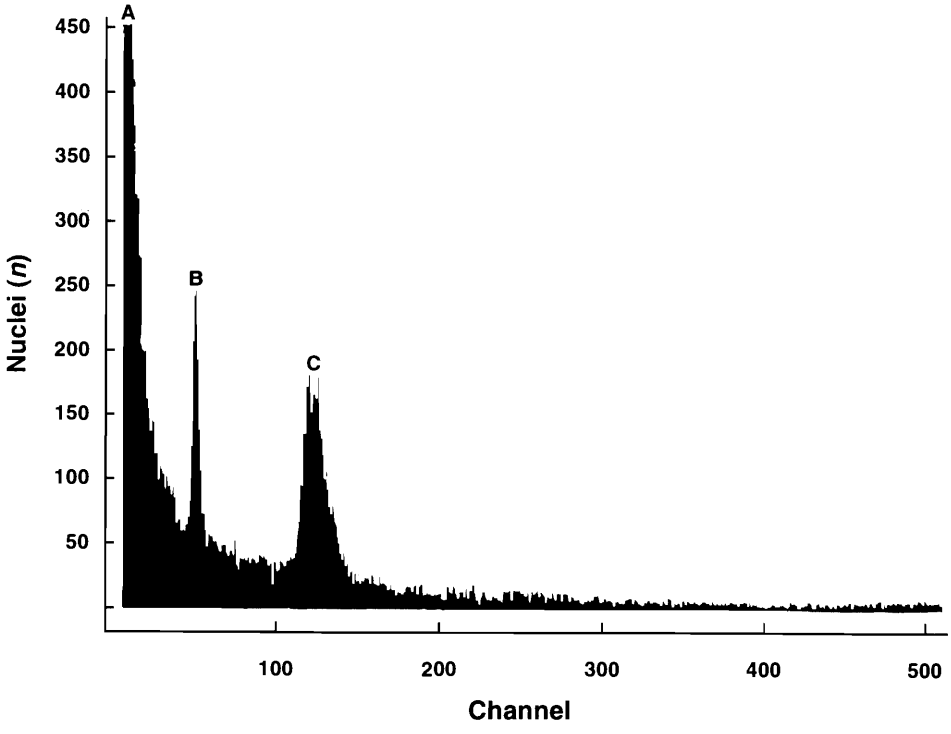
Fig. 1. Flow cytometry of *Hordeum euclaston* leaf nuclei. The data are displayed as a histogram of numbers of nuclei per relative fluorescence channel (n = 512) on a linear scale. Peak A represents cell debris, peak B, CRBC nuclei, and peak C, the G_1-phase leaf nuclei. A total of 2×10^4 nuclei were counted.



distributed on every barley chromosome and comprise almost 7% of the total genome (Suoniemi et al. 1996), making it a major component of the repetitive DNA fraction. Retrotransposon copy numbers (Joseph et al. 1990; Pearce et al. 1996) can vary considerably from genome to genome, and genome size in plants can vary more than 170-fold (Arumuganathan and Earle 1991*a*), even differing within a single species (Cullis and Cleary 1986; Bennett and Bennett 1992; Rayburn et al. 1993). The only report on the genome sizes of wild *Hordeum* was made some 25 years ago (Bennett and Smith 1971), where little (4%) variation was detected. In order to explore the role of *BARE*-like retrotransposons in genome diversification in *Hordeum*, we first looked again at genome sizes in this genus. We report here the nuclear DNA content, determined by flow cytometry, of 35 accessions of the genus *Hordeum*, from a total of 19 species, including specimens of 2 cultivars and 2 landraces of *Hordeum vulgare* ssp. *vulgare*, as well as samples of 12 *Hordeum vulgare* ssp. *spontaneum* populations.

Materials and methods

Intact nuclei of the *Hordeum* accessions were prepared from frozen (−80°C) or fresh 10-day-old seedling leaves as previously described (Arumuganathan and Earle 1991*b*), except that 4′,6-diamidino-2-phenylindole (DAPI) at 2 μg/mL (Rayburn 1993) was substituted for propidium iodide.

Nuclei were analyzed by flow cytometry on a Partec PAS II flow cytometer (Partec AG, Arlesheim, Switzerland), equipped with a high pressure mercury lamp (HBO 100 W, Osram, Augsburg, Germany), installed at VTT Biotechnology and Food Research, Espoo, Finland. The instrument was aligned and calibrated according to the manufacturer's instructions prior to proceeding with the measurements. Nuclear DNA content was estimated by the fluorescence of the nuclei with DAPI relative to nuclei of chicken red blood cells (CRBC) or rainbow trout red blood cells (RTRBC). Fluorescence was measured on a linear scale over 512 channels. A DNA content of 2C = 2.33 pg for CRBC nuclei (Galbraith et al. 1983) was used to determine the DNA content of RTRBC (2C = 4.98 ± 0.01 pg), after which either one served as the internal standard for the *Hordeum* samples. For each sample, the DAPI fluorescence of 2×10^4 nuclei was measured.

Results

Typical raw data, in this case for *Hordeum euclaston*, are graphed in Fig. 1. The histogram represents DAPI-fluorescence intensity distributed over the 512 channels. The strong spike in the lowest channels, as well as the background along the baseline, is caused by fluorescing particles of plant cell debris and has been reported earlier (Arumuganathan and Earle 1991*a*). This background increased the statistical variation in the data collected for some samples. Only two nuclear fluorescence peaks were detected, one for the CRBC, and the other for the *Hordeum* nuclei, both for G_1-phase cells. No G_2+M phase peaks were observed, perhaps owing to the background and to the fragility of G_2 and M nuclei.

Differences in nuclear DNA content were found among both the *Hordeum* species as a whole (Table 1) and among populations of *H. vulgare* ssp. *spontaneum* (Table 2). Values

Table 1. Nuclear DNA content and origins of *Hordeum* accessions.

Species	Accession	Origin	2*n*	2C±SD/pg[a]	Genome	Source[b]
H. euclaston	H 1132	Buenos Aires, Argentina	14	5.69±0.06	H	RvB
H. pusillum	H 1906	U.S.A.	14	5.83±0.10	H	RvB
H. brachyanterum ssp. *californicum*	H 1942	U.S.A.	14	7.04±0.08	H	RvB
H. erectifolium	H 1150	Buenos Aires, Argentina	14	7.58±0.08	H	RvB
H. bogdanii	H 4014	Pakistan	14	7.70±0.10	H	RvB
H. muticum	H 958	Bolivia	14	7.81±0.21	H	RvB
H. bogdanii	H 7065	Qinghai, China	14	7.81±0.09	H	RvB
H. stenostachys	H 1108	Argentina	14	7.94±0.02	H	RvB
H. marinum ssp. *gussoneanum*	H 155	Greece	14	8.08±0.06	X	RvB
H. patagonicum ssp. *santacrucense*	H 1240	Chubut, Argentina	14	8.10±0.10	H	RvB
H. roshevitzii	H 7039	Qinghai, China	14	8.32±0.21	H	RvB
H. murinum ssp. *glaucum*	H 801	Mazanderan, Iran	14	8.58±0.14	Y	RvB
H. patagonicum ssp. *patagonicum*	H 1319	Argentina	14	8.79±0.16	H	RvB
H. vulgare ssp. *spontaneum* ($\bar{x}$, *N*=12)	SCI 77-1	Israel	14	9.37±0.36	I	HA
H. vulgare ssp. *vulgare* (cv. Bonus)	CI 3947	Sweden	14	9.41±0.05	I	RvB
H. depressum	H 2089	U.S.A.	28	13.13±0.08	HH	RvB
H. bulbosum	H 136	Armenia	28	14.80±0.36	II	NJ
H. jubatum	H 4159	China	28	15.26±0.12	HH	RvB
H. murinum ssp. *leporinum*	H 509	West Estepona, Spain	28	17.03±0.05	YY	RvB
H. murinum ssp. *leporinum*	H 796	West Azarbaijan, Iran	42	17.17±0.20	YYY	RvB
H. murinum ssp. *murinum*	H 217	Berlin, Germany	28	18.36±0.24	YY	RvB

[a]Measurements of three independent isolations.
[b]HA, Hannu Ahokas; RvB, Roland von Bothmer; NJ, Niels Jacobsen; EN, Eviatar Nevo.

from 5.69 pg/2C (*H. euclaston*) to 18.36 pg/4C (*Hordeum murinum* ssp. *murinum*) were measured. The smallest *Hordeum* genomes measured were *H. euclaston* and *Hordeum pusillum* in genome group H, being discontinuous from the range of genomes greater than 7 pg/2C. Despite its unusual genome size, the other characters of *H. euclaston* examined place it squarely in a large group of South American diploid *Hordeum* species (Jørgensen 1986; Doebley et al. 1992; Svitashev et al. 1994). The species *Hordeum patagonicum*, composed of distinct but hybridizing components, has been separated into subspecies (von Bothmer et al. 1995). Despite their meiotic compatibility, for the accessions examined, the genome of *Hordeum patagonicum* ssp. *santacrucense* is 8% smaller than that of ssp. *patagonicum* (statistically significant at the 1% level).

Of the tetraploid accessions investigated, the smallest genome was only 72% of the size of the largest. Just as *Hordeum brachyanterum* ssp. *californicum* was among the smallest diploid genomes examined, *Hordeum depressum* was among the smallest tetraploid genomes examined. The tetraploid *H. depressum* (13.13 pg) is held to be phylogenetically close to *H. brachyanterum* ssp. *californicum* (7.04 pg) (Jørgensen 1986; Doebley et al. 1992; Svitashev et al. 1994), which more generally occurs as a tetraploid (ssp. *brachyanterum*) than as a diploid cytotype (von Bothmer et al. 1995). The basic genome (one-half) of *H. depressum* is nevertheless 7% smaller than that of the diploid *H. brachyanterum*. The species *Hordeum murinum* occurs as diploid, tetraploid, and hexaploid subspecies. The basic genome (one-half) of the tetraploid subspecies *Hordeum murinum* ssp. *leporinum* (8.52 pg) is equivalent in size to that of the diploid subspecies *Hordeum murinum* ssp. *glaucum* (2C = 8.58 pg), but significantly smaller than the basic genome of the tetraploid ssp. *murinum* (9.18 pg). Surprisingly, the basic genome (one-third) of the hexaploid accession for ssp. *leporinum* (5.72 pg) is of the same order as the smallest of the diploid genomes analyzed.

Considerable variation was found as well within *H. vulgare* ssp. *vulgare* and ssp. *spontaneum* (Table 2), the diploid nuclear DNA content ranging from 7.92 pg (Indian landrace) to 9.41 pg (East-African landrace). The nuclear DNA content of the 12 accessions of *H. vulgare* ssp. *spontaneum* ranged from 8.27 to 9.37 pg. By comparison, the two geographically separated accessions of *Hordeum bogdanii* were not significantly different in nuclear DNA content. Our measurements for cultivated barley (9.06 pg for cv. Bomi and 9.41 pg for cv. Bonus) are smaller than the 10.10 pg determined earlier by flow cytometry (Arumuganathan and Earle 1991*a*), which is smaller by the same amount than the values derived by Feulgen microdensitometry: 10.7 pg, cv. Algerie 48; 10.9 pg, cv. Gilgit 7; 10.9 pg, cv. Proctor; 10.9 pg, cv. Swanneck; and 11.1 pg, cv. Sultan (Bennett and Smith 1976). Although the cultivars we examined were not identical to those reported earlier, the disparity is more likely to stem from systematic methodological differences. Both sets of measurements depart substantially from the first determinations of *H. vulgare* genome size, on average 13.3 ± 0.2 pg/2C for 5 cultivars (Bennett and Smith 1971). Among the accessions of *H. vulgare*, three groups differing in genome size can be distinguished (statistical significance 0.05; noted in Table 2). Subspecies *spontaneum* accessions had been collected along several transects representative of the ecological and

Table 2. Nuclear DNA content and origins of *H. vulgare* accessions.

Subspecies	Accession	Origin[a]	2*n*	2C±SD/pg[b]	Source[c]
vulgare, landrace	CI 3947	East Africa	14	7.92±0.21	HA
spontaneum	20-48	Sédé Boqér, Negev desert, Israel	14	8.27±0.01	EN
spontaneum	SCI 77-1	Upper Galilee, Israel	14	8.36±0.06	EN
spontaneum	25-34	Atlit, coastal, Israel	14	8.37±0.18	EN
spontaneum	1-27	Mount Hermon (el. 1530 m), Golan, Israel	14	8.66±0.29	EN
spontaneum	14-6	Talpiyyot (el. 800 m), Judean Mountains, Israel	14	8.81±0.12	EN
spontaneum	9-40	Mount Meron (el. 1150 m), Upper Galilee, Israel	14	8.88±0.15	EN
spontaneum	22-53	Mehola (el. −150 m), Jordan Rift Valley, Israel	14	8.90±0.14	EN
spontaneum	26-2	Caesarea, coastal, Israel	14	8.95±0.02	EN
spontaneum	H 3174	China	14	9.02±0.20	EN
spontaneum	31-22	HaMachtésh HaGadol, Negev Desert, Israel	14	9.02±0.11	EN
vulgare cv. Bomi		Denmark	14	9.06±0.18	RvB
spontaneum	18-27	Revivim, Negev desert, Israel	14	9.35±0.08	EN
spontaneum	11-17	Damon (el. 425 m), Mount Carmel, Israel	14	9.37±0.13	EN
vulgare, landrace	CI 1090	India	14	9.41±0.16	HA
vulgare cv. Bonus		Sweden	14	9.41±0.05	HD

[a]el., elevation.
[b]Measurements of three independent isolations. Grouped accessions identical with 0.05 significance.
[c]HA, Hannu Ahokas; RvB, Roland von Bothmer; HD, Hans Doll; EN, Eviatar Nevo.

geological range of the subspecies (Nevo et al. 1979, 1986). However, the pattern of genome size variation found here failed to correlate with any environmental gradient (E. Nevo, personal communication). Genome size in cultivated barley varies at least as much as in ssp. *spontaneum*, and the range of variation includes both the smallest and largest *H. vulgare* accession genomes examined. The genome of the single tetraploid *H. bulbosum* population examined (Table 1) was considerably less than twice the size of the smallest *H. vulgare* genome. This contrasts with the results for *H. murinum*, where both tetraploid genomes examined were roughly double that of the diploid.

Discussion

We have examined 35 accessions from 19 *Hordeum* species and measured genome sizes from 5.69 to 9.41 pg for the G_1 nuclei of the diploids and from 13.13 to 18.36 pg for those of the tetraploids. Previously determined genome sizes in the Poaceae (Arumuganathan and Earle 1991*a*) range from 0.78 pg (*Oryza longistaminata*, African rice) to 33.09 pg (*Triticum aestivum* (2*n* = 6*x*), wheat). In contrast, the only previous report for wild *Hordeum* species (Bennett and Smith 1971) found less than 4% difference in the size of the largest and smallest *Hordeum* genomes examined against the 1.7-fold difference reported here. Furthermore, the largest of the *H. vulgare* genomes measured (cv. Bonus) was 18% larger than the smallest (an East African landrace), corresponding to a difference of 1.49 pg or 1.36×10^9 base pairs of DNA.

The variation in genome size extended across the four genome types of the species. Although an I accession was the largest of the diploid genomes measured, the I, H, X, and Y genome sizes overlapped. Furthermore, the genome size did not correlate with the compatibility of the diploid species in meiotic pairing (von Bothmer et al. 1986). *Hordeum pusillum* (5.83 pg), for example, paired fairly well with *Hordeum stenostachys* (7.94 pg), despite the large difference in genome size, as did the latter with *H. euclaston* (5.69 pg). However, *H. brachyanterum* ssp. *californicum* (7.04 pg) paired much less well with the more closely sized *Hordeum muticum* (7.78 pg). This suggests that components of the genome leading to gross changes in size (presumably repetitive DNA) are not synonymous with those associated with meiotic pairing (Irick 1994). In addition, the relationships among the diploid species observed in Giemsa C-banding patterns (Linde-Laursen et al. 1992) do follow differences in genome size, for example, between *H. pusillum* (5.83 pg) and *H. brachyanterum* ssp. *californicum* (7.04 pg).

The variations in genome size, moreover, are not congruent with relationships within the genus based on isozyme data (Jørgensen 1986), where *H. pusillum* (5.83 pg) is allied to *H. pategonicum* (8.10 and 8.79 pg). The same is true for phylogenetic analyses based on restriction fragment length polymorphism (RFLP) analyses of repetitive DNA (Svitashev et al. 1994) that place *H. bogdanii* (7.70 and 7.81 pg) together with *Hordeum roshevitzii* (8.32 pg), which has a significantly larger genome ($P < 0.05$). This may be contrasted with *Vaccinium* (blueberry), where genome size and phylogenetic relationships were parallel (Costich et al. 1993).

For barley, the *H. vulgare* ssp. *spontaneum* samples examined varied considerably in genome size, and were taken from populations representative of the environmental range in which the plant grows. The subspecies is genetically diverse, and environmental factors have been well correlated to genetic variation (Nevo 1992; Nevo et al.

1986; Saghai Maroof et al. 1990). Furthermore, the populations divide into three geographic groups by the RFLP pattern of their cpDNA (Neale et al. 1986). However, links between genome size and either locale or environment are not apparent from our data. For example, accessions from Sede Boqer and Revivim, representing marginal desert populations separated by not more than 30 kilometres, fall near the extremities of the genome size distribution for the subspecies, at 8.27 and 9.35 pg, respectively. While data from single accessions may not be definitive for entire populations, the difference between these two accessions is nevertheless 9.86×10^8 bp, which within a population would imply unprecedented genomic flux. For the cultivated barleys, both cultivars and landraces, the genomes ranged from the smallest to the largest measured for the species, and did not reflect the pattern seen with the grass *Milium effusum* (Bennett and Bennett 1992), where cultivated populations had genomes highly significantly larger than populations in the wild.

In summary, both diploid and tetraploid *Hordeum* species exhibit considerable genome size variation, which does not at first sight appear correlated with proposed phylogenetic relationships, meiotic compatibility, or environmental factors. Genome size has been linked to environmental adaptation for the annual grasses (Grime and Mowforth 1982; Ceccarelli et al. 1992), and has been proposed more broadly to be under natural selection (Price et al. 1981; Srivastava and Lavania 1991; Jasleński and Bazzaz 1995). Our data suggest that the genome may change in size within *Hordeum* on a timescale shorter than that for speciation. In this way, the balance of selection, founder effects, and microclimatic conditions, acting even on populations, may obscure broader environmental or evolutionary patterns. Stable changes in genome size can occur in a single generation, at least in *Linum usitatissimum* (flax; Price et al. 1981). Rapid alterations in genome size have been correlated to changes in the quantity of repetitive DNA (Cullis and Cleary 1986; Ceccarelli et al. 1992). In barley, simple sequence repeats (SSRs) have been shown both to be highly polymorphic and to change in frequency at rates as high as 0.016 per generation (Saghai Maroof et al. 1994). As independently replicating and inserting components of the plant genome, retrotransposons offer a potential mechanism for increasing genome size. In this regard, the highly repetitive ($>10^5$ copies per genome) *del* retrotransposon appears to have undergone bursts of propagation during the evolution of the *Lilium* genus (Joseph et al. 1990). Such bursts may be related to genomic or physiological stress, both of which are known to activate retrotransposons in a variety of organisms (Bradshaw and McEntee 1989; Pouteau et al. 1994). We are currently examining whether the prevalence of *BARE*-like retrotransposons can be correlated with genome size in *Hordeum*.

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