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Genome size variation in diploid and tetraploid wild wheats

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Abstract

Background and aims Intra- and interspecific variations of C-values and the relationship between habitat factors and genome size were studied in natural populations of diploid and tetraploid wild wheats.

Methodology The 1C nuclear DNA content of 376 individual plants representing 41 populations of diploid and tetraploid wild wheats was determined by flow cytometry (FCM) and correlated with geographical and bioclimate variables.

Principal results Based on analysis of variance, significant differences between diploid and tetraploid *Triticum* species were found. Differences among populations of *T. boeoticum* and *T. dicoccoides* were also statistically significant and argue for isolation between populations, except for *T. araraticum*. However, the variation among individuals of the same population was not statistically significant. Maximum genome size differences among populations for *T. boeoticum* (0.143 pg; 2.32 %), *T. dicoccoides* (0.314 pg; 2.49 %) and *T. araraticum* (0.116 pg; 0.98 %) argue for genome constancy in these species. There was no significant correlation between intra-population variance and geographical and bioclimate variables for *T. boeoticum* and *T. dicoccoides*. In contrast to the limited genome size variation at the intraspecific level, the interspecific variation was large: ~0.5 pg/1C (8 %) at the diploid level (*T. boeoticum* vs. *T. urartu*) and ~1 pg/1C (9.7 %) at the tetraploid level (*T. dicoccoides* vs. *T. araraticum*).

Conclusions Low intraspecific genome size variation occurs in diploid and tetraploid wild wheats, and this limited variation is not correlated with geographical and climate variables. However, interspecific variation is significant at the diploid and tetraploid level. It can be concluded that the genome size of wild self-fertilizing *Triticum* species is generally stable, despite the presence of many potentially active retroelements. In natural habitats, it is very difficult to distinguish wild wheats from each other. However, all four species can be distinguished easily, quickly and unambiguously by using the FCM technique.

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Introduction

The term genome size refers to the DNA content of the unreplicated reduced nucleus, irrespective of the ploidy level of the taxon, and it is expressed as C value in picograms (Swift, 1950). Since it became possible to measure the DNA content of a single nucleus (1950s), various researchers have reported interspecific variation among different species (Swift, 1950; Price *et al.*, 1981; Bennett and Leitch, 1995, 2005). The 1C nuclear DNA amount of plant species with the same ploidy level differs by several orders of magnitude, from 0.0648 pg/1C for *Genlisea margaretae* Hutch (Greilhuber *et al.*, 2006) to 132.45 pg/1C for *Trillium camschatcense* Ker Gawler (Zonneveld, 2010). More recently, it was found that differences in genome size among species are predominantly associated with differences in the amount of repetitive sequences. Particularly, retrotransposons play a dominant role in genome size differences, and most of the variation in genome size in plants can be ascribed to differential accumulation of retrotransposons (Bennetzen, 2000, 2007; Feuillet and Keller, 2002). The occurrence and extent of genome size variation below the species level are still controversial and not satisfactorily analysed yet. Intraspecific variation in plants has been reported for numerous species and was attributed to differences in chromosome number, chromosome size (polyploidy, aneuploidy, B chromosomes, sex chromosomes) and inherent undetected cryptic species (Greilhuber, 1998; Gregory, 2005). However, evidence for intraspecific genome size variation other than chromosome polymorphism and cryptic taxonomic variation is rare and still controversial. A high degree of genome constancy, which is in agreement with the initial notion of constancy in DNA content within individuals and species (Swift, 1950), has been found in many species, including the base calibration standard for estimating C values, *Allium cepa* L. (Bennett *et al.*, 2000). On the other hand, in several earlier studies, intraspecific variation often based on densitometry or cytofluorometry techniques has been observed, but these results could not be confirmed by subsequent flow cytometry (FCM) analyses that used exactly the same plant material. Therefore, in most cases, intraspecific variation has been explained by taxonomic misclassification or technical artefacts such as suboptimal staining and insufficient standardization (reviewed by Greilhuber, 1998, 2005). However, it seems plausible that genome size may diverge in populations, even in the face of limited gene flow (Kron *et al.*, 2007).

Wheat is one of the principal cereal crops in the world. There are two wild diploid *Triticum* species: *T. boeoticum* Boiss. (A^bA^b) and *T. urartu* Thum. ex Gandil. (A^uA^u). These species are separated by crossing barriers (Johnson and Dhaliwal, 1976), and differ in their plant morphology (Gandilian, 1972; Dorofeev *et al.*, 1979) and biochemical and molecular marker loci (Johnson, 1975; Kilian *et al.*, 2007*a*; Konovalov *et al.*, 2010). *Triticum boeoticum* has been considered to be the progenitor of cultivated diploid einkorn wheat, *T. monococcum* L. There are also two wild tetraploid *Triticum* species: *Triticum dicoccoides* (Körn. ex Aschers. & Graebn.) Schweinf. (BBA^uA^u) and *T. araraticum* Jakubz. (GGA^uA^u). *Triticum dicoccoides* is the primary wild-type form and has given rise to several tetraploid wheat taxa that are now cultivated. Similarly, *T. araraticum* is the wild-type progenitor of *T. timopheevii* Menabde et Ericzjan (GGA^uA^u). The A genome of *T. dicoccoides* and *T. araraticum* was contributed by the diploid wheat *T. urartu* (Dvorak *et al.*, 1993). The diploid and tetraploid wild wheats are important germplasm sources for cultivated wheat improvement.

Interspecific variability in nuclear DNA content has been reported in *Triticum* L. and the related genus *Aegilops* L. Furuta *et al.* (1977) measured the nuclear DNA content in 43 accessions of the genus *Aegilops* and confirmed that intra- and interspecific variation existed. Recently, Eilam *et al.* (2007) reported that within the diploid *Aegilops* species, the 1C DNA amount ranged from 4.84 pg in *Ae. markgrafii* (Greuter) Hammer (= *Ae.* caudata L.) to 7.52 pg in *Ae. sharonensis* Eig. The nuclear DNA content of *Ae. geniculata* Roth (= *Ae. ovata* L.) was measured at 9.23 pg (Furuta, 1970), and *Ae. neglecta* Req. ex Bertol. at 16.35 pg of 1C DNA (Maranon and Grubb, 1993). Rees and Walters (1965) and Nishikawa and Furuta (1978) have indicated that the nuclear DNA content of *T. timopheevii* is less than that of *T. turgidum* L. 1C DNA amounts of 11.30 pg for *T. timopheevii* (Rees and Walters, 1965), 10.05 pg for *T. araraticum* (Hülgenhof *et al.*, 1988) and 12.28 pg for *T. durum* Desf. (Bennett and Smith, 1976) have been reported. Recently, Eilam *et al.* (2008) studied *T. araraticum*, *T. timopheevii*, *T. dicoccoides* and *T. durum*, and the nuclear DNA content was found to be 11.82, 11.87, 12.84 and 12.91 pg, respectively.

In order to make wild wheat sampling more representative, we studied wild wheat populations from different geographical regions. The present study was carried out on 376 plants representing 41 well-characterized populations belonging to two diploid and two tetraploid wild wheat species collected recently in Southeast Turkey. The objectives of this study were (i) to determine the nuclear DNA content (1C) using FCM, (ii) to assess the magnitude of intraspecific variation and (iii) to study the correlation between the mean nuclear DNA content per population and their geographical origins and 19 bioclimate variables.

Materials and methods

Experimental material

The first step was to sample diploid and tetraploid wild wheats from Southeast Turkey. Collecting missions were carried out in 2004 and 2005. During the missions, 26 populations of *T. boeoticum*, 1 population of *T. urartu*, 10 populations of *T. dicoccoides* and 4 populations of *T. araraticum* were sampled. Later, all these populations were planted in a nethouse, morphologically re-identified and selfed in 2006. Altogether, 376 plants representing 41 populations belonging to 4 species were analysed in this study (Table 1). Collection sites are shown in Additional information, Fig. S1.

Information on current climate data was obtained from the WorldClim database (Hijmans *et al.*, 2005; www.worldclim.org). Climate data included monthly mean variables of minimum and maximum temperature and precipitation. Based on these data, 19 so-called 'bioclimate' variables were calculated (Hijmans *et al.*, 2005) and were more useful than monthly values, since they were independent of latitudinal variation. The climate data are presented in Additional information, Table S1.

Genome size determination by FCM

Nuclear DNA content analysis was carried out on 6–21 plants for each population. The procedures described by Arumuganathan and Earle (1991) were used to determine the DNA content per nucleus. Briefly, the procedure consists of preparing suspensions of intact nuclei by chopping plant tissues and lysing protoplasts in an $MgSO_4$ buffer mixed with DNA standards and staining with propidium iodide (PI) in a solution containing DNase-free RNase. Fluorescence intensities of the stained nuclei are measured by a flow cytometer CYTOMICS FC 500 (Beckman Coulter, Inc., Fullerton, CA, USA). The values for nuclear DNA content are estimated by comparing fluorescence intensities of the nuclei of the test population with those of an appropriate internal DNA standard that is included with the tissue being tested. We used barley (*Hordeum vulgare* L.) cultivar Sladoran as the internal standard. It is a diploid ($2x = 14$) species that has a 1C complement of DNA of 5.325 pg per nucleus (Tuna *et al.*, 2001). Specifically, for the flow cytometer analysis, 50 mg of fresh leaf tissue were excised from 3- to 4-week-old healthy seedlings and placed on ice in a sterile plastic Petri dish. Twenty milligrams of fresh leaf tissue from barley were added to the Petri dish as standard. Tissue was chopped into 0.25–1 mm segments in 1 mL of solution A [24 mL of $MgSO_4$ buffer (ice cold); 25 mg of dithiothreitol; 500 μL of PI stock (5.0 mg of PI in 1.0 mL of double-distilled H_2O); 625 μL of Triton X-100 stock (1.0 g of Triton X-100 in 10 mL of double-distilled H_2O)]. The solution and tissue were filtered through a 30-μm nylon mesh into a microcentrifuge tube and centrifuged at high speed (13 000 r.p.m.) for ~15–20 s. The supernatant was discarded; the pellet was resuspended in 400 μL of solution B (7.5 mL of solution A; 17.5 μL of DNase-free RNase) and incubated for 20 min at 37 °C before flow cytometric analysis. Samples stained with PI were excited with a 15-mW argon ion laser at 488 nm. Red PI fluorescence area signals (FL2A) from nuclei were collected in the FL2 channel. Mean DNA content per sample was based on analysis of 1000 nuclei per sample. The nuclear DNA content was determined using the flow cytometer CYTOMICS FC 500 at the Central Laboratory of the Medical School of Trakya University. The analysis was repeated if the variation coefficient of the sample was >2.5.

Statistical analysis

Analysis of variance (ANOVA) was carried out to evaluate whether the differences within and among populations of diploid and tetraploid wild wheats were significant or not. In those cases in which ANOVA revealed significant differences, Student's test was performed. Correlations between mean nuclear DNA content and geographical information (altitude, latitude, longitude) as well as 19 bioclimate variables were estimated with Spearman rank correlations. All statistical analyses were carried out using JMP® 5.0 Statistical Discovery software (SAS Institute, Inc., Cary, NC, USA; http:www.jmp.com).

Results

The mean nuclear DNA content of natural populations belonging to four wild wheat species is presented in Table 2. Based on ANOVA, significant differences between diploid and tetraploid *Triticum* species were found. Furthermore, significant variations among populations of *T. boeoticum* and *T. dicoccoides*, but not *T. araraticum,* were found (Table 2). However, variation among individuals of the same population was not statistically significant (data not shown).

Mean nuclear DNA content per population ranged from 6.167 to 6.310 pg/1C for *T. boeoticum*, from 12.603 to 12.917 pg/1C for *T. dicoccoides* and from 11.613 to 11.729 pg/1C for *T. araraticum*. For the one population of *T. urartu* studied, we detected a mean nuclear DNA content of 5.784 pg. The highest genome size value for *T. boeoticum* was observed for the Türkoğlu population (BOE-53, 6.310 pg), followed by BOE-6, BOE-33 and BOE-16; the smallest value was noted for the Narlı population (BOE-56, 6.167 pg). In *T. dicoccoides*, the largest genome size was found for DIC-17 (12.917 pg), a

Table 1 List of diploid and tetraploid wild wheat populations studied and their collection sites

Population name	Collection no.	Locality	Altitude (m)	Latitude (°N)	Longitude (°E)
T. boeoticum (A^bA^b)					
BOE-1	2004-6-21-3	12 km SSW from Çamardı to Pozantı	1200	37°43′49″	35°01′03″
BOE-3	2004-6-29-2	20 km SW from Diyarbakır to Ovadağ	760	37°49′36″	40°14′51″
BOE-6	2004-6-29-8	18.5 NW from Ovadağto Pirinçlik	920	37°49′17″	39°59′34″
BOE-9	2004-6-29-11	2.9 km NE from Karabahçe to Pirinçlik	1300	37°49′12″	39°46′29″
BOE-11	2004-6-29-13	41.2 km SW from Pirinçlik	1250	37°46′42″	39°44′50″
BOE-13	2004-6-30-2	4.6 km SW from Karabahçe	1180	37°46′19″	39°44′03″
BOE-15	2004-6-30-4	21.7 km SW from Karabahçe	1235	37°42′51″	37°44′03″
BOE-16	2004-6-30-5	28.1 km SW from Karabahçe	1170	37°39′49″	39°42′49″
BOE-17	2004-6-30-6	33.7 km SW from Karabahçe	1150	37°38′18″	37°42′37″
BOE-18	2004-6-30-7	37.9 km SW from Karabahçe	1180	37°36′27″	39°43′41″
BOE-19	2004-6-30-8	41.6 km SW from Karabahçe	1170	37°35′08″	39°44′36″
BOE-20	2004-6-30-9	48.7 km SW from Karabahçe	1030	37°33′09″	39°42′06″
BOE-22	2004-6-30-10	27.6 km SW from Karacadağ	950	37°37′40″	39°33′40″
BOE-24	2004-7-1-1	27.1 km SE from Diyarbakır to Bismil	650	37°47′58″	40°25′35″
BOE-25	2004-7-1-2	42.2 km SE from Diyarbakır to Bismil	600	37°50′02″	40°33′51″
BOE-26	2004-7-1-3	9 km SE from Diyarbakır to Bismil	580	37°51′03″	40°45′15″
BOE-31	2004-7-3-4	44.5 km W from Adıyaman to Besni	710	37°42′21″	37°54′55″
BOE-32	2004-7-3-9	17.5 km SW from Araban to Gaziantep	900	37°21′57″	37°32′30″
BOE-33	2004-7-3-11	17.5 km W from Gaziantep to Nurdağ	1010	37°10′14″	37°12′12″
BOE-38	2005-6-27-1	11 km NW from Beyşehir to Yalvaç	1150	37°46′27″	31°39′51″
BOE-46	2005-7-5-3	42 km E from Türkoğlu to Gaziantep	710	37°19′31″	37°09′28″
BOE-48	2005-7-5-7	57 km SE from Türkoğlu to Gaziantep	860	37°15′59″	37°14′00″
BOE-49	2005-7-5-8	60 km SE from Türkoğlu	840	37°17′33″	37°14′42″
BOE-51	2005-7-5-10	63 km SE from Türkoğlu	840	37°18′53″	37°15′41″
BOE-53	2005-7-5-11	72 km SE from Türkoğlu	800	37°19′46″	37°16′29″
BOE-56	2005-7-5-16	39 km ESE from Narlı	760	37°17′06″	37°17′39″
T. urartu (A^uA^u)					
URA-8	2004-6-29-10	20 km SW from Pirinçlik	1260	37°50′40″	39°47′58″
T. dicoccoides (AABB)					
DIC-3	2004-6-29-7-4	12.9 km NW from Ovadağ to Pirinçlik	1007	37°47′31″	39°57′18″
DIC-5	2004-6-29-9-4	20.1 km SW from Pirinçlik	1080	37°52′02″	39°51′05″
DIC-6	2004-6-29-10	20 km SW from Pirinçlik	1260	37°50′40″	39°47′58″
DIC-7	2004-6-29-11	2.9 km NE from Karabahçe to Pirinçlik	1300	37°49′12″	39°46′29″
DIC-17	2004-6-30-10	27.6 km SW from Karacadağ	950	37°37′40″	39°33′40″
DIC-19	2004-7-2-6	30.2 km SW from Çermik to Siverek	800	38°00′56″	39°22′11″
DIC-29	2005-7-5-11	72 km SE from Türkoğlu	800	37°19′46″	37°16′29″
DIC-30	2005-7-5-14	33 km ESE from Narlı	780	37°20′25″	37°17′54″
DIC-31	2005-7-5-15	34 km ESE from Narlı	780	37°20′12″	37°17′53″
DIC-33	2005-7-5-16	39 km ESE from Narlı	760	37°17′06″	37°17′39″

Continued

Table 1 ***Continued***

Population name	Collection no.	Locality	Altitude (m)	Latitude (°N)	Longitude (°E)
T. araraticum (AAGG)					
ARA-21	2004-7-3-11	17.5 km W from Gaziantep to Nurdağ	1010	37°10′14″	37°12′12″
ARA-22	2005-7-5-3	42 km E from Türkoğlu to Gaziantep	710	37°19′31″	37°09′28″
ARA-23	2005-7-5-9	61 km SE from Türkoğlu (SW of Karadağ)	840	37°17′51″	37°14′55″
ARA-24	2005-7-5-10	63 km SE from Türkoğlu (SW of Karadağ)	840	37°18′53″	37°15′41″

population from Karacadağ/Diyarbakır, closely followed by populations from Ovadağ/Pirinçlik/Diyarbakır (DIC-3, 12.890 pg) and Türkoğlu/Kahramanmaraş (DIC-29, 12.863 pg); the lowest genome size was discovered in population DIC-30 (12.603 pg), located in the Kartal-Kardağ region. In the case of *T. araraticum*, the maximum genome size was noted for one population from Gaziantep (ARA-22, 11.729 pg); however, genome size differences in *T. araraticum* populations were not significantly different from each other. Small genome size differences between the most extreme populations per species, BOE-53 vs. BOE-56 (0.143 pg, 2.31 %), DIC-17 vs. DIC-30 (0.314 pg, 2.49 %), and ARA-22 vs. ARA-21 (0.116 pg, 0.99 %), were detected. This indicates a narrow variation among populations.

In contrast to limited genome size variation at the intraspecific level, interspecific variation was large, with an average nuclear DNA amount ranging in diploid wild wheats from 5.784 pg/1C in *T. urartu* to 6.247 pg/1C in *T. boeoticum*. For wild tetraploid wheats, the average nuclear DNA amount ranged from 11.680 pg/1C in *T. araraticum* to 12.813 pg/1C in *T. dicoccoides*.

To determine the association between mean nuclear DNA content per population and their collection site-specific geographical parameters, the non-parametric Spearman test was used. We found no significant correlation between nuclear DNA amount and all studied geographical/bioclimate data in *T. boeoticum* and *T. dicoccoides* (Table 3). However, for *T. araraticum*, the nuclear DNA content was positively or negatively correlated with all bioclimate data except altitude, annual mean temperature, maximum temperature of warmest month, mean temperature of coldest quarter, precipitation of wettest month, precipitation seasonality and precipitation of driest quarter (Table 3).

Discussion

The main goal of the present study was to assess the extent of intra- and interspecific genome size variation in diploid and tetraploid wild wheat populations sampled recently in Turkey, and to determine whether the mean genome size per population is correlated with geographical and bioclimate variables. To determine the nuclear DNA content, we used the FCM technique. Early studies showed that FCM can be employed successfully for determining ploidy level and genome size in Triticeae species (Tuna *et al.*, 2001, 2005, 2006). For FCM, the choice of standard with a known DNA content is important to estimate the DNA content of unknown samples exactly. Therefore, we used barley (*H. vulgare*, cultivar Sladoran, 5.325 pg/1C) as an internal reference standard. Barley was selected as it has an appropriate DNA content for all wheat species analysed. Using this standard, we found that FCM is a reliable and highly sensitive method for detecting the differences in the small nuclear DNA amount in wild wheat species. Nuclear DNA contents (in pg/1C) found in this study were consistent with previously published values for wheat (Bennett and Smith, 1976; Özkan *et al.*, 2003). However, discrepancies were observed compared with previous work of Rees and Walters (1965), Furuta *et al.* (1986) and Eilam *et al.* (2007). The genome size difference of ~5 % can probably be explained by the use of different instruments and techniques (Fuelgen vs. FCM) and different internal standards as reported by Dolezel and Bartos (2005).

Interspecific variation

Triticum dicoccoides and *T. araraticum* are morphologically very similar and have partly overlapping distribution areas (Kilian *et al.*, 2009). Similarly, *T. boeoticum* and *T. urartu* are nearly indistinguishable in their natural habitats. It is not surprising that confusion sometimes arises during sampling, propagating and investigating germplasm collections. However, based on molecular markers and wheat genome-specific re-sequencing, all four wild wheat species can be recognized easily (Kilian *et al.*, 2007*b*; Konovalov *et al.*, 2010). Variance analyses showed that significant differences among diploid and tetraploid *Triticum* species can be detected. Similarly, Eilam *et al.* (2007) observed significant differences in genome size at the interspecific level in diploid and tetraploid *Triticum* species. In our study at the diploid level

Table 2 Nuclear DNA content in diploid and tetraploid wild wheat populations

Population name	Number of individuals studied	Mean 1C value (pg)	SD (±)	Student's test grouping (*P*: 0.01)[a]
T. boeoticum (A^bA^b)				
BOE-53	6	6.310	0.041	A
BOE-6	6	6.287	0.034	AB
BOE-33	12	6.284	0.052	AB
BOE-16	6	6.283	0.005	ABC
BOE-38	9	6.279	0.091	ABC
BOE-48	6	6.276	0.039	ABC
BOE-24	6	6.273	0.031	ABC
BOE-3	18	6.271	0.033	ABC
BOE-25	18	6.263	0.044	BCD
BOE-49	6	6.260	0.044	BCDE
BOE-31	6	6.260	0.017	BCDE
BOE-1	6	6.260	0.014	BCDE
BOE-15	15	6.259	0.039	BCDE
BOE-46	9	6.256	0.032	BCDE
BOE-9	6	6.253	0.005	BCDEF
BOE-19	12	6.248	0.047	BCDEF
BOE-22	14	6.244	0.075	CDEF
BOE-26	6	6.237	0.075	CDEFGH
BOE-13	12	6.236	0.061	DEFG
BOE-51	6	6.220	0.017	EFFGHI
BOE-20	21	6.211	0.047	GHI
BOE-18	6	6.207	0.027	GHIJ
BOE-17	6	6.197	0.019	GHIJ
BOE-32	6	6.187	0.019	HIJ
BOE-11	6	6.183	0.019	IJ
BOE-56	6	6.167	0.026	J
Mean		6.247		
T. urartu (A^uA^u)				
URA-8	12	5.784	0.042	
Mean		5.784		
T. dicoccoides (AABB)				
DIC-17	6	12.917	0.013	A
DIC-3	6	12.890	0.047	AB
DIC-29	6	12.863	0.049	ABC
DIC-19	6	12.858	0.067	ABC
DIC-31	18	12.856	0.092	ABC
DIC-6	9	12.808	0.087	BC
DIC-7	9	12.786	0.055	BCD
DIC-5	9	12.776	0.097	CD
DIC-33	15	12.717	0.108	D
DIC-30	6	12.603	0.241	E
Mean		12.813		
T. araraticum (AAGG)				
ARA-22	9	11.729	0.091	
ARA-24	12	11.700	0.139	
ARA-23	12	11.678	0.055	
ARA-21	6	11.613	0.085	
Mean		11.680		

[a]Populations with different letters are significantly different from each other according to Student's test at $P < 0.01$.

T. urartu has ~0.5 pg/1C (8 %) less nuclear DNA content when compared with *T. boeoticum*. These two species diverged ~3 million years ago (Huang *et al.*, 2002; Dvorak and Akhunow, 2005; Chalupska *et al.*, 2008), probably from a common ancestor. Differences in genome size are expected to be mainly due to copy number differences of retroelements that occurred since deviation from the common ancestor. Especially the *Jeli* retrotransposon family, which belongs to the *Gypsy* class, was found to be A-genome specific and participated due to different transpositional activities in wheat A genome speciation (Konovalov *et al.*, 2010).

Our results for wild tetraploid wheats are similar to those of Rees and Walters (1965), Nishikawa and Furuka (1978) and Eilam *et al.* (2007), who reported that the genome size of *T. araraticum* is smaller than that of *T. dicoccoides*. We found that *T. araraticum* has more than 1 pg/1C (9.7 %) less nuclear DNA content compared with *T. dicoccoides*. Dvorak and Akhunov (2005) estimated the origin of *T. dicoccoides* to have occurred ~0.36 million years ago. The age of formation of *T. araraticum* is still unkown and cannot be concluded based on genome size. The balance of retroelement accumulation and removal may vary between species and over time, but is nevertheless maintained. Several studies have pointed out that *T. dicoccoides* is more diverse than *T. araraticum*;

Table 3 Spearman rank correlations between nuclear DNA content and altitude, latitude, longitude and 19 bioclimatic variables in diploid and tetraploid wild wheats

Variables	Nuclear DNA content		
	T. boeoticum	*T. dicoccoides*	*T. araraticum*
Altitude (m)	−0.208	0.202	0.316
Latitude (°N)	0.105	−0.103	−0.948[a]
Longitude (°E)	−0.093	0.139	1.000[a]
Annual mean temperature	−0.042	0.365	0.316
Mean diurnal range	0.105	−0.122	0.800
Isothermality	0.238	−0.073	1.000[a]
Temperature seasonality	−0.207	−0.024	0.948[a]
Max. temperature of warmest month	−0.125	0.365	0.200
Min. temperature of coldest month	−0.093	−0.049	1.000[a]
Temperature annual range	−0.071	0.189	1.000[a]
Mean temperature of wettest quarter	−0.021	0.061	1.000[a]
Mean temperature of driest quarter	−0.092	0.413	1.000[a]
Mean temperature of warmest quarter	−0.087	0.413	−0.200
Mean temperature of coldest quarter	−0.031	−0.061	1.000[a]
Annual precipitation	−0.186	0.061	1.000[a]
Precipitation of wettest month	−0.165	−0.509	−0.200
Precipitation of driest month	0.037	−0.479	1.000[a]
Precipitation seasonality	−0.073	−0.292	0.400
Precipitation of wettest quarter	−0.141	−0.365	1.000[a]
Precipitation of driest quarter	0.093	−0.380	−0.200
Precipitation of warmest quarter	0.069	0.019	1.000[a]
Precipitation of coldest quarter	−0.126	−0.3647	−0.948[a]

[a]Relationships are significant at <0.01.

it has therefore been concluded that *T. dicoccoides* was formed earlier than *T. araraticum*. Restriction fragment length polymorphism results obtained from Mori *et al.*, (1995) showed larger intraspecific variation (at the DNA level) for *T. dicoccoides* than for *T. araraticum*. Similar conclusions have been made based on cytogenetic data (Badaeva *et al.*, 1990, 1995).

Our present study provides further evidence for considerable nuclear DNA content differences between *T. dicoccoides* and *T. araraticum*, and between *T. urartu* and *T. boeoticum*. Therefore, these four species can easily be distinguished from each other in early growth stage by comparing their DNA content by FCM.

Intraspecific variation

Intraspecific variation for nuclear DNA content has been reviewed by Greilhuber (2005). Muray (2005) and Greilhuber (2005) suggested that most of the previous results reported for different species could be artefacts, and intraspecific variation in genome size can be small and non-significant. Recently, limited genome size variation was also reported in populations of *Sesleria albicans* Kit. ex Schult. (Lysak *et al.*, 2000). For wheat and wheat wild relatives, Furuta *et al.* (1977) measured the nuclear DNA content of 43 accessions belonging to section Sitopsis of *Aegilops* and confirmed that intra- and interspecific variation existed. Furuta *et al.* (1978) also reported that there is no appreciable difference in nuclear DNA content among accessions of *T. boeoticum*, *T. monococcum* and *T. urartu*. Furuta *et al.* (1975) reported a considerable intraspecific variation in nuclear DNA content among four varieties of *Ae. tauschii* Coss. In our comprehensive study of 376 individuals, we found low genome size variation at the intraspecific level for diploid and tetraploid

wild wheats. The largest genome contained ~2.32, 2.49 and 0.98 % more DNA than the smallest in *T. boeoticum*, *T. dicoccoides* and *T. araraticum*, respectively.

Besides the frequent wild stands of *T. boeoticum* sampled during our collection trips in 2004–2006, we found few populations of *T. dicoccoides*, four populations of *T. araraticum* and only one population of *T. urartu*. At the moment, we cannot correlate our genome size data with the wild einkorn races recently described by Kilian *et al.* (2007*a*); however, our data represent the genome size diversity/range for the whole *T. boeoticum* group. Population BOE-38, collected 11 km northwest of Beyşehir to Yalvaç, shows the largest standard deviation. Based on our recent studies, we expected the wild einkorn race gamma (the most diverse wild einkorn race) at this collection site (Kilian *et al.*, 2007*a*). More wild populations from the whole natural distribution range per species should be studied, and the recent distribution of wild einkorn races should be considered.

Similar evidence for small intraspecific genome size variation in the diploid *Aegilops* and *Triticum* species comes from Eilam *et al.* (2007), using FCM. Our results are also in accord with the conclusions of Greilhuber (2005) and Muray (2005), who reported that plant species exhibit very little intraspecific variation in genome size. The limited genome size variation found here at the intraspecific level of diploid and tetraploid wheats could be related to self-pollination. For instance, Eilam *et al.* (2007) reported that cross-pollinating Triticeae species such as *Ae. speltoides* Tausch, *Ae. mutica* Boiss. (= *Amblyopyrum muticum* (Boiss.) Eig) and *Secale montanum* Guss. resulted in higher variation in nuclear DNA content when compared with self-pollinating species. Predominantly self-pollinated *Triticum* populations show very stable genome sizes and harbour long-persisting haplotypes due to limited recombination events and well-controlled retroelement activities.

Correlation between genome size and collection site-specific parameters

Bennett (1987) suggested that interspecific variation in DNA content has adaptive significance and is correlated with environment and geographical distribution. Eco-geographically correlated intraspecific genome size variability has been reported for several Poaceae species such as *Poa annua* L. (Grime, 1983), *Milium effusum* L. (Bennett and Bennett, 1992), *Dactylis glomerata* (Reeves *et al.*, 1998), *Dasypyrum villosum* (Caceres *et al.*, 1998) and *Hordeum spontaneum* (Vicient *et al.*, 1999; Kalendar *et al.*, 2000). *Zea mays* L. cultivars in North America exhibited a decline in nuclear DNA content with increasing latitude (Rayburn *et al.*, 1989). Similarly, a negative correlation between nuclear DNA content and latitude was found in European populations of *Festuca arundinacea* Schreb (Ceccarelli *et al.*, 1992). Creber *et al.* (1994) found a significant negative correlation between DNA C-value and altitude among eight natural populations of *Dactylis glomerata*. Furthermore, Schmuths *et al.* (2004) reported a significant positive correlation between genome size and longitude, and a significant negative correlation between genome size and latitude in *Arabidopsis thaliana*. The environment in which an organism grows seems to influence the DNA content (Johnston *et al.*, 1996). It has been concluded that genome size variation is an adaptation to different environments. However, results were not straightforward and the relationship between intraspecific variation and environment is still not completely resolved. For instance, Teoh and Rees (1976) and Creber *et al.* (1994) did not find a consistent relationship between nuclear DNA content and latitude in *Picea glauca* (Moench) Voss and *D. glomerata*. Similar results were also reported for the relationship between altitude and nuclear DNA content for the same species. Kankanpaa *et al.* (1996) reported that the pattern of genome size variation in *H. spontaneum* was not correlated with environmental factors. Ceccarelli *et al.* (1992) did not find any correlation between genome size, latitude, longitude and altitude in *Vicia faba* L. In the current study, we detected a mean genome size difference of 0.013 pg/1C between the most southern (BOE-33) and the most northern wild einkorn populations (BOE-3) (~75 km), whereas the difference between the most western (BOE-38) and the most eastern (BOE-26) population (~770 km) was 0.042 pg/1C. Therefore, no significant correlation was detected between genome size and latitude and longitude.

Populations collected from the same geographical region harboured the largest and smallest mean genome sizes. The largest genome size content was found for BOE-53 (6.310 pg/1C) from Türkoğlu (Kartal-Karadag region) and for BOE-6 (6.287 pg/1C) from Ovadag (Karacadag region). The smallest genome sizes were found in BOE-56 (6.167 pg/1C) from Narlı (Kartal-Karadag region) and in BOE-11 (6.183 pg/2C) from Pirinçlik (Karacadag region). Similar to the above-mentioned examples, we did not find any significant correlation between three geographical (altitude, latitude and longitude) or 19 bioclimate variables and genome size for *T. boeoticum* and *T. dicoccoides*; *T. araraticum* was the exception. No conclusion can be made for *T. urartu* because only one population was investigated. The genome size of wild self-fertilizing *Triticum* species is generally stable, despite the presence of many potentially active retroelements (Charles *et al.*, 2008). This suggests that retroelement activities in these species

are well controlled and/or balanced by removal via recombination (Grover and Wendel, 2010; Tenaillon *et al.*, 2010). The correlation of *T. araraticum* genome size with some collection site-specific geographical parameters is interesting in this respect. To understand the intraspecific variation and resolve the influence of environment on genome size within plant species, more measurements of natural populations from the whole natural distribution area are required for all four wild wheat species studied here. Future studies also have to take into account the subdivision of wild *Triticum* species into wild races (Özkan *et al.*, 2005; Kilian *et al.*, 2007*a*; Luo *et al.*, 2007).

In conclusion, low but statistically significant intraspecific genome size variation was found in diploid *T. boeoticum* and tetraploid *T. dicoccoides* wild wheats, and this limited variation was not correlated with geographical and climate variables. In contrast to limited genome size variation at the intraspecific level, interspecific variation was large: ~0.5 pg/1C (8 %) at the diploid level (*T. boeoticum* vs. *T. urartu*) and ~1 pg/1C (9.7 %) at the tetraploid level (*T. dicoccoides* vs. *T. araraticum*). In natural habitats, it is very difficult to distinguish these wild wheats from each other, especially in the early stages of development due to similar morphology. However, all four species can be distinguished easily, quickly and unambiguously by using the FCM technique. This can be useful for identifying wild wheat species in laboratories. FCM estimates the nuclear DNA amount per plant on the basis of thousands of nuclei and yields a narrow distribution of DNA intensities. This technique enables robust and precise analysis that should be carried out under standard conditions in more Triticeae species.

Conclusions and forward look

We studied genome size variation in natural populations of wild diploid and tetraploid wheats. Based on ANOVA, we found that inter-population differences were significant at both ploidy levels whereas intra-population differences were not significant. Maximum genome size differences among populations for *T. boeoticum* (0.143 pg; 2.32 %), *T. dicoccoides* (0.314 pg; 2.49 %) and *T. araraticum* (0.116 pg; 0.98 %) argue for genome constancy in these species. In contrast to the limited genome size variation at the intraspecific level, the interspecific variation was large: ~0.5 pg/1C at diploid level (*T. boeoticum* vs. *T. urartu*) and ~1 pg/1C at the tetraploid level (*T. dicoccoides* vs. *T. araraticum*). Low intraspecific genome size variation occurs within diploid and tetraploid wild wheats, and this limited variation is not correlated with geographical or climate variables. We conclude that the genome size of wild self-fertilizing *Triticum* species is generally stable, despite the presence of many potentially active retroelements. This suggests that retroelement activities in these species are well controlled and/or balanced by removal via recombination. In natural habitats, it is very difficult to distinguish wild wheats from each other especially in their early stages of their development due to their similar morphology. However, all four species can be distinguished easily, quickly and unambiguously by using the FCM technique.

Additional information

The following additional information is available in the online version of this article –

Figure S1 presents Geographical Information System (GIS) based collection sites for all 41 wild wheat populations (*T. boeoticum*, red; *T. urartu*, green; *T. dicoccoides*, blue; *T. araraticum*, black).

Table S1 summarizes the climate data per collection site.

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Contributions by the authors

H.Ö. and M.T. undertook the experimental work and laboratory analyses; all authors contributed to the planning of the research and wrote the manuscript.

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Conflict of interest statement

None declared.

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