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\(^b\)A\(^b\)) and T. urartu Thum. ex Gandil. (A\(^a\)A\(^a\)). 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; Kilián et al., 2007a; 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 (Körrn. ex Aschers. & Graebn.) Schweinf. (BBA\(^A\)) and T. araraticum Jakubz. (GGA\(^A\)). 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 Ercıçjan (GGA\(^A\)). 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. urartu 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 bioclimatic 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 MgSO4 buffer mixed with DNA standards and staining with propidium iodide (PI) in a solution containing DNase-free RNase. Fluorescence intensities of the nuclei of stained nuclei are measured by a 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 bioclimatic 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 Türkog˘lu 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 (AABB) | | | | | |
| 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 Ovadağ to Pirinçlik | 920 | 37°49′17″ | 39°59′34″ |
| BOE-9 | 2004-6-29-11 | 2.9 km NE from Karabağçe 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 Karabağçe | 1180 | 37°46′19″ | 39°44′03″ |
| BOE-15 | 2004-6-30-4 | 21.7 km SW from Karabağçe | 1235 | 37°42′51″ | 37°44′03″ |
| BOE-16 | 2004-6-30-5 | 28.1 km SW from Karabağçe | 1170 | 37°39′49″ | 39°42′49″ |
| BOE-17 | 2004-6-30-6 | 33.7 km SW from Karabağçe | 1150 | 37°38′18″ | 37°42′37″ |
| BOE-18 | 2004-6-30-7 | 37.9 km SW from Karabağçe | 1180 | 37°36′27″ | 39°43′41″ |
| BOE-19 | 2004-6-30-8 | 41.6 km SW from Karabağçe | 1170 | 37°35′08″ | 39°44′36″ |
| BOE-20 | 2004-6-30-9 | 48.7 km SW from Karabağçe | 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 Beştepe to Yalova | 1150 | 37°46′27″ | 31°39′51″ |
| BOE-46 | 2005-7-5-3 | 42 km E from Türköglü to Gaziantep | 710 | 37°19′31″ | 37°09′28″ |
| BOE-48 | 2005-7-5-7 | 57 km SE from Türköglü to Gaziantep | 860 | 37°15′59″ | 37°14′00″ |
| BOE-49 | 2005-7-5-8 | 60 km SE from Türköglü | 840 | 37°17′33″ | 37°14′42″ |
| BOE-51 | 2005-7-5-10 | 63 km SE from Türköglü | 840 | 37°18′53″ | 37°15′41″ |
| BOE-53 | 2005-7-5-11 | 72 km SE from Türköglü | 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 (AAAA) | | | | | |
| 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 Karabağçe 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 Türköglü | 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**
population from Karacadag/Diyarbakir, closely followed by populations from Ovada/Pirinci/Diyarbakir (DIC-3, 12.890 pg) and Turkoğu/Kahramanmaras (DIC-29, 12.863 pg); the lowest genome size was discovered in population DIC-30 (12.603 pg), located in the Karte/Kardag region. In the case of T. araraticum, the maximum genome size was noted for one population from Gaziantepe (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 Elam 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 Dalezel 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., 2007b; Konovalov et al., 2010). Variance analyses showed that significant differences among diploid and tetraploid Triticum species can be detected. Similarly, Elam 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

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**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 Gaziantepe to Nurdag | 1010 | 37°10′14″ | 37°12′12″ |
| ARA-22 | 2005-7-5-3 | 42 km E from Turkoğu to Gaziantepe | 710 | 37°19′31″ | 37°09′28″ |
| ARA-23 | 2005-7-5-9 | 61 km SE from Turkoğu (SW of Karadağ) | 840 | 37°17′51″ | 37°14′55″ |
| ARA-24 | 2005-7-5-10 | 63 km SE from Turkoğu (SW of Karadağ) | 840 | 37°18′53″ | 37°15′41″ |
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 Akhunov, 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 unknown 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 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) |
|----------------|-------------------------------|--------------------|--------|----------------------------------|
| **T. boeoticum (AABB)** | | | | |
| 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 | BCDE |
| 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 | GHJJ |
| BOE-17 | 6 | 6.197 | 0.019 | GHJJ |
| 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^2A^2)** | | | | |
| 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 |

*Populations with different letters are significantly different from each other according to Student's test at P < 0.01.*
it has therefore been concluded that \textit{T. dicoccoides} was formed earlier than \textit{T. araraticum}. Restriction fragment length polymorphism results obtained from Mori et al. (1995) showed larger intraspecific variation (at the DNA level) for \textit{T. dicoccoides} than for \textit{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 \textit{T. dicoccoides} and \textit{T. araraticum}, and between \textit{T. urartu} and \textit{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 \textit{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 \textit{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 \textit{T. boeoticum}, \textit{T. monococcum} and \textit{T. urartu}. Furuta et al. (1975) reported a considerable intraspecific variation in nuclear DNA content among four varieties of \textit{Ae. tauschii} Coss. In our comprehensive study of 376 individuals, we found low genome size variation at the intraspecific level for diploid and tetraploid

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

| Variables                                | \textit{T. boeoticum} | \textit{T. dicoccoides} | \textit{T. araraticum} |
|------------------------------------------|-----------------------|------------------------|-----------------------|
| Altitude (m)                             | -0.208                | 0.202                  | 0.316                 |
| Latitude (°N)                            | 0.105                 | -0.103                 | -0.948*               |
| Longitude (°E)                           | -0.093                | 0.139                  | 1.000*                |
| 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*                |
| Temperature seasonality                  | -0.207                | -0.024                 | 0.948*                |
| Max. temperature of warmest month        | -0.125                | 0.365                  | 0.200                 |
| Min. temperature of coldest month        | -0.093                | -0.049                 | 1.000*                |
| Temperature annual range                 | -0.071                | 0.189                  | 1.000*                |
| Mean temperature of wettest quarter      | -0.021                | 0.061                  | 1.000*                |
| Mean temperature of driest quarter       | -0.092                | 0.413                  | 1.000*                |
| Mean temperature of warmest quarter      | -0.087                | 0.413                  | -0.200                |
| Mean temperature of coldest quarter      | -0.031                | -0.061                 | 1.000*                |
| Annual precipitation                     | -0.186                | 0.061                  | 1.000*                |
| Precipitation of wettest month           | -0.165                | -0.509                 | -0.200                |
| Precipitation of driest month            | 0.037                 | -0.479                 | 1.000*                |
| Precipitation seasonality                | -0.073                | -0.292                 | 0.400                 |
| Precipitation of wettest quarter         | -0.141                | -0.365                 | 1.000*                |
| Precipitation of driest quarter          | 0.093                 | -0.380                 | -0.200                |
| Precipitation of warmest quarter         | 0.069                 | 0.019                  | 1.000*                |
| Precipitation of coldest quarter         | -0.126                | -0.3647                | -0.948*               |

*Relationships are significant at < 0.01.
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. (2007a); however, our data represent the genome size diversity/range for the whole T. boeoticum group. Population BOE-38, collected 11 km northwest of Beys¸ehir to Yalvac¸, 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., 2007a). 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. Ecogeographically 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 (Vicent 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. Kankapoa 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 Turko˘glu (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 Narli (Kartal-Karadag region) and in BOE-11 (6.183 pg/2C) from Pirinc¸lik (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; Tenaille 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., 2007a; 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: \( \sim 0.5 \) pg/1C (8%) at the diploid level (T. boeoticum vs. T. urartu) and \( \sim 1 \) 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: \( \sim 0.5 \) pg/1C at diploid level (T. boeoticum vs. T. urartu) and \( \sim 1 \) 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.O. 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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