Genome size variation in the genus *Carthamus* L. (Asteraceae, Cardueae): systematic implications and additive changes during allopolyploidization.

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Genome size variation in the genus *Carthamus* L. (Asteraceae, Cardueae): systematic implications and additive changes during allopolyploidization.

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• *Background and Aims:* Plant genome size is an important biological characteristic, embracing a host of interesting relationships, including systematics and ecology or distribution. Currently, there is no information regarding nuclear DNA content for any *Carthamus* species. In addition to improving this knowledge base, our research focuses on the study of interspecific variation and its implications for the infrageneric classification of this group. We also address genome size variation in the process of allopolyploid formation.

• *Methods:* Nuclear DNA samples from 34 populations of 16 species of the genus *Carthamus* were assessed by flow cytometry based on propidium iodide staining.

• *Key Results:* The 2C values ranged from 2.26 pg for *Carthamus leucocaulos* to 7.46 pg for *C. turkestanicus*, and monoploid genome size (1Cx-value) ranged from 1.13 pg in *C. leucocaulos* to 1.53 pg in *C. alexandrinus*. Mean genome sizes differed significantly, based on sectional classification. Both allopolyploid species (*C. creticus* and *C. turkestanicus*) exhibited nuclear DNA contents in accordance with the sum of the putative parental C-values (in one case with a slight reduction, frequent in polyploids), supporting their hybrid origin.

• *Conclusions:* Genome size represents a useful tool in delimiting systematic relationships between closely related species. A considerable reduction in monoploid genome size, possibly due to the hybrid formation, is also reported within these taxa.

Key words: Allopolyploidization, *Carthamus*, Compositae, C-value, DNA content, flow cytometry, genome size, interspecific hybrids, systematics.

INTRODUCTION

As currently circumscribed (Vilatersana *et al.*, 2005), *Carthamus* includes 18 species in two sections, *Carthamus* and *Atractylis* Rchb. Its distribution is centred in the east of the Mediterranean basin. Some species (*C. creticus* L., *C. lanatus* L. and *C. leucocaulos* Sibth. & Sm.) have colonized other Mediterranean regions, including Argentina, Australia, California and South Africa, where they can be invasive (Knowles and Ashri, 1958; Ashri and Knowles, 1960; Hanelt, 1963; Estilai and Knowles, 1978). *Carthamus tinctorius* L. (safflower) is widely cultivated for a variety of uses including oil extraction (Hanelt, 1963) and as a saffron substitute.

Section *Atractylis*, includes an interesting group of allopolyploid species. Much early work, based on morphology, karyology, experimental hybridizations and isozyme studies (Ashri and Knowles, 1960; Harvey and Knowles, 1965; Khidir and Knowles, 1970*a*, *b*; Efron *et al.*, 1973) postulated that *C. creticus* originated from *C. lanatus* and *C. leucocaulos*, and *C. turkestanicus* Popov originated from *C. lanatus* and *C. glaucus* M. Bieb ssp. *glaucus*. Some researchers regarded *C. lanatus* as an interspecific hybrid between one x=10 ancestor and another x=12 (Ashri and Knowles, 1960). However, it is also possible that *C. lanatus* is an autopolyploid (Vilatersana, unpubl. res.) originating from an x=11 ancestor, such as *C. divaricatus* Beg. and Vaccari (Estilai and Knowles, 1976). The latter is a Libyan species that has not been studied in the present work and that appears to be higly variable. The present study addresses nearly all of the species of *Carthamus* encompassing its distribution throughout the Mediterranean basin.

Three basic chromosome numbers occur in *Carthamus* (x=10, 11 and 12), excluding the allotetraploids, which behave as diploids (there are no multivalents at the meiosis; Khidir and Knowles 1970*a*, *b*), with 2n=64.

The amount of nuclear DNA plays an important role in systematics (Kellogg, 1998; Leitch *et al.*, 1998), and although originally it was primarily linked to the ecological and physiological conditions of an organism, it has recently received increased focus within this field. Since 1950, when the term C-value was coined by Swift (as applied to the amount of DNA in the unreplicated haploid or gametic nucleus of an individual), considerable scientific effort has been made, not only to increase information related to plant C-values (Bennett and Leitch, 2004), but also to understand both the tremendous differences in DNA amounts among various organisms, known as the C-value enigma (Gregory, 2001, 2005) and the molecular mechanisms leading to increases or decreases in genome size (Petrov *et al.*, 2000; Bennetzen *et al.*, 2005).

Numerous studies on nuclear DNA content in allopolyploids have been conducted (Gerstel and Burns, 1966; Buitendijk *et al.*, 1997; Comai, 2000; Bennetzen, 2002; Liu and Wendel, 2002; Ozkan *et al.*, 2003; Siško *et al.*, 2003; Bureš *et al.*, 2004). It now seems apparent that in allopolyploids nuclear DNA content either corresponds to approximately the sum of the parental genome sizes or is non-additive, with a smaller amount of nuclear DNA for the hybrid than expected. On the other hand, changes in genome size within a narrow group of species are believed to be a true indicator of the ongoing processes of speciation or genetic divergence (Price, 1976; Murray, 2005).

The main goals of this study were: a) to assess the degree of variation (particularly interspecific variation) in nuclear DNA content; b) to investigate the connection (if any) between nuclear DNA content and the infrageneric classification of *Carthamus*; c) to document the changes in genome size resulting from allopolyploidization; and d) to contribute data on the C-values for this genus, since there are no previous studies on these species.

MATERIAL AND METHODS

Plant material

Table 1 shows the provenance of all material investigated. *Petunia hybrida* Vilm. 'PxPc6' (2C=2.85 pg) and *Pisum sativum* L. 'Express Long' (2C=8.37 pg) were used as internal standards for flow cytometric measurements (Marie and Brown, 1993). The seeds were provided by the Institut des Sciences du Végétal, Gif-sur-Yvette (France). Voucher specimens are preserved in the herbarium BC.

DNA content assessment

Fresh young leaves from the plants studied were co-chopped with an internal standard in 600 µl of Galbraith's buffer (Galbraith *et al.*, 1983) supplemented with 100 µg/ml ribonuclease A (RNase A, Boehringer, Meylan, France) using a razor blade in a plastic Petri dish. To ensure peak identification, the amount of the target species leaf (about 3 cm^2) was approximately twice that of the standard. Additionally, a sample containing only the standard was first prepared and analysed to determine its peak position. Nuclei were filtered through a 30-µm nylon filter in order to eliminate cell debris before adding 60 µg/ml of propidium iodide (Sigma-Aldrich Química, Alcobendas, Madrid, Spain). Samples were kept on ice for 20 min before measurement. Five individuals per species were analysed (except *C. ruber*, marked in the table with an asterisk). Two samples from each individual were extracted and measured independently. Fluorescence analysis was carried out using an Epics XL flow cytometer (Coulter Corporation, Hialeah, Florida). The instrument was set up in the standard configuration: excitation of the sample was conducted using a standard 488 nm air-cooled argon-ion laser at 15 mW power. Forward scatter (FSC), side scatter (SSC) and red (620 nm) fluorescence for propidium iodide were then acquired. Optical alignment was based on the optimized signal from 10 nm fluorescent beads (Immunocheck, Epics Division). Time was

used as a control for the stability of the instrument. Red fluorescence was projected on a 1024 monoparametrical histogram. Aggregates were excluded, with single cells gated by area vs. peak fluorescence signal. The total nuclear DNA content was calculated by multiplying the known DNA content of the standard by the quotient between the 2C peak positions of the target species and the standard in the histogram of fluorescence intensities, under the assumption that there is a linear correlation between the fluorescent signals from stained nuclei of the unknown specimen, the known internal standard, and DNA content.

Statistical analyses

The means and standard deviations (SD) were calculated from the means of individual plants. Analysis of variance (ANOVA) was carried out to evaluate whether the differences among sections were significant or not. In those cases in which ANOVA revealed significant differences, a least significant difference (LSD) test was performed. Statgraphics Plus 5.0 (Statistical Graphics Corp.) was used for the statistical analysis.

ANOVA was performed using 2C values and monoploid genome size as dependent variables (1Cx, according to the recently proposed terms for genome size in Greilhuber *et al.*, 2005).

RESULTS

Data on nuclear DNA content and other karyological features are presented in Table 2. The 2C values ranged from 2.26 pg for *Carthamus leucocaulos* to 7.46 pg for *C. turkestanicus*, and monoploid genome size (1Cx-value) ranged from 1.13 pg in *C. leucocaulos* to 1.53 pg in *C. alexandrinus*. The analyses were of good quality [mean half peak coefficient of variation (HPCV) = 4.54%]. According to both the literature and the Plant DNA C-values Database (http://www.rbgkew.org.uk/cval/homepage.html; release 3.0, Bennett and Leitch, 2004), this is the first study on genome size in the genus *Carthamus*.

DISCUSSION

Systematic implications for infrageneric classification

The means of 2C values were significantly different between the two sections, *Carthamus* (2.70 pg) and *Atractylis* (4.33 pg), considered by Vilatersana *et al.* (2005) (P=0.0103). When the allopolyploids were omitted from the analysis, the means were no longer significantly different (P=0.1009). This suggests that these differences in genome size might be attributable to the presence of putative hybrids, with ploidy levels of 4x and 6x, since all other species are diploid (see Table 2). To prevent this effect, that is, to avoid the bias due to inclusion of data from species with different ploidy levels, the analyses were carried out using the 1Cx-value (monoploid genome size) as a variable. Means of 1Cx values (1.32 pg for *Atractylis* and 1.35 pg for *Carthamus*) between two sections are not significantly different (P=0.4711). This seemingly results from the low monoploid genome size of the allopolyploid taxa, lowering the mean of the section. However, when these putative hybrids are eliminated from the analysis, the means still remain not significantly different (P=0.1076).

Considered as a whole, these results lead to the conclusion that differences in genome size within this species group go further than those due to formation of allopolyploids. These

results also suggest that, in addition to polyploidy, other differential features are present in their genome.

The dendrogram shown in Fig. 1 illustrates the differentiation among three clusters. Clusters A and C include the species of section *Atractylis*. One (C) is formed by the allopolyploid species. Sectional classification should not be constructed on the basis of hybrid characteristics, i.e. on the assumption that allopolyploids form a separate clade only because they are polyploid and of hybrid origin, and not for possessing characteristics sufficiently different from the remaining species to constitute an entirely independent section. However, this group also includes *C. leucocaulos* and *C. nitidus*, both from section *Atractylis,* but not allopolyploid. The former is a species with an insular distribution (Greek islands), a fact possibly related to a reduction in genome size as compared with the species of cluster A, where they should be included. This reduction may result from colonization pressures (Garnatje *et al.*, unpubl. res., Suda *et al.*, 2003), supporting the hypothesis that small C-values were an evolutionary advantage under the pressures of insular selection. According to Estilai and Knowles (1978), *C. leucocaulos* has a morphological appearance rather different from most of the remaining species in this genus although, it is quite similar to *C. nitidus*. The second, *C. nitidus*, has been regarded as a "link species" between sections *Atractylis* and *Carthamus*, (Vilatersana *et al.*, 2000).

Thus, cluster C includes the species of section *Atractylis* with a lower monoploid DNA amount. This finding could reflect the process of allopolyploid hybrid formation in section *Atractylis* and the decrease in monoploid genome size that this phenomenon leads to, as Ozkan *et al.* (2003) noted in *Aegilops-Triticum,* whereby the DNA loss detected during allopolyploidization may represent a pre-programmed adaptive response, as a mechanism which could stabilize polyploid genomes, to the genomic stress resulting from hybridization and allopolyploidy.

The other cluster (B) corresponds to the section *Carthamus*, apart from *C. dentatus* ssp. *ruber* and *C. tenuis*, which appear within this section *Carthamus* cluster, although they have previously been placed in section *Atractylis*. Although the explanation for this remains unclear, it is possible that both of these species are related to *C. nitidus*, with which they share a more basal position in the phylogeny (Vilatersana *et al.*, unpublished data). This would also agree with Leitch *et al.* (1998), who found that most primitive species have smaller nuclear DNA contents.

Moreover, the case of the populations studied of *C. lanatus* shows that insularity could also explain the reduction in genome size. Continental species have more DNA than those from islands and within these latter, the population from Formentera, the smallest island and consequently the island subject to higher selection constraints, has a significantly (P=0.0019) lower nuclear DNA amount than the population from Crete (4.62 vs. 4.75, a difference of 2.81%).

Cytogenetic implications

The ANOVA results demonstrate that in both cases the means of total nuclear DNA content (2C values) differ significantly in relation to chromosome number and ploidy level ($P \leq 0.0001$). When the ANOVA is performed using the monoploid genome size (1Cx), significant differences result when the independent variable is either the ploidy level (P=0.0005) or the chromosome number ($P \leq 0.0001$), as expected.

The multiple range test (LSD) shows that all of the means are significantly different between the four chromosome numbers, except those between 2n=44 and 2n=64. Monoploid genome size decreases with increasing chromosome number. Mean 1Cx values are significantly different between diploids and tetraploids, as well as between diploids and hexaploids, but are not significant between the two groups of polyploids.

Hybrid species

A number of researchers (Khidir and Knowles, 1970a; Estilai and Knowles, 1978; Vilatersana *et al.*, 2005,) support the hypothesis that *C. creticus* (2n=64) is an allopolyploid derived from *C. lanatus* (2n=44) and *C. leucocaulos* (2n=20). The sum of the 2C values for *C. lanatus* (4.73 pg) and *C. leucocaulos* (2.26 pg) results in 6.99 pg, almost the same as the 2C value for *C. creticus* (6.98 pg). When analysing the origins of *C. turkestanicus* (2n=64), an allopolyploid derived from *C. lanatus* (2n=44) and *C. glaucus* ssp. *glaucus* (2n=20), the sum of the 2C values of the parental species was 7.73 pg, whereas the mean of the four *C. turkestanicus* populations was 7.35 pg (7.29, 7.31, 7.32, and 7.46). These putative hybrids, *C. creticus* and *C. turkestanicus*, are regarded as stabilized, and although of polyploid origin, they currently behave as diploids. In both cases, nuclear DNA amounts of the hybrid species fell slightly below the sum of the genome sizes of the parental species. The genome size of *C. creticus* nearly coincides with the sum of those of *C. lanatus* and *C. leucocaulos*, a finding consistent with the hypothesis that these species were its progenitors. This would not, however, exclude other possible parents. In the case of *C. turkestanicus*, its less-than-expected nuclear DNA content could be explained in terms of non-additive changes in genome size as discussed by Ozkan *et al.* (2003).

These results are consistent with studies on genome size in hybrid species; natural hybrids with a lower nuclear DNA amount than the sum of those of the parents, heve been recorded in the genus *Cirsium* (Bureš *et al.*, 2004). A similar situation has been found in artificial hybrids produced by embryo rescue in the genus *Cucurbita* (Šiško *et al.*, 2003).

For the most part, *Carthamus* sp. exhibited low nuclear DNA amounts compared with the plant DNA C-values recorded to date (Plant DNA C-values Database, Bennett and Leitch, 2004). The success of weeds has been linked to small genome size, which, among other advantages, helps them to establish quickly and develop rapidly throughout their life cycle

(Bennett *et al.*, 1998). Supporting this hypothesis, all these species are annuals and weeds. Conversely, some of the species of hybrid origin, particularly *C. creticus* and *C. lanatus*, have relatively high nuclear DNA contents and also display a more aggressive invasive character, colonizing areas of Australia and the United States (Peirce, 1992).

Concluding remarks

Analyses of genome size in this genus do not provide additional evidence for recognition of two (*Atractylis* and *Carthamus*) sections, although they shows that the species of sect. *Carthamus* form a distinct cluster. Putative hybrid taxa, however, are clearly differentiated from the remaining species due to their decreased monoploid genome size, probably a consequence of allopolyploidization. We have also verified in these species that 1Cx values decrease with increasing ploidy levels, and that those taxa of presumed allopolyploid origin exhibit a total nuclear DNA content more or less equal to, or a little less than, the sum of the parental species. Finally, the most invasive *Carthamus* sp. exhibit an increased genome size but a decreased chromosome number, with respect to the other taxa of the genus. From the perspective of genome size study, it would be of great interest to see whether the patterns of DNA content variation in allopolyploids, weeds and island colonizers demonstrated in this study are also evident within other plant groups.

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1 TABLE 1. *Origin of the material studied (vouchers are in the herbarium BC).*

Species	Origin of materials
Sect. *Atractylis*	
Carthamus alexandrinus (Boiss. & Heldr.) Asch.	EGYPT, Alexandria: between El Amiriya and Bourg-el-Arab, *Susanna 1835 and Vilatersana*, 7 Jun. 1998. EGYPT, Alexandria: 10 km from Bourg-el-Arab, *Susanna 1843 and Vilatersana*, 7 Jun. 1998. EGYPT, Alexandria: 106 km East of Marsa Matruh, *Susanna 1858 and Vilatersana*, 8 Jun. 1998.
Carthamus anatolicus (Boiss.) G. Samuelsson in Rech. f.	ISRAEL, Messilot: near Shehulot. Institut für Pflanzengenetik und Kulturpflanzenforschung, Gatersleben. Cart 43/76. ISRAEL, Kefar Shammai. Institut für Pflanzengenetik und Kulturpflanzenforschung, Gatersleben. Cart 53/76.
Carthamus boissieri Halácsy	GREECE, Crete: Rethymnon, road between Asomatos and Moni Preveli, *Vilatersana 30*, 7 Jul. 1996. GREECE, Crete: Hania, Drapanon Peninsula, *Vilatersana 36*, 9 Jul. 1996. GREECE. Institut für Pflanzengenetik und Kulturpflanzenforschung, Gatersleben. Cart 85/99.
Carthamus creticus L.	MOROCCO, Al Hoceima: 38 km S of Al Hoceima on the road to Nador, *Garnatje, Susanna 1772 and Vilatersana*, 15 Jun. 1997. EGYPT, Alexandria: near El Amiriya, *Susanna 1851 and Vilatersana*, 7 Jun. 1998.
Carthamus dentatus Vahl ssp. *ruber* (Link) Hanelt	GREECE, Crete: Rethymnon, road N-97 between Rotosi and Mesohorio, *Vilatersana 44*, 14 Jul. 1996.
Carthamus glaucus M. Bieb. ssp. *glaucus*	TURKEY, Ahar Dağ: Tekeyatağ, 1500 m. *Ertuğrul, Garcia-Jacas, Susanna 2338 and Uysal*, 4 Aug. 2002.
Carthamus lanatus L.	GREECE, Crete: Rethymnon, between road N-77 and necropolis Minois, *Vilatersana 27*, 7 Jul. 1996. SPAIN, Soria: between Morcuera and Montejo de Tiermes. *Garcia-Jacas and Susanna 2444B*, 15 Aug. 2003. ITALY, Calabria: road SS-106, km 256, near Neto river, *Carretero, Pignone, Sonante and Vilatersana 207*, 22 Jul. 2003. SPAIN, Balearic Islands: Formentera, *Garnatje and Vilatersana 413*, 18 Apr. 2005.

Carthamus lanatus L. ssp. *montanus* (Pomel) Gahand et Maire.	TUNISIA, Gulf of Tunis: Cedria Plage, Institut für Pflanzengenetik und Kulturpflanzenforschung, Gatersleben. Cart 84/95.
Carthamus leucocaulos Sibth. & Sm.	GREECE, Crete: Hania, base of Mount Hrissokalitissas, *Vilatersana 40*, 11 Jul. 1996.
Carthamus nitidus Boiss.	ISRAEL: Negev Desert, Dead Sea, *R. Levy*, Sep. 1997.
Carthamus tenuis (Boiss. & Blanche) Bornm.	ISRAEL: Jordan Valley, *R. Levy*, Sep. 1997.
Carthamus turkestanicus Popov	ARMENIA, Ararat: near Surenavan along a water conduction 1 km from the road, *Fajvush, Gabrielyan, Garcia-Jacas, Guara, Hovannisyan, Susanna 1532, Tamanyan and Vallès*, 19 Aug. 1995. ARMENIA, Ekhegnazdor: near Agarakadzor, *Fajvush, Gabrielyan, Garcia-Jacas, Guara, Hovannisyan, Susanna 1551N, Tamanyan and Vallès*, 20 Aug. 1995. IRAN, Azarbayjan-e-Shargui: 35 km from Tabriz on the road to Ahar, *Garcia-Jacas, Mozaffarian, Susanna 1656 and Vallès*, 5 Aug. 1996. UZBEKISTAN, Tashkent: between Jizak and Tashkent, *Kapustina, Khassanov, Susanna 2064B and Vallès*, 8 Oct. 1999.
Sect. *Carthamus*	
Carthamus gypsicola Iljin	ARMENIA, Ararat: Vedi, *Fajvush, Gabrielyan, Garcia-Jacas, Guara, Hovannisyan, Susanna 1579, Tamanyan and Vallès*, 25 Aug. 1995.
Carthamus oxyacantha M. Bieb.	IRAN, Tehran: Sorkhehesar near Tehran, *Garcia-Jacas, Mozaffarian, Susanna 1626 and Vallès*, 2 Aug. 1996. IRAN, Azarbayjan-e-Gharbi: 30 km from Khoy on the road to Orumiyeh, *Garcia-Jacas, Mozaffarian, Susanna 1689 and Vallès*, 2 Aug. 1996.
Carthamus palaestinus Eig	ISRAEL. USDA, Western Regional Plant Introduction Station. Pullman, Washington PI 235663.
Carthamus persicus Desf. ex Willd.	LEBANON. USDA, Western Regional Plant Introduction Station. Pullman, Washington PI 243151. TURKEY, Elaziğ: road from Elaziğ to Bingöl. *Ertuğrul, Garcia-Jacas, Susanna 2358 and Uysal*, 6 Aug. 2002.
Carthamus tinctorius L.	KAZAKHSTAN, Irsu: 1 km from Rayerka, near of Aksu Canyon. *Ivaschenko, Susanna 2190 and Vallès*, 30 Aug. 2000.

SLOVENIA, Ljubljana: Botanical Garden.
SPAIN, Huesca: *S. Castroviejo*, 20 Sep. 1984.
UZBEKISTAN, Samarkand: between Samarkand and Bukhara, *Khassanov*, Nov. 1999.

TABLE 2. *Nuclear DNA content and the other karyological features of the populations studied.*

Taxa	2C ± SD (pg)[1]	2C (Mbp)[2]	2n[3]	Ploidy level	1Cx[4]	Standard[5]
Sect. *Atractylis*						
C. alexandrinus, S-1835	3.02 ± 0.20	2953.56	20	2x	1.51	*Pisum*
C. alexandrinus, S-1843	2.99 ± 0.04	2924.22	20	2x	1.50	*Pisum*
C. alexandrinus, S-1858	3.06 ± 0.11	2992.68	20	2x	1.53	*Pisum*
C. anatolicus, 53/76	2.96 ± 0.03	2894.88	20	2x	1.48	*Pisum*
C. anatolicus, 43/76	2.99 ± 0.06	2924.22	20	2x	1.50	*Pisum*
C. boissieri, V-30	2.89 ± 0.03	2826.42	20	2x	1.45	*Pisum*
C. boissieri, V-36	2.94 ± 0.01	2875.32	20	2x	1.47	*Pisum*
C. boissieri, Greece	2.95 ± 0.18	2885.10	20	2x	1.48	*Pisum*
C. creticus, S-1772	7.06 ± 0.11	6904.68	64	6x	1.18	*Petunia*
C. creticus, S-1851	6.89 ± 0.07	6738.42	64	6x	1.15	*Petunia*
C. dentatus subsp. *ruber*	2.70*	2640.60	20	2x	1.35	*Pisum*
C. glaucus subsp. *glaucus*	3.00 ± 0.08	2934.00	20	2x	1.50	*Pisum*
C. lanatus, V-27	4.75 ± 0.05	4645.50	44	4x	1.19	*Petunia*
C. lanatus, V-207	4.76 ± 0.08	4655.28	44	4x	1.19	*Pisum*
C. lanatus, S-2444B	4.80 ± 0.07	4694.40	44	4x	1.20	*Pisum*
C. lanatus, V-413	4.62±0.02	4518.36	44	4x	1.16	*Pisum*
C. lanatus subsp. *montanus*	4.83 ± 0.06	4723.74	44	4x	1.21	*Pisum*
C. leucocaulos	2.26 ± 0.02	2210.28	20	2x	1.13	*Pisum*
C. nitidus	2.44 ± 0.04	2386.32	24	2x	1.22	*Pisum*
C. tenuis	2.74 ± 0.07	2679.72	20	2x	1.37	*Pisum*
C. turkestanicus, S-1532	7.32 ± 0.11	7158.96	64	6x	1.22	*Petunia*
C. turkestanicus, S-1551N	7.46 ± 0.17	7295.88	64	6x	1.24	*Pisum*
C. turkestanicus, S-1656	7.29 ± 0.05	7129.62	64	6x	1.22	*Petunia*
C. turkestanicus, S-2064B	7.31 ± 0.11	7149.18	64	6x	1.22	*Petunia*
Sect. *Carthamus*						

C. gypsicola	2.71 ± 0.06	2650.38	24	2x	1.36	*Pisum*
C. oxyacantha, S-1626	2.58 ± 0.02	2523.24	24	2x	1.29	*Pisum*
C. oxyacantha, S-1689	2.62 ± 0.06	2562.36	24	2x	1.31	*Pisum*
C. palaestinus	2.82 ± 0.06	2757.96	24	2x	1.41	*Pisum*
C. persicus, Lebanon	2.65 ± 0.08	2591.70	24	2x	1.33	*Pisum*
C. persicus, S-2358	2.65 ± 0.06	2591.70	24	2x	1.33	*Pisum*
C. tinctorius, S-2190	2.77 ± 0.04	2709.06	24	2x	1.39	*Pisum*
C. tinctorius, Uzbekistan	2.76 ± 0.07	2699.28	24	2x	1.38	*Pisum*
C. tinctorius, Huesca	2.79 ± 0.05	2728.62	24	2x	1.40	*Pisum*
C. tinctorius, Ljubljana	2.68 ± 0.04	2621.04	24	2x	1.34	*Pisum*

[1]2C nuclear DNA content (mean value ± standard deviation of 10 samples). [2]1 pg = 978 Mbp (Doležel *et al.*, 2003). [3]Somatic chromosome number.[4]Monoploid genome size (2C value divided by ploidy level) [5]Internal standard used in each case (see text for details regarding *Pisum* and *Petunia*).
*Only one individual was measured.

FIGURE 1. Nearest Neighbor Method dendrogram based on Cx values, showing the squared Euclidean distance of the *Carthamus* taxa analysed.