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Ploidy Levels, Relative Genome Sizes, and Base Pair Composition in *Cotoneaster*

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Abstract. **The genus *Cotoneaster* (Rosaceae, Maloideae) is highly diverse, containing ≈400 species. Like other maloids, there is a high frequency of naturally occurring polyploids within the genus, with most species being tetraploid or triploid. Apomixis is also prevalent and is associated with polyploidy. The objective of this study was to estimate genome sizes and infer ploidy levels for species that had not previously been investigated as well as compare estimates using two fluorochromes and determine base pair (bp) composition. Chromosome counts of seven species confirmed ploidy levels estimated from flow cytometric analysis of nuclei stained with 4′,6-diamidino-2-phenylindole (DAPI). Monoploid (1Cx) genome sizes ranged from 0.71 to 0.96 pg. Differences in monoploid genome size were not related to current taxonomic treatment, indicating that while chromosome sizes may vary among species, there are no clear differences related to subgeneric groups. A comparison of DAPI and propidium iodide (PI) showed a difference in DNA staining in *Cotoneaster* comparable to other rosaceous species. Base pair composition (AT%) in *Cotoneaster* ranged from 58.4% to 60.8%, which led to overestimation of genome size estimates in many cases—assuming the estimates of the DNA intercalator are accurate. Our findings will inform breeders with regard to the reproductive behavior of potential parents and may be used to confirm hybrids from interploid crosses.**

Cotoneaster is a genus of woody plants composed of ≈400 species that range in habit from tight, impenetrable groundcovers to airy shrubs and medium-sized trees. The center of species diversity is the Himalayas and mountains of Yunnan and Sichuan provinces of China. The distribution encompasses the temperate zones of Eurasia and Northern Africa. The northern end of the range stretches from Spain to Siberia, and the southern limit extends from Morocco to the southern tip of India and South Korea (Fryer and Hylmö, 2009).

Although there are hundreds of species of *Cotoneaster*, a relatively small percentage are commonly grown in ornamental landscapes, as illustrated by Dirr (2009) listing only 14. These species were selected for their multiseason interest from flowers, fruit, and plant habit. In the 2014 Census of Horticultural Specialties (U.S. Department of Agriculture, 2014), *Cotoneaster* sales were estimated to exceed $7 million in the United States, although the value is likely greater because this figure accounted only for sales of *Cotoneasters* classified as "broadleaf evergreens" and many species are deciduous or semievergreen depending on climate and environmental factors.

Cotoneaster is a member of Rosaceae, subfamily Maloideae, and appears to be most closely related to *Pyracantha* (firethorn) and *Heteromeles* (christmas berry) (Robertson et al., 1991; Rohrer et al., 1992). Taxonomy at the family level is complicated, with interspecific and intergeneric hybridization being common. Interspecific hybrids of several species of *Cotoneaster* have been reported, and *Cotoneaster melanocarpus* has reportedly hybridized with *Sorbus acuparia* ssp. *siberica* to form the intergeneric hybrid ×*Sorbocotoneaster* (Fryer and Hylmö, 2009). Within *Cotoneaster*, there are two subgenera, *Chaenopetalum* and *Cotoneaster*, which are primarily defined by floral morphology. These subgenera have been further divided into 11 sections based on botanical characteristics, and further dissected into 37 series based on botanical characteristics and geographic origins of the species (Flinck and Hylmö, 1966). However, keys associated with this treatment are ambiguous and often of limited use for species identification. We are collaborating with Hoyt Arboretum (Portland, OR) to identify and evaluate our germplasm collection, with little success in identifying unknown samples.

The base chromosome number of Maloideae is 17 and is thought to be of allopolyploid origin—perhaps derived from a hybridization event between other subfamilies in Rosaceae [Rosoideae (x = 7, 8, 9), Spiraeoideae (x = 9), Amygaloideae (x = 8)] followed by a whole genome doubling event (Dickson et al., 1992; Sax, 1954). *Cotoneaster* species show a ploidy series, with estimates of 70% tetraploid ($2n = 4x = 68$), 15% triploid ($2n = 3x = 51$), and 10% diploid ($2n = 2x = 34$), and the remaining species of greater ploidy level (Fryer and Hylmö, 2009). Apomixis is common in *Cotoneaster* and appears to be associated with polyploidy, as the tetraploids and triploids are frequently obligate or rarely facultative apomicts, while diploid progeny are sexually derived (Bartish et al., 2001; Czapik, 1996; Hjelmqvist, 1962; Nybom and Bartish, 2007).

Because apomixis is so common in polyploid *Cotoneaster*, knowledge of ploidy level is essential for breeders to design

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crosses with hopes of hybrid seed, as the female must be a sexually fertile diploid. In addition, information on ploidy level, genome size, and bp composition may give taxonomists and phylogeneticists insight to the evolution and organization of the genus and related taxa. Previous reports of genome sizes in *Cotoneaster* are limited; therefore, our goals were to determine relative genome sizes and produce ploidy estimates across a wide selection of *Cotoneaster* including its breadth of taxonomic groups.

Materials and Methods

Plant material. Germplasm was collected through various means including whole plants from nurseries, cuttings from gardens and arboreta, and seeds from gardens around the world participating in Index Seminum (Table 1). The latter formed the bulk of our collection. Plants were maintained in containers or in field plots at Oregon State University and all were assigned accession numbers.

Genome sizing. Holoploid (2C) genome sizes were determined by flow cytometry (CyFlow PA; Partec, Münster, Germany) and comparison of mean relative fluorescence of the sample against an internal standard, *Pisum sativum* 'Ctirad', with a known genome size of 8.76 pg (Greilhuber et al., 2007). Two different fluorochromes were used. A total of 67 accessions representing 65 species were sampled across the two subgenera and all 11 sections using flow cytometric analysis of nuclei stained with DAPI (CyStain ultraviolet Precise P; Partec). A subset of 17 taxa was also prepared with PI (CyStain ultraviolet Absolute P; Partec). Nuclei of each sample and our standard were concurrently prepared by chopping with a double-sided razor blade in extraction buffer (CyStain ultraviolet Precise P Nuclei Extraction Buffer; Partec) for ≈90 s before being filtered through a 50-µm nylon mesh filter (CellTrics®; Partec) and stained with either fluorochrome. For PI-stained samples, RNase was included to ensure staining of DNA exclusively. *Cotoneaster* samples were prepared using 4 cm of rapidly growing terminal stem tissue including vegetative buds and we used 1 cm^2 of fresh pea leaf tissue. DAPI-stained samples were incubated in darkness for 5 to 10 min before analysis and PI-stained samples were incubated in darkness for at least 30 min on ice. Three replicates of each accession were prepared for both DAPI and PI. A minimum of 3000 particles were analyzed for each sample. Sample runs were rejected if the coefficient of variation (CV) was greater than 7%.

Holoploid DNA content (2C) was calculated as DNA content of standard × (mean fluorescence value of sample/mean fluorescence of standard). Then, analysis of variance and means separation by Tukey's honestly significant difference was performed, with ploidy levels inferred from mean separation. Monoploid genome sizes were calculated by dividing each sample's 2C genome size by inferred ploidy. Analysis of variance was then conducted on monoploid genome size by accession and then taxonomic division to test for significant differences among subgenera or sections; mean separation was performed as described above when the model was significant ($\alpha = 0.05$). For the subset of 17 accessions that were examined with both DAPI and PI, genome size estimates using each fluorochrome were compared using a *t* test ($\alpha = 0.05$) separately for each accession. Base pair composition was calculated as AT% = AT% for internal standard × {[(fluorescence sample, DAPI)/(fluorescence internal standard, PI/fluorescence sample, PI)]$^{(1/\text{binding length})}$} (Godelle et al., 1993). AT% of *P. sativum* 'Ctirad' is 61.50% and has a binding length of ≈3.5 bp (Meister and Barrow, 2007).

Cytology. Chromosomes were counted for seven species, five of which were included in the genome sizing, and two additional species *Cotoneaster hebephyllus* and *Cotoneaster poluninii*. Somatic cells were collected from actively growing root tips, which grew freely from the bottom of their containers into sand. Roots were treated with 0.003 M 8-hydroxyquinoline for 2 h at 4 °C and fixed in Carnoy's solution [6 absolute ethanol : 3 chloroform : 1 glacial acetic acid (by volume)] overnight. Root tips were stored in 70% ethanol at 4 °C until prepared for chromosome counts (Goldblatt and Gentry, 1979). Chromosomes were examined by root tip squashes with modified carbol fuchsin, at ×63 to ×100 magnification (Axio imager.A1; Zeiss, Thornwood, NY) and images were collected using a monochromatic CCD camera (AxioCam MRm; Zeiss). A minimum of three cells were counted for each species.

Results and Discussion

Relative 2C genome sizes for 67 accessions determined by flow cytometry with DAPI ranged from 1.52 pg (*Cotoneaster frigidus*) to 4.71 pg (*Cotoneaster kweitschoviensis*) (Table 2). The 2C genome sizes showed marked divisions, which were used to assign ploidy level. Of the 67 accessions, 10 (15%) were diploids ($2n = 2x = 34$) with 2C values ranging from 1.52 to 1.73 pg, 5 (9%) were triploids ($2n = 3x = 51$) with 2C values ranging from 2.14 to 2.58 pg, 51 (76%) were tetraploids ($2n = 4x = 68$) with 2C values ranging from 2.88 to 3.34 pg, and 1 (1.5%) accession was hexaploid ($2n = 6x = 102$) with a 2C value of 4.71 pg (Table 2). Regarding relative percentage of ploidy levels, our findings generally agree with previous reports including Kroon (1975) who reported 3 diploid, 3 triploid, and 23 tetraploid species among the 28 studied. Ours is the first report of ploidy estimation for 13 of the species. However, one diploid species, *Cotoneaster juratana*, is not a valid species and we have been unable to confirm its identity. One assumption was a mislabeling of *Cotoneaster juranus*, which has only been reported as a tetraploid (Fryer and Hylmö, 2009). Unfortunately, morphology of the plant labeled as *C. juratana* did not match the description of *C. juranus* and has since been lost from our collection. We sampled two accessions of *Cotoneaster adpressus* and *Cotoneaster acutifolius* and both showed ploidy series. *Cotoneaster adpressus* 10-0157 was tetraploid and the other, 'Tom Thumb', was triploid. Previous reports for *C. adpressus* indicated that it was either diploid or triploid (Sax, 1954; Zeilinga, 1964), making ours the first report of tetraploidy for the species. *Cotoneaseter acutifolius* 09–0047 was diploid and *C. acutifolius* 10–0126 was triploid. Our results are consistent with Sax (1954) who reported diploids, triploids, and tetraploids for *C. acutifolius.* This is in contrast to Zeilinga (1964) who reported that all species in their study were diploid or tetraploid and only cultivars were found to be triploid. This provides evidence that 'Tom Thumb' and *C. acutifolius* 10-0126 are hybrids. *Cotoneaster ×watereri* 10-106 was a triploid in contrast to previous reports indicating this hybrid species to be diploid (Fryer and Hylmö, 2009). This species arose as a hybrid of *C. frigidus* × *Cotoneaster salicifolius*, which generally are both regarded as diploids, though Sax (1954) also reported triploidy in *C. salicifolius*. It is unclear how this triploid accession arose, but possibilities include a previously unknown tetraploid cytotype of one of the parent species,

Table 1. Source and collection information for 67 *Cotoneaster* accessions.

Taxon	OSU accession	Source[z]	Collection information and/or IPEN number as available[y]
Cotoneaster ×suecicus 'Coral Beauty'	10-0166	NWREC	
Cotoneaster ×watereri	10-0106	ISATU	XX-0-AJUDA-15-605
Cotoneaster acutifolius	09-0047	Mlyňany Arboretum	From expedition to China in 1960 but without information if collected in some botanical institution or in wild
C. acutifolius	10-0126	ZBPT	BG Shanghai/China, Gansu Province, Mt. Maiji, alt. 1,000–1,600 m
Cotoneaster adpressus	10-0124	Blue Heron Farm Nursery	
C. adpressus	10-0157	Horticultural Society of Iceland	
Cotoneaster albokermesinus	09-0065	Strasbourg	[XX-0-STR-1993021 G I S Hylmö, Bertil, 1260 Bjuv (SE) 1993] OR [XX-0-STR-1992093 G B S Botanical Garden Bulgarian Academy of Sciences, Sofia (BG) 1992]
Cotoneaster apiculatus	10-0006	Rogów Arboretum	Wild collected in China: no further data available
Cotoneaster applanatus	09-0067	Strasbourg	
Cotoneaster arbusculus	09-0068	Strasbourg	XX-0-STR-1991006 G B S Arboretum de Chèvreloup (MNHN), 78150 Rocquencourt (FR) 1991
Cotoneaster armenus	09-0069	Strasbourg	XX-0-STR-1977023 G B S Botanical Garden Agricultural University
Cotoneaster astrophoros	11-0034	Dublin	
Cotoneaster atrovirens	09-0072	Strasbourg	Z B S Göteborg Botanical Garden (SE) 1984 China Sichuan, Kangding toward Cheto Smith, Harry (SE) HS 12918 1934
Cotoneaster bacillaris	09-0073	Strasbourg	XX-0-STR-1993050 G B S Botanical Garden Mendel University, Brno (CZ) 1993
Cotoneaster boisianus	09-0074	Strasbourg	XX-0-JENA-7821428 G B S Botanischer Garten Friedrich-SchillerUniversität Jena (DE) 1978
Cotoneaster braydi	09-0076	Strasbourg	[CN-0-STR-1991007 Z B P Fryer, Jeanette, Rumsey Gardens, PO8 0PD Waterlooville (UK) 1991 China SW Si-Kiang,Maoniu, 3300 m Smith, Harry (SE) HS 12624] OR [XX-0-STR-1992089 G B S Ness Botanic Gardens, University of Liverpool (UK) University of Liverpool 1992] OR [XX-0 STR-1993008 G B S Arboretum National des Barres, 45290Nogent-sur-Vernisson (FR) 1993]
Cotoneaster buxifolius	09-0077	Strasbourg	XX-0-STR-1989036 G B S Botanical Garden University Torino (IT) 1989
Cotoneaster canescens	09-0079	Strasbourg	SE-0-STR-1993011 Z I S Hylmö, Bertil, 1260 Bjuv (SE) 1993 Sweden Oland, Gotland, Borgholm
Cotoneaster cashmiriensis	09-0080	Strasbourg	XX-0-JENA-7727230 G B S Botanischer Garten Friedrich-SchillerUniversität Jena (DE) 1980
Cotoneaster chungtiensis	09-0082	Strasbourg	CN-0-STR-1996004 Z B P Fryer, Jeanette, Rumsey Gardens, PO8 0PD Waterlooville (UK) 1996 China Yunnan, Zhongdian
Cotoneaster cinerascens	09-0083	Strasbourg	XX-0-STR-1991008 G B P Fryer, Jeanette, Rumsey Gardens, PO8 0PD Waterlooville (UK) 1991 HY 1883
Cotoneaster cochleatus	09-0085	Strasbourg	
Cotoneaster commixtus	09-0084	Strasbourg	XX-0-STR-1972008 G B S Botanical Garden of Comenius University Bratislava (SK) 1973
Cotoneaster congestus	10-0088	Forestfarm Nursery	
Cotoneaster cooperi[x]	11-0064	Dublin	2001.2840 (OS, Vacratot)
Cotoneaster daliensis	10-0129	ZBPT	BG Strasbourg/BG Ness/China, Yunnan, Dali, Tsang Shan, road to Longquan Peak, alt. 3,500 m
Cotoneaster dammeri	11-0039	Dublin	Not in 2016 seminus
Cotoneaster dielsianus	09-0013	Hohenheim	XX-0-HOH-WS-33-10538
Cotoneaster divaricatus	10-0089	Forestfarm Nursery	
Cotoneaster fangianus	10-0008	Rogów Arboretum	Alnarp Agricultural University, Uppsala, Sweden
Cotoneaster fastigiatus	10-0130	ZBPT	Strasbourg
Cotoneaster frigidus	09-0045	Mlyňany Arboretum	
Cotoneaster ganghobaensis	10-0131	ZBPT	Strasbourg/B. Hylmö/China, Yunnan, Gang-Ho-Ba, Brickell et Leslie

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Table 1. Continued.

Taxon	OSU accession	Source[z]	Collection information and/or IPEN number as available[y]
Cotoneaster genitanus	10-0132	ZBPT	Strasbourg/BG Brno 1992
Cotoneaster henryanus	09-0017	Hohenheim	XX-0-HOH-EG-B-040-10540
Cotoneaster horizontalis	10-0104	ISATU	XX-0-AJUDA-15-594
Cotoneaster hypocarpus	10-0133	ZBPT	Strasbourg/J. Fryer/China, Yunnan, Yulong Shan
Cotoneaster ignavus	10-0010	Rogów Arboretum	China: Tian-Shan
Cotoneaster integerrimus	10-0011	Rogów Arboretum	
Cotoneaster juratana[w]	10-0171	JFAS	Lautaret, 1,900 m
Cotoneaster kweitschoviensis	10-0134	ZBPT	Arboretum Nový Dvůr/BG Jena/China, Guizhou
Cotoneaster lacteus	10-0108	ISATU	XX-0-AJUDA-14-597
Cotoneaster lidjiangensis	10-0135	ZBPT	Arboretum Nový Dvůr/BG Dublin 1939.004501 RB
Cotoneaster lucidus	09-0018	Hohenheim	
Cotoneaster melanocarpus	10-0012	Rogów Arboretum	Wild collected in Hungary: no further data available
Cotoneaster milkedandaensis	10-0174	Hoyt Arboretum	
Cotoneaster niger	09-0051	Mlyňany Arboretum	As specimen 218/61 (introduced in 1961 from Tharandt Forstbotanischen Garten)
C. niger	10-0105	ISATU	XX-0-AJUDA-15-599
Cotoneaster nitens	09-0052	Mlyňany Arboretum	As specimen 229/61 from Botanischer Garten Köln-Riehl
Cotoneaster obscurus	10-0110	ISATU	XX-0-AJUDA-15-600
Cotoneaster pannosus	10-0102	ISATU	XX-0-AJUDA-14-601
Cotoneaster popovii[v]	09-0081	Strasbourg	[XX-0-STR-1970009 G B S Chisinau Botanical Garden, Academy of Sciences of Moldova (MD) 1971] OR [XX-0-STR-1975008 G B S Forest Steppe Experimental Plant Breeding Station, Lipetsk (RU) 1975]
Cotoneaster preacox	10-0013	Rogów Arboretum	
Cotoneaster procumbens	10-0137	ZBPT	Arboretum Nový Dvůr/BG Ness/India, Himachal Province, Simla to Kulu Valley, Karang
Cotoneaster qungbixiensis	10-0138	ZBPT	Strasbourg/J. Fryer/China, Yunnan, Cang Shan
Cotoneaster roseus	10-0015	Rogów Arboretum	Strasbourg
Cotoneaster rubens	10-0016	Rogów Arboretum	
Cotoneaster salicifolius var. *floccosus*	09-0022	Hohenheim	XX-0-HOH-EG-B-058-16063
Cotoneaster shansiensis	11-0197	USNA	NA:69292
Cotoneaster sikagensis	10-0095	Forest Farm Nursery	
Cotoneaster simonsii	09-0023	Hohenheim	XX-0-HOH-WS-24-5149
Cotoneaster splendens	09-0024	Hohenheim	
Cotoneaster sternianus	09-0025	Hohenheim	XX-0-HOH-EG-B-062-13658
Cotoneaster thymifolius	10-0122	Blue Heron Farm Nursery	
Cotoneaster turbinatus	10-0096	Forest Farm Nursery	
Cotoneaster vandelaarrii	10-0139	ZBPT	Strasbourg/BG Wageningen/China, Yunnan, Hua-Hong, above 2,000 m
Cotoneaster zabelii	09-0027	Hohenheim	XX-0-HOH-EG-B-055-13659

[z]North Willamette Research and Extension Center, Aurora, OR (NWREC); Institute of Superior Agronomy Technical University in Lisbon, Portugal (ISATU); Mlyňany Arboretum at the Slovak Academy of Sciences, Tesárske Mlyňany, Slovakia (Mlyňany Arboretum); Zoological and Botanical Garden of Pilsen, Pilsen, Czech Republic (ZBTP); Blue Heron Farm Nursery, Corvallis, OR; Horticultural Society of Iceland, Reykjavik, Iceland; Botanical Garden, University of Strausbourg, Strausbourg, France (Strasbourg); Rogów Arboretum of Warsaw University of Life Sciences, Warsaw, Poland (Rogów Arboretum); National Botanic Gardens, Dublin, Ireland (Dublin); Forestfarm Nursery, Williams, OR; Botanical Garden of the University of Hohenheim, Germany (Hohenheim); Joseph Fourier Alpine Station of Grenoble University, Col du Lautaret, Grenoble, France (JFAS); Hoyt Arboretum, Portland, OR; U.S. National Arboretum, Washington, DC (USNA).

[y]Wild collections, accession, International Plant Exchange Number (IPEN), and/or other source information reported from gardens on their Index Seminum listing or through direct communication.

[x]*Cotoneaster cooperi* was received as *Cotoneaster griffithii*, which is a synonym.

[w]*Cotoneaster juratana* is not a valid species and we can locate no additional information regarding synonymy.

[v]*Cotoneaster popovii* was received as *Cotoneaster chailaricus*, which is a synonym.

Table 2. Holoploid (2C) and monoploid (1Cx) genome sizes of 67 accessions of *Cotoneaster* determined by flow cytometric analysis of nuclei stained with 4′,6-diamidino-2-phenylindole (DAPI) using *Pisum sativum* 'Ctirad' (2C = 8.76 pg) as an internal standard, inferred ploidy levels based on flow cytometry data, previously reported ploidy, and mean 1Cx value of 10 taxonomic sections studied.

Subgenus	Section	Species or subspecific taxon	Accession	2C (pg)	1Cx (pg)	Inferred ploidy[z]	Reported ploidy[y]	Mean section 1Cx (pg)
Chaenopetalum								
	Alpigeni							0.77
		Cotoneaster ×suecicus 'Coral Beauty'	10-0166	1.53	0.77	2	2	
		Cotoneaster buxifolius	09-0077	2.88	0.72	4[x]	–	
		Cotoneaster cashmiriensis	09-0080	3.06	0.77	4	–	
		Cotoneaster cochleatus	09-0085	3.01	0.75	4	4	
		Cotoneaster congestus	10-0088	2.28	0.76	3	2, 3[w]	
		Cotoneaster dammeri	11-0039	1.73	0.87	2	2, 2[v], 2[u], 2[t]	
		Cotoneaster lidjiangensis	10-0135	3.05	0.76	4[x]	–	
		Cotoneaster procumbens	10-0137	3.09	0.77	4	2[w], 4	
		Cotoneaster thymifolius	10-0122	1.55	0.78	2	2	
	Chaenopetalum							0.81
		Cotoneaster ×watereri	10-0106	2.58	0.86	3	2	
		Cotoneaster bacillaris	09-0073	2.41	0.80	3	2, 3[s], 4[u]	
		Cotoneaster frigidus	09-0045	1.52	0.76	2	2, 2[t]	
	Densiflori							0.80
		Cotoneaster henryanus	09-0017	1.57	0.78	2[x]	2, 2[u]	
		Cotoneaster lacteus	10-0108	3.31	0.83	4	4	
		Cotoneaster pannosus	10-0102	3.06	0.77	4	4	
		Cotoneaster salicifolius var. *floccosus*	09-0022	1.60	0.80	2	2[u], 2[t], 3[u]	
		Cotoneaster turbinatus	10-0096	3.25	0.81	4	4	
	Multiflori							0.78
		Cotoneaster albokermesinus	09-0065	3.08	0.77	4	–	
		Cotoneaster arbusculus	09-0068	3.16	0.79	4	–	
		Cotoneaster astrophoros	11-0034	2.93	0.73	4	3, 4, 5	
		Cotoneaster cooperi	11-0064	1.64	0.82	2	2	
Cotoneaster								
	Acutifolii							0.78
		Cotoneaster acutifolius	09-0047	1.72	0.86	2	2[u], 3[u], 4[s]	
		Cotoneaster acutifolius	10-0126	2.14	0.71	3	2[u], 3[u]	
		Cotoneaster boisianus	09-0074	3.28	0.82	4	4	
		Cotoneaster cinerascens	09-0083	3.13	0.78	4	3, 4[s]	
		Cotoneaster daliensis	10-0129	3.16	0.79	4	4	
		Cotoneaster lucidus	09-0018	2.99	0.75	4	3[u], 4, 4[t]	
		Cotoneaster obscurus	10-0110	3.05	0.76	4	4	
		Cotoneaster sikagensis	10-0095	3.21	0.80	4	3[s]	
	Adpressi							0.79
		Cotoneaster adpressus	10-0157	3.25	0.81	4	2, 2[t], 3[u]	
		Cotoneaster adpressus	10-0124	2.45	0.82	3	2, 2[t], 3[u]	

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Table 2. Continued.

Subgenus	Section	Species or subspecific taxon	Accession	2C (pg)	1Cx (pg)	Inferred ploidy[z]	Reported ploidy[y]	Mean section 1Cx (pg)
		'Tom Thumb'						
		Cotoneaster apiculatus	10-0006	3.30	0.82	4	3[u], 4[s]	
		Cotoneaster atrovirens	09-0072	3.13	0.78	4	–	
		Cotoneaster divaricatus	10-0089	3.05	0.76	4	3[u], 4[v], 4[t]	
		Cotoneaster ganghobaensis	10-0131	3.05	0.76	4	3	
		Cotoneaster horizontalis	10-0104	3.07	0.77	4	4, 4[v], 4[u], 4[t]	
		Cotoneaster melanocarpus	10-0012	2.95	0.74	4	4[s]	
		Cotoneaster milkedandaensis	10-0174	3.34	0.84	4	2	
		Cotoneaster nitens	09-0052	3.06	0.77	4	3[u], 4[s]	
		Cotoneaster praecox	10-0013	3.23	0.81	4	4	
		Cotoneaster simonsii	09-0023	3.18	0.80	4	4, 4[t]	
	Cotoneaster							0.75
		Cotoneaster armenus	09-0069	3.00	0.75	4	4	
		Cotoneaster canescens	09-0079	3.07	0.77	4	4	
		Cotoneaster commixtus	09-0084	2.98	0.75	4	–	
		Cotoneaster fangianus	10-0008	3.00	0.75	4	–	
		Cotoneaster gentianus	10-0132	2.92	0.73	4[x]	–	
		Cotoneaster ignavus	10-0010	2.95	0.74	4	3[u], 4[s]	
		Cotoneaster integerrimus	10-0011	3.03	0.76	4	2[s], 3[u], 4[s]	
		Cotoneaster popovii	09-0081	3.20	0.80	4	–	
		Cotoneaster niger	09-0051	2.90	0.72	4	4[r]	
		Cotoneaster niger	10-0105	1.65	0.82	2	4[s]	
		Cotoneaster shansiensis	11-0197	2.92	0.73	4	3[u], 4, 4[t]	
		Cotoneaster zabelii	09-0027	2.90	0.73	4	3[u], 4, 4[t]	
	Franchetioides							0.78
		Cotoneaster applanatus	09-0067	3.03	0.76	4	4	
		Cotoneaster braydi	09-0076	3.04	0.76	4	4	
		Cotoneaster chungtiensis	09-0082	3.03	0.76	4	3	
		Cotoneaster dielsianus	09-0013	3.11	0.78	4	3[u], 4, 4[s,t]	
		Cotoneaster fastigiatus	10-0130	3.18	0.80	4	–	
		Cotoneaster hypocarpus	10-0133	3.19	0.80	4	4	
		Cotoneaster qungbixiensis	10-0138	3.12	0.78	4	4	
		Cotoneaster splendens	09-0024	3.03	0.76	4	4[s]	
		Cotoneaster sternianus	09-0025	3.23	0.81	4	4, 4[t]	
	Megalocarpi							0.75
		Cotoneaster roseus	10-0015	3.01	0.75	4	3[u], 4[s]	
	Rokujodaisanensis							0.77
		Cotoneaster kweitschoviensis	10-0134	4.71	0.78	6	4	
		Cotoneaster vandelaarrii	10-0139	3.06	0.76	4[x]	4	
	Sanguinei							0.77
		Cotoneaster rubens	10-0016	3.10	0.77	4	3[u]	
Subgenus unknown								
	Section unknown							0.79

Continued next page

Table 2. Continued.

Subgenus	Section	Species or subspecific taxon	Accession	2C (pg)	1Cx (pg)	Inferred ploidy[z]	Reported ploidy[y]	Mean section 1Cx (pg)
		Cotoneaster juratana	10-0171	1.58	0.79	2	–	
				0.32	0.09			NS
		Tukey's HSD minimum significant difference ($\alpha = 0.05$)						

HSD = honestly significant difference.
[z]Ploidy inferred from 2C genome size divided by mean 1Cx genome size (0.778).
[y]Reported ploidy level from Fryer and Hylmö (2009) unless otherwise noted, "–" signifies no previous report of ploidy level.
[x]Ploidy level confirmed by chromosome count.
[w]Darlington and Wylie, 1956.
[v]Jedrzejczyk and Sliwinska, 2010.
[u]Sax, 1954.
[t]Zeilinga, 1964.
[s]Kroon, 1975.
[r]Nybom and Bartish, 2007.

unreduced gamete production in one of the progenitors, an apomictic seedling, or even a self-pollinated seedling from an unreported triploid cytotype of *C. salicifolius*, as triploids often are regarded as facultative apomicts. Another alternative is that the parent plant from which we received seed was diploid and it was accidentally pollinated by a tetraploid, resulting in triploid progeny. There is some evidence against obligate apomixis in *C.* ×*watereri*, as Fryer and Hylmö (2009) describe five cultivars, a level of diversity that would not be expected if the species was an obligate apomict. Both Zeilinga (1964) and Kroon (1975) reported that all species were diploid or tetraploid and triploids only arise through hybridization. To determine if that assertion is supported by our study, a detailed morphological or molecular investigation of species and hybrids would be required, which is beyond the scope of the current research. In five other species, our data indicated a different cytotype than reported by Fryer and Hylmö (2009). These include *Cotoneaster ganghobaensis*, *C. kweitschoviensis*, *Cotoneaster milkedandaensis*, *Cotoneaster niger*, and *Cotoneaster sikagensis*. The differing cytotypes we report do not suggest consistent error in either direction (over or underestimates) and include two 4*x* that were reportedly 3*x*, one 6*x* previously reported as 4*x*, one 4*x* previously reported as 2*x*, and one 2*x* previously reported as 4*x* (Table 2). A larger screening with more accessions representing each species would likely uncover more ploidy series within other species as well as identifying more examples for which new findings would differ from previous reports. Discrepancies in ploidy between previous papers and our findings are due to testing different sources or accessions of material, some of which may have arisen from hybridization.

When looking across taxonomic divisions, variation in ploidy level was observed in the subgenera and within many sections (Table 2). The 1Cx genome size ranged from 0.71 pg of DNA for *C. acutifolius* (10-0126) to 0.87 pg of DNA for *Cotoneaster dammeri*. When compared across all taxa, monoploid genome sizes showed a detectable difference ($P < 0.05$); however, when these differences were examined by taxonomic division, they were insignificant ($P \geq 0.05$). Since we did not observe differences between taxonomic groups, we infer that our mean monoploid genome size can be used to calibrate our ploidy estimations in future investigations. In addition, when compared with other genera like *Magnolia*, which has observable differences in monoploid genome size between taxonomic sections (Parris et al., 2010), *Cotoneaster* has undergone relatively little divergence in chromosome size.

DAPI genome size estimates generally were larger than PI with differences ranging from 0.03 to 0.14 pg (Table 3). Fifteen of 17 accessions were different ($P < 0.05$) using respective fluorochromes and four accessions had higher significance ($P < 0.0001$). Doležel et al. (1992) reported significant differences ($P < 0.01$) for five genera in four families including four genera for which DAPI overestimated genome size from 11% to 30% compared with PI and one species for which DAPI underestimated genome size by nearly 27%. Our calculations show that bp composition in *Cotoneaster* ranges from 58.4% to 60.8% AT (Table 3), which agrees with estimates of 59.2% to 61.1% AT for three genera from Rosaceae (Meister and Barrow, 2002). We found no relationship between ploidy level (thus genome size) and AT%, a finding consistent with previous reports in Rosaceae (Meister and Barrow, 2002). Interestingly, we observed a trend that the lower the AT%, the greater the overestimation of genome size using DAPI compared with PI.

Table 3. Monoploid (1Cx) genome sizes determined by analysis of nuclei stained with 4′,6-diamidino-2-phenylindole (DAPI) or propidium iodide (PI) using *Pisum sativum* 'Ctirad' (2C = 8.76 pg) as an internal standard, the difference between genome size estimates between fluorochromes and bp composition of 17 taxa of *Cotoneaster*.

Taxa	Accession	Ploidy	1Cx (pg) DAPI	1Cx (pg) PI	*P* value[z]	DAPI – PI	AT (%)
Cotoneaster ×*suecicus* 'Coral Beauty'	10-0166	2	0.77	0.74	0.096	0.03	60.8
Cotoneaster adpressus	10-0157	4	0.81	0.75	0.052	0.06	60.2
Cotoneaster applanatus	09-0067	4	0.76	0.68	0.021	0.08	59.7
Cotoneaster arbusculus	09-0068	4	0.79	0.71	0.001	0.08	59.7
Cotoneaster bacillaris	09-0073	3	0.80	0.69	0.004	0.11	58.9
Cotoneaster boisianus	09-0074	4	0.82	0.68	<0.001	0.14	58.4
Cotoneaster canescens	09-0079	4	0.77	0.64	<0.001	0.13	58.4
Cotoneaster chungtiensis	09-0082	4	0.76	0.70	<0.001	0.05	60.2
Cotoneaster cinerascens	09-0083	4	0.78	0.68	0.003	0.11	59.0
Cotoneaster cochleatus	09-0085	4	0.75	0.69	0.018	0.07	59.9
Cotoneaster frigidus	09-0045	2	0.76	0.70	0.032	0.06	60.1
Cotoneaster milkedandaensis	10-0174	4	0.84	0.74	0.011	0.10	59.3
Cotoneaster roseus	10-0015	4	0.75	0.70	0.049	0.05	60.2
Cotoneaster sikagensis	10-0095	4	0.80	0.70	0.002	0.11	59.0
Cotoneaster splendens	09-0024	4	0.76	0.69	0.025	0.07	59.9
Cotoneaster thymifolius	10-0122	2	0.78	0.72	0.017	0.06	60.2
Cotoneaster zabelii	09-0027	4	0.73	0.64	<0.001	0.08	59.5

[z]1Cx genome size estimates within taxon were compared between fluorochromes using a paired *t* test ($\alpha = 0.05$).

Even though Parris et al. (2010) found that DAPI underestimated genome size for *Magnolia*, both of our studies show the same trend: increasing AT% results in a lower DAPI estimate compared with PI. Although the trends were similar, we observed overestimation using DAPI, whereas Parris et al. (2010) observed underestimation, even though AT% was similar, albeit higher in their study. A possible source of variation in our study was using different types of tissue in our samples (young stems and vegetative buds) and pea standard (young, expanded leaves). It is possible this resulted in variation in chromatin structure, which would affect the amount of unstainable DNA (Doležel et al., 1992).

Although our choice of fluorochrome influenced genome size estimates, most differences were not large enough to affect ploidy estimation. However, there could be some confusion for several species included in our analysis. For instance, *Cotoneaster boisianus* showed a 0.14 pg difference in monoploid genome size estimate between fluorochromes. Using the estimate from PI of 2.72, the inferred ploidy level would calculate as 3.5*x*. Without additional information provided from cytological analysis, accurate ploidy assignment may be challenging and sample readings could erroneously be interpreted as aneuploid. DAPI is less expensive, uses less toxic compounds, and often resulted in lower CV for mean nuclei fluorescence than PI for *Cotoneaster*. Overall, we consider DAPI acceptable for our purposes in an applied breeding program.

From our chromosome counts in seven species, we found one diploid accession and six tetraploid accessions (4*x* *Cotoneaster vandelarii* not shown; Fig. 1). These ploidy estimates matched our results from flow cytometry for the five species that were examined by both methods. Overall, when our results were compared with literature for both cytology and flow cytometry, most were in agreement. Where there is conflict in the reports, the conflict may be from the way that the ploidy reports have been generated. Sax (1954) conducted estimations via chromosome counting in pollen mother cells and Zeilinga (1964) found several conflicting reports when root tips were examined. Zeilinga suggested that with polyploids, the chromosome pairing observed in pollen mother cells was crowded and led to confusion in counting. With a relatively high base chromosome number and common occurrence of polyploidy (102 chromosomes in hexaploids), it is possible that previous chromosome counts included errors. Determining ploidy level by counting chromosomes in *Cotoneaster* was time consuming and difficult, while flow cytometry proved to be much faster and accurate.

Thus far, taxonomic organization in *Cotoneaster* has largely failed to incorporate molecular data and has relied on morphology and species provenance, although a report by Bartish et al. (2001) using randomly amplified polymorphic DNA supported the recognition of subgenera. Because of the number of species within the genus and the difficulty in organizing *Cotoneaster*, we hope fundamental information on genome size, ploidy level, and bp composition may give others insight to the relationship among the species. However, the results of this study do not show a relationship of chromosome size to current taxonomic organization, as monoploid genome sizes did not appear to be linked to taxonomic division.

This work may be useful to breeders for predicting success of interspecific hybridization and fertility of F_1 populations. Along with other factors, similarity in chromosome size contributes to functional meiosis and bivalent pairing between genomes. In this study, monoploid genome sizes varied up to 23% among species. In *Rudbeckia*, a hybrid was recovered when there was a difference in genome size of >300% (Palmer et al., 2009). The much smaller range in *Cotoneaster* suggests variation in monoploid genome size, thus chromosome size, is not expected to hinder interspecific hybridization. Furthermore, we have successfully performed several intersubgeneric and interploidy crosses including *Cotoneaster* ×*suecicus* 'Coral Beauty' (2*x*, 2C = 1.53 pg, subgenus *Chaenopetalum*) × *Cotoneaster splendens* (4*x*, 2C = 3.03 pg, subgenus *Cotoneaster*) that resulted in a triploid hybrid, which was confirmed using flow cytometry (2C = 2.42 pg), thus supporting broad compatibility in the genus. Breeding programs should conduct ploidy analysis for each accession included in a germplasm

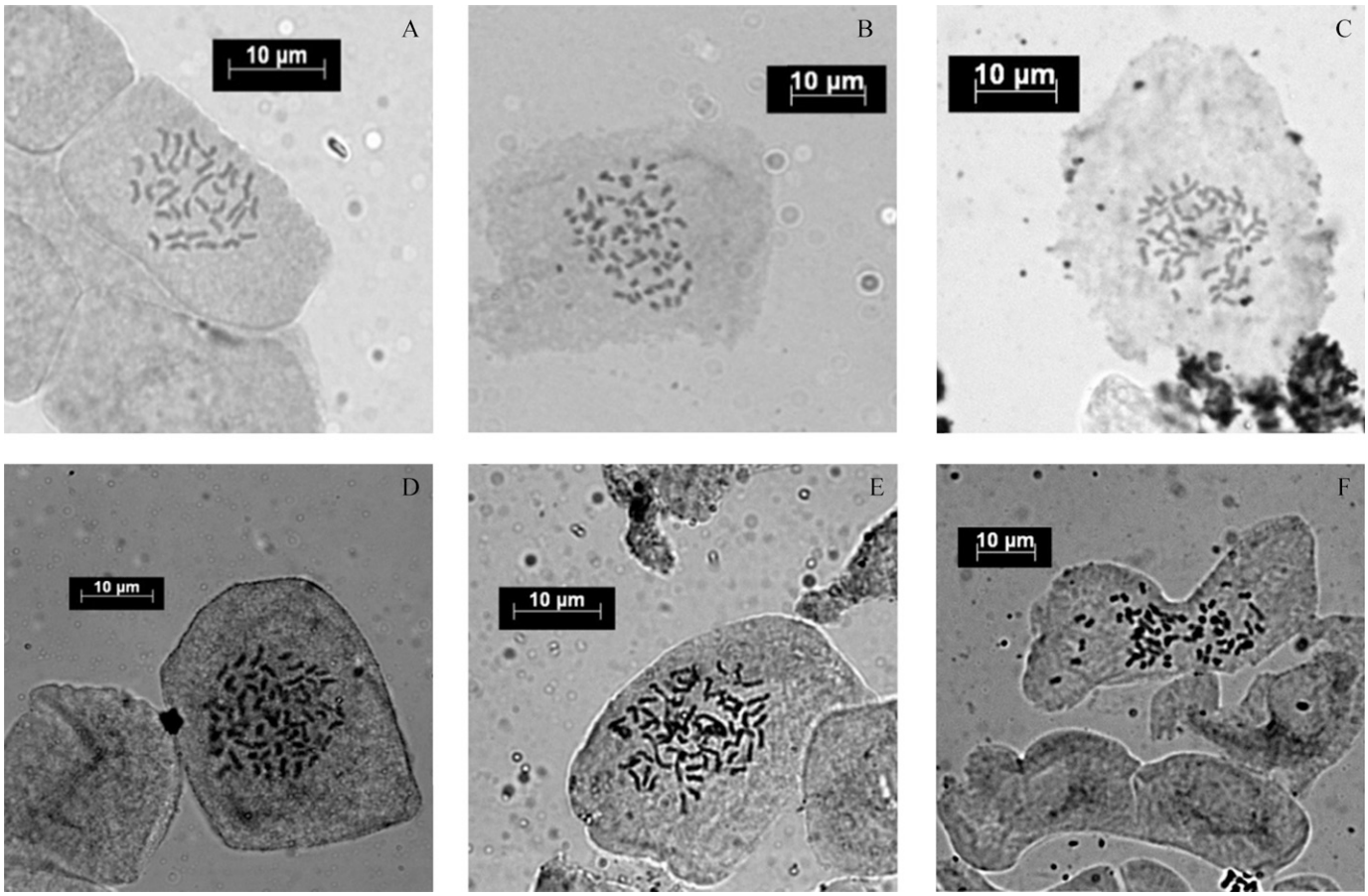

Fig. 1. Photomicrographs of six *Cotoneaster* accessions including (**A**) a diploid ($2n = 2x = 34$) *Cotoneaster henryanus*, and (**B**) five tetraploids ($2n = 4x = 68$) *Cotoneaster buxifolius*, (**C**) *Cotoneaster vandelarii*, (**D**) *Cotoneaster hebephyllus*, (**E**) *Cotoneaster lidjiangensis*, and (**F**) *Cotoneaster poluninii*. Cells were prepared from root tips and chromosomes were stained using modified carbol fuchsin.

collection without relying on previous reports. We expect the broader the survey among species from various sources, the more examples of ploidy series will be found. Nevertheless, we have demonstrated the utility of apomictic polyploids for breeding when used as pollen parents.

Taxonomy of *Cotoneaster* is challenging due to morphological similarities, the propensity for hybridization, and the presence of apomixis. The tangled issue of separating and correctly identifying these species is emphasized by Dirr (2009) who stated, "*Cotoneaster* identification is not easy with 400 species, many possibly the result of hybridization, subsequent apomixis, which leads to microspecies that essentially reproduce vegetatively via seed." Our study relied heavily on material obtained through Index Seminum and here we present the material labeled as we received it. Because of the presence of apomixis, particularly among tetraploids, we have strong confidence in the identification of most species presented. It is worth noting that materials in our study were not wild collected as one may expect in a classical floristic study but our findings should be quite relevant to applied plant breeders or others studying cultivated material of *Cotoneaster* or Maloideae.

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