

Chromosome number and genome size variation in *Colocasia* (Araceae) from China

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Abstract Chromosome number and genome size are important cytological characters that significantly influence various organismal traits. We investigated chromosome number and genome size variation in 73 accessions belonging to four *Colocasia* species from China. Five different chromosome counts ($2n=26, 28, 38, 42,$ and 56) were found, the largest one representing a new record in *Colocasia*. The basic chromosome numbers are $x=13, 14,$ and $19,$ corresponding to $2x, 3x,$ and $4x$ cytotypes. Yunnan Province, China is considered the center of *Colocasia* polyploid origin. The $2C$ values in our accessions ranged from 3.29 pg in *C. gigantea* to 12.51 pg in *C. esculenta*. All species exhibit inter- and intraspecific chromosomal variation. Differences in DNA content among the *Colocasia* species

seem to have occurred by chromosomal gain under similar habitats. Polyploidization also obviously contributes to $2C$ value variation.

Keywords Chromosome number · *Colocasia* · Genome size · Polyploidy

Introduction

The genus *Colocasia* Schott (Araceae) contains taxa of edible, medicinal, ornamental, and cultural importance, of which taro (*Colocasia esculenta* (L.) Schott) is probably the best known (Ahmed 2014; Das and Das 2014; Matthews 2006). It comprises about 20 species, mainly distributed in tropical and subtropical Asia and Oceania (Li and Boyce 2010), with six species endemic to Yunnan Province, China. Yunnan appears to be the main diversity center for the genus (Cao and Long 2004; Li and Boyce 2010).

The relevance of karyological study to the knowledge of the systematics and evolution of *Colocasia* has long been noted. Previous cytological investigation has reported the basic chromosome number to be $x=14,$ and to be constant throughout the genus (Yang et al. 2003). Other studies have claimed that chromosomal variation corresponds to different ploidy levels plus several aneuploids (Cao and Long 2004; Chakraborty and Bhattacharya 1984; Huang et al. 2012; Kuruvilla and Singh 1981; Petersen 1989; Sreekumari and Mathew 1991a, b). Furthermore, some authors claim that, in addition to constancy of basic chromosome number, *Colocasia* species also display a morphological uniformity of chromosomes and a homogeneous karyotype arrangement (Kuruvilla and Singh 1981). However, others have found enough interspecific karyotype differences to allow species characterization (Coates et al. 1988; Yang

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et al. 2003). Such discrepancies have also been observed at the intraspecific level, mainly in the widely studied *C. esculenta* (L.) Schott. (Das and Das 2014; Nakayama et al. 2008; Parvin et al. 2008; Sreekumari and Mathew 1991a, b). Previous karyological studies have predominantly concentrated on *Colocasia* members from regions outside of China, and have been limited to chromosome number; thus there is a paucity of data for Chinese species so far.

In addition to chromosome number and ploidy level, genome size (nuclear DNA content) also provides useful information in many fields of plant biology, including systematics, evolution, and conservation (Bennett and Leitch 2005). Nuclear DNA content has previously been reported for only two *Colocasia* species. Bennett MD and Smith JB (unpublished data 1987) determined $2C=6.65$ pg in *C. antiquorum*, and Baum A, Kenton AY and Bennett MD (pers. commun. 1989) found $2C=8.15$ pg in *C. esculenta* (L.) Schott. *Colocasia esculenta* was also investigated by Das and Das (2014), who observed $4C$ values ranging from 7.24 to 18.24 pg in 10 cultivars. In our present study, four species of *Colocasia* from China were examined in greater detail than so in prior reports. We report the variation in ploidy level, chromosome number and morphology, and DNA content among different populations.

Materials and methods

Plant material

In total, 73 individual plants belonging to four *Colocasia* species from China were included in our study (Table 1; Fig. 1). The number of individuals per species varied from five to 44. Living plants were cultivated in a greenhouse at the Kunming Institute of Botany. Voucher specimens were deposited in Herbarium, Kunming Institute of Botany, Chinese Academy of Sciences.

Chromosome number

Root tips were collected from each individual and pretreated with a solution of 0.002 mol/L 8-hydroxyquinoline at 20–21 °C for 4–5 h. After fixation for 50 min by Carnoy solution (3:1 ethanol:acetic acid) at 4 °C, the root tips were dissociated in a mixture of 1 N HCl and 45% acetic acid (1:1) at 60 °C for 30 s, stained with 1% acetic orcein for 2–3 h and squashed on a glass slide (Wang et al. 2013).

Chromosome numbers were determined for each accession from at least 50 cells of at least two seedlings for mitotic observations. Mitotic interphase nuclei and prophase chromosomes preparations followed Tanaka (1971, 1977, 1987), and the designation of the centromeric

position follows Levan et al. (1964). Karyotype asymmetry was classified according to Stebbins (1971).

Determining 2C values

About 0.5 cm² of leaf material was finely diced using a new razor blade in a Petri dish containing 1500–2000 µL of WPB nuclear solution buffer (0.2 mol L⁻¹ Tris-HCl, 4 mmol L⁻¹ MgCl₂·6H₂O, 2 mmol L⁻¹ EDTA Na₂·2H₂O, 86 mmol L⁻¹ NaCl, 10 mmol L⁻¹ Na₂S₂O₅, 1% PVP-10, 1% (v/v) Triton X-100, pH 7.5) (Tian et al. 2011). The nuclear suspension was then filtered through disposable filters (30 µm) to remove cell debris, and then stained with 150 µL propidium iodide (PI) [50 µg mL⁻¹; including RNase (500 µg mL⁻¹)] for 10 min. Samples were analyzed on a CyFlow Space (Partec, Münster, Germany) flow cytometer equipped with a blue laser operating at 488 nm. At least 5000 nuclei were measured for all samples. FlowMax ver. 2.82 was used to analyze the resulting histograms. *Z. mays* (B73) ($2C=5.43$ pg) was chosen as an internal standard. The $2C$ value of a sample was calculated as follows: (mean of sample peak/mean of standard peak) × $2C$ value (pg) of the standard species (Table 1).

Results

Chromosome number and ploidy level

Chromosome number and ploidy level for all samples are listed in Table 1. All accessions were karyologically analyzed (supplementary figure). Five different chromosome numbers were identified ($2n=26, 28, 38, 42,$ and 56). Most of these are $x=14$, which we regard to be the genuine basic chromosome number. Plants with 26 and 38 somatic chromosomes have previously been found in three *Colocasia* species (Onwueme 1978; Chaudhuri and Sharma 1979); *C. gigantea* and *C. esculenta* have $2n=26$, and *C. antiquorum* has $2n=38$. It appears that two other basic numbers ($x=13$ and 19) have also occurred in the genus.

Genome size variation

Genome size was analyzed in 73 accessions of the genus *Colocasia* (Table 1). The $2C$ DNA content ranged from 3.29 pg for the diploid population of *C. gigantea* to 12.51 pg for the triploid sample of *C. esculenta*. The $1Cx$ value, which indicates DNA content per genome, ranged from 1.55 pg in *C. antiquorum* to 5.72 pg in *C. antiquorum*. Diploid species showed $2C$ values between 3.29 and 11.43 pg. Triploid species ranged from 4.65 to 12.51 pg, and the only tetraploid taxon (*C. esculenta*) had a $2C$ value of 6.56 pg. The dispersion diagram in Fig. 2 shows the

Table 1 Voucher information, cytological characteristics, DNA content for *Colocasia* accessions used in this study

No.	Taxon	Locality	Voucher	Ratio LC/SC	CL (µm)	Mean ± SD	AI	Type	Karyotype formula	2C value (pg)	1Cx value (pg)
1	<i>C. fallax</i>	Ganba Village, Dadu'gang Country-side, Jinghong, Yunnan	1-1	2.21	2.68 (±0.33)		1.83	2B	2n = 3x = 42 = 3M + 29m + 9sm + 1st	7.19	2.40
2		Mengxing-Mengyuan road, Mengla, Xishuangbanna, Yunnan	18-5	2.41	2.75 (±0.49)		1.70	1B	2n = 3x = 42 = 2M + 36m + 4sm	6.59	2.20
3		Xinzhai, Mengku Town, Shuangjiang, Lincang, Yunnan	111	3.21	3.46 (±0.78)		2.34	2B	2n = 3x = 42 = 1M + 36m + 5sm	8.19	2.73
4		Ji'nuoshan Village, Jinghong, Yunnan	10-3	2.73	3.67 (±0.90)		2.90	2B	2n = 2x = 28 = 21m + 7sm	6.10	3.05
5		Dongfeng Farm, Man'na'nu, Menggong Town, Jinghong, Yunnan	61	2.01	2.53 (±0.44)		1.73	1B	2n = 2x = 28 = 1M + 23m + 4sm	4.44	2.22
6		Reservoir, Baixiang Mountain, Mengban Town, Mengla, Xishuangbanna, Yunnan	53-5	2.08	3.76 (±0.73)		2.83	2B	2n = 2x = 28 = 3M + 20m + 5sm	4.17	2.09
7		Menglun-Mengyuan road, Mengla, Xishuangbanna, Yunnan	13-1	2.30	3.19 (±0.60)		2.39	2B	2n = 2x = 28 = 5M + 20m + 3sm	7.16	3.58
8		Mengxing-Mengyuan road, Mengla, Xishuangbanna, Yunnan	27-1	1.83	2.93 (±0.45)		1.26	1A	2n = 2x = 28 = 5M + 23m	5.18	2.59
9		Mengla-Bupan road, Mengla, Xishuangbanna, Yunnan	33-4	2.01	2.89 (±0.59)		2.40	2B	2n = 2x = 28 = 1M + 20m + 7sm	4.88	2.44
10		Mangonyun Road, Jinghong, Yunnan	71-5	3.00	3.70 (±0.93)		3.17	2B	2n = 2x = 28 = 2M + 20m + 6sm	4.84	2.42
11		Guangming Village, Mengban Town, Mengla, Xishuangbanna, Yunnan	48-10	2.09	3.44 (±0.62)		1.58	1B	2n = 2x = 28 = 2M + 24m + 2sm	5.72	2.86
12	<i>C. gigantea</i>	Mengxing, Mengla, Xishuangbanna, Yunnan	16-7	2.16	3.49 (±0.61)		2.23	2B	2n = 2x = 28 = 1 + 21m + 6sm	3.29	1.65
13		Bupan Village, Mengla, Xishuangbanna, Yunnan	39-1	3.02	3.72 (±0.63)		2.65	2B	2n = 2x = 28 = 4M + 14m + 10sm	5.55	2.78
14		Ganba Village, Dadugang Country-side, Jinghong, Yunnan	2-11	2.28	3.36 (±0.55)		1.62	2B	2n = 2x = 28 = 3M + 21m + 4sm	6.75	3.38
15		Dongfeng Farm, Man'na'nu, Menggong Town, Jinghong, Yunnan	64-2	1.81	4.01 (±0.64)		1.94	2A	2n = 2x = 28 = 5M + 20m + 3sm	6.31	3.16
16		Heping Village, Mengxian Town, Ning'er, Pu'er, Yunnan	119-1	2.00	3.79 (±0.73)		1.80	2B	2n = 2x = 26 = 23m + 3sm	4.82	2.41
17	<i>C. esculenta</i>	Jinuoshan Countryside, Jinghong, Yunnan	3-6	1.86	3.57 (±0.58)		2.22	2A	2n = 3x = 42 = 5M + 28m + 9sm	6.54	2.18
18		Menglong-Ga'sa Town, Jinghong, Yunnan	70-3	2.00	3.24 (±0.57)		2.83	2B	2n = 3x = 42 = 8M + 22m + 12sm	7.59	2.53
19		Mengsong Village, Damenglong, Jinghong, Yunnan	74-16	2.54	3.67 (±0.86)		2.24	2B	2n = 3x = 42 = 1M + 36m + 5sm	9.82	3.27
20		Manjiaiao Village, Mengsong, Menggong Town, Jinghong, Yunnan	82-1	2.08	3.21 (±0.59)		2.23	2B	2n = 2x = 28 = 17m + 11sm	5.48	2.74

Table 1 (continued)

No.	Taxon	Locality	Voucher	Ratio LC/SC	CL (μm)	Mean \pm SD	AI	Type	Karyotype formula	2C value (pg)	1Cx value (pg)
21		Guangming Village, Mengban Town, Mengla, Xishuangbanna, Yunnan	44-1	1.62	3.09 (± 0.39)	1.26 (± 0.39)	1A	2n=2x=28=1M+23m+4sm	5.49	2.75	
22		Guangming Village, Mengban Town, Mengla, Xishuangbanna, Yunnan	51-6	2.19	3.16 (± 0.59)	2.49 (± 0.59)	2B	2n=3x=42=2M+31m+9sm	6.55	2.18	
23		Kami Village, Mengban Town, Mengla, Xishuangbanna, Yunnan	56-8	2.81	3.67 (± 0.75)	2.09 (± 0.75)	2B	2n=3x=42=1M+33m+8sm	6.22	2.07	
24		Man'en Village, Mengzhe Town, Menghai, Xishuangbanna, Yunnan	86-6	1.85	3.50 (± 0.59)	1.49 (± 0.59)	1A	2n=2x=28=24m+4sm	5.24	2.62	
25		Huimin Countryside, Lancang, Pu'er, Yunnan	94-4	2.08	3.03 (± 0.49)	1.72 (± 0.49)	2B	2n=3x=42=5M+34m+3sm	7.41	2.47	
26		Ayunzhai, Lancang, Pu'er, Yunnan	97-2	2.18	3.06 (± 0.56)	1.69 (± 0.56)	1B	2n=3x=42=2M+35m+5sm	6.75	2.25	
27		Mohei Town, Ning'er, Pu'er, Yunnan	127-7	2.19	3.14 (± 0.58)	2.12 (± 0.58)	2B	2n=3x=42=1M+31m+10sm	7.81	2.60	
28		Shifodong Village, Gengma, Lincang, Yunnan	107-6	2.27	3.99 (± 0.80)	3.05 (± 0.80)	2B	2n=2x=28=4M+17m+7sm	6.13	3.07	
29		Menglong-Ga'sa Town, Jinghong, Yunnan	69-2	1.86	3.61 (± 0.57)	2.09 (± 0.57)	2A	2n=2x=28=16m+12sm	5.68	2.84	
30		Dongfeng Farm, Man'na'nu, Menggong, Jinghong, Yunnan	65-5	2.09	3.14 (± 0.62)	1.64 (± 0.62)	2B	2n=2x=28=3M+25m	4.44	2.22	
31		Manjiaojiao Village, Mengsong, Menggong Town, Jinghong, Yunnan	80-1	2.07	3.96 (± 0.79)	2.62 (± 0.79)	2B	2n=3x=42=3M+34m+5sm	7.62	2.54	
32		Jinuo Mountain Countryside, Jinghong, Yunnan	8-6	2.73	3.74 (± 0.70)	2.98 (± 0.70)	2B	2n=3x=42=3M+21m+18sm	5.57	1.86	
33		Jinuo Mountain Countryside, Jinghong, Yunnan	9-3	2.41	3.18 (± 0.57)	1.54 (± 0.57)	2B	2n=2x=26=3M+23m	5.09	2.55	
34		Man'en Village, Mengzhe Town, Menghai, Xishuangbanna, Yunnan	88-9	2.31	3.78 (± 0.76)	2.27 (± 0.76)	1B	2n=2x=28=2M+21m+5sm	6.43	3.22	
35		Man'en Village, Mengzhe Town, Menghai, Xishuangbanna, Yunnan	85-17	2.00	3.23 (± 0.53)	1.78 (± 0.53)	2B	2n=3x=42=3M+34m+5sm	6.83	2.28	
36		Xinghuo Village, Menghai, Xishuangbanna, Yunnan	92-1	2.01	2.70 (± 0.48)	1.70 (± 0.48)	1B	2n=2x=28=4M+21m+3sm	5.70	2.85	
37		Mengxing-Mengyuan road, Mengla, Xishuangbanna, Yunnan	21-2	2.00	3.97 (± 0.72)	2.84 (± 0.72)	2B	2n=2x=28=1M+19m+8sm	5.46	2.73	
38		Mengxing-Mengyuan road, Mengla, Xishuangbanna, Yunnan	17-1	2.87	2.46 (± 0.62)	3.22 (± 0.62)	2B	2n=3x=42=5M+28m+9sm	5.55	1.85	
39		Mengxing-Mengyuan road, Mengla, Xishuangbanna, Yunnan	19-3	2.37	3.19 (± 0.68)	2.43 (± 0.68)	2B	2n=3x=42=4M+31m+7sm	6.62	2.21	
40		Mengla Farm, Mengla, Xishuangbanna, Yunnan	28-4	2.14	3.55 (± 0.68)	1.79 (± 0.68)	2B	2n=2x=28=1M+26m+1sm	5.32	2.66	
41		Bupan Village, Mengla, Xishuangbanna, Yunnan	43-2	2.17	3.41 (± 0.67)	1.91 (± 0.67)	2B	2n=2x=28=5M+21m+2sm	7.02	3.51	

Table 1 (continued)

No.	Taxon	Locality	Voucher	Ratio LC/SC	CL (µm)	Mean ± SD	AI	Type	Karyotype formula	2C value (pg)	1Cx value (pg)
42		Bupan Village, Mengla, Xishuangbanna, Yunnan	36-8	2.50	3.52 (±0.76)	2.57	2B	2n = 3x = 42 = 3M + 32m + 7sm	10.27	3.42	
43		Kami Village, Mengban Town, Mengla, Xishuangbanna, Yunnan	55-2	3.08	4.59 (±1.09)	3.85	2B	2n = 2x = 28 = 1M + 17m + 10sm	5.75	2.79	
44		Guangming Village, Mengban Town, Mengla, Xishuangbanna, Yunnan	52-4	2.51	2.94 (±0.67)	2.99	2B	2n = 3x = 42 = 2M + 30m + 10sm	7.18	2.39	
45		Mengla, Xishuangbanna, Yunnan	60-1	2.13	2.61 (±0.45)	2.21	2B	2n = 3x = 42 = 5M + 27m + 10sm	7.19	2.40	
46		Heping Village, Mengxian Town, Ning'er, Pu'er, Yunnan	121-3	2.31	3.11 (±0.65)	2.37	2B	2n = 2x = 28 = 5M + 20m + 3sm	4.89	2.45	
47		Heping Village, Mengxian Town, Ning'er, Pu'er, Yunnan	122-5	1.84	2.92 (±0.47)	2.43	2A	2n = 3x = 42 = 2M + 28m + 12sm	12.51	4.17	
48		Tabacco, Heping Village, Mengxian Town, Ning'er, Pu'er, Yunnan	124-2	2.75	3.80 (±0.91)	4.30	2B	2n = 3x = 42 = 5M + 24m + 13sm	9.63	3.21	
49		Huimin Countryside, Lancang, Pu'er, Yunnan	93-5	2.91	3.45 (±0.66)	1.90	1B	2n = 3x = 42 = 2M + 32m + 8sm	6.02	2.01	
50		Tangsheng Village, Menglang Town, Lancang, Pu'er, Yunnan	95-3	2.31	2.75 (±0.49)	1.82	1B	2n = 3x = 42 = 5M + 34m + 3sm	5.08	1.69	
51		Xiaoxinzhai, Xiayun Countryside, Lancang, Pu'er, Yunnan	98-3	2.17	3.25 (±0.66)	2.21	2B	2n = 3x = 42 = 3M + 36m + 3sm	7.73	2.58	
52		Xiaoxinzhai, Xiayun Countryside, Lancang, Pu'er, Yunnan	99-7	2.11	2.53 (±0.48)	1.76	2B	2n = 4x = 56 = 5M + 47m + 4sm	6.56	1.64	
53		Wuliang Mountain Town, Nanrun, Dali, Yunnan	116-1	2.07	3.64 (±0.61)	2.14	2B	2n = 3x = 42 = 1M + 31m + 10sm	7.53	2.51	
54		Gonglang, Wuliang Mountain Town, Nanrun, Dali, Yunnan	115-10	1.93	3.44 (±0.61)	1.96	2A	2n = 2x = 28 = 1M + 22m + 5sm	5.81	2.91	
55		Yinjie Town, Midu, Dali, Yunnan	118-4	2.84	3.43 (±0.72)	3.01	2B	2n = 3x = 42 = 1M + 33m + 7sm + 1st	8.78	2.93	
56		Gengma, Lincang, Yunnan	103-1	2.08	3.32 (±0.68)	2.55	2B	2n = 2x = 28 = 19m + 9sm	5.77	2.89	
57		Genggang Road, Gengma, Lincang, Yunnan	105-2	2.09	3.28 (±0.57)	2.05	2B	2n = 3x = 42 = 3M + 32m + 7sm	9.41	3.14	
58		Mankan Village, Gengma, Lincang, Yunnan	106-3	2.10	3.07 (±0.54)	2.10	2B	2n = 3x = 42 = 2M + 32m + 8sm	5.05	1.68	
59		Xinzhai, Mengku Town, Shuangjiang, Lincang, Yunnan	110-1	1.93	3.35 (±0.53)	1.60	2A	2n = 3x = 42 = 33m + 9sm	9.70	3.23	
60		Jianshui, Honghe, Yunnan	BT-08-6	2.21	2.97 (±0.50)	1.84	1B	2n = 3x = 42 = 3M + 32m + 7sm	7.62	2.54	

Table 1 (continued)

No.	Taxon	Locality	Voucher	Ratio LC/SC	CL (μm)	Mean \pm SD	AI	Type	Karyotype formula	2C value (pg)	1Cx value (pg)
61	<i>C. antiquorum</i>	Mengxing-Mengyuan road, Mengla, Xishuangbanna, Yunnan	22-1	2.61	3.23 (± 0.74)	2.68	2B	$2n = 3x = 42 = 4M + 32m + 6sm$	7.60	2.53	
62		Jinuo Mountain Countryside, Jinghong, Yunnan	5-3	2.51	2.98 (± 0.67)	2.41	2B	$2n = 3x = 42 = 3M + 32m + 7sm$	5.64	1.88	
63		Mengxing-Mengyuan road, Mengla, Xishuangbanna, Yunnan	20-7	2.50	3.04 (± 0.69)	2.73	2B	$2n = 3x = 42 = 30m + 12sm$	7.63	2.54	
64		Mengxing-Mengyuan road, Mengla, Xishuangbanna, Yunnan	24-5	2.31	3.02 (± 0.64)	2.21	1B	$2n = 2x = 28 = 8M + 19m + 1sm$	5.29	2.65	
65		Mengxing-Mengyuan road, Mengla, Xishuangbanna, Yunnan	26-2	2.34	3.21 (± 0.81)	3.25	2B	$2n = 2x = 28 = 4M + 20m + 4sm$	4.75	2.38	
66		Mengla-Bupan Village road, Mengla, Xishuangbanna, Yunnan	32-4	2.11	2.83 (± 0.47)	1.11	1B	$2n = 2x = 28 = 4M + 24m$	4.21	2.11	
67		Kami Village, Mengban Town, Mengla, Xishuangbanna, Yunnan	58-2	2.14	3.96 (± 0.69)	2.73	2B	$2n = 3x = 42 = 4M + 29m + 8sm + 1st$	6.83	2.28	
68		Kami Village, Mengban Town, Mengla, Xishuangbanna, Yunnan	59-3	3.27	2.66 (± 0.89)	4.31	2B	$2n = 3x = 42 = 9M + 28m + 5sm$	4.65	1.55	
69		Yongde, Cangyuan, Lincang, Yunnan	100-2	1.86	3.63 (± 0.58)	1.90	1A	$2n = 2x = 28 = 1M + 20m + 7sm$	4.69	2.35	
70		Xinzhai, Mengku Town, Shuangjiang, Lincang, Yunnan	109-1	2.34	3.65 (± 0.74)	1.78	1B	$2n = 3x = 42 = 8M + 32m + 2sm$	9.00	3.00	
71		Yunda Road, Menglong-Gasa Town, Jinghong, Yunnan	67-2	1.64	3.19 (± 0.46)	1.63	1A	$2n = 2x = 28 = 5M + 20m + 3sm$	5.79	2.90	
72		Jinuo Mountain Countryside, Jinghong, Yunnan	6-2	2.25	3.28 (± 0.60)	2.14	1B	$2n = 3x = 42 = 5M + 30m + 7sm$	8.64	2.88	
73		Heping Village, Mengxian Town, Ning'er, Pu'er, Yunnan	123-1	1.91	3.01 (± 0.48)	1.73	1B	$2n = 2x = 38 = 6M + 29m + 3sm$	11.43	5.72	

AI asymmetry index according to Paszko (2006), CL mean length of chromosome, LC longest chromosome length, SC shortest chromosome length, SD standard deviation, M median, m metacentric, sm submetacentric, st subtetacentric, Type classification of karyotypes in relation to their degree of asymmetry according to Stebbins (1971)

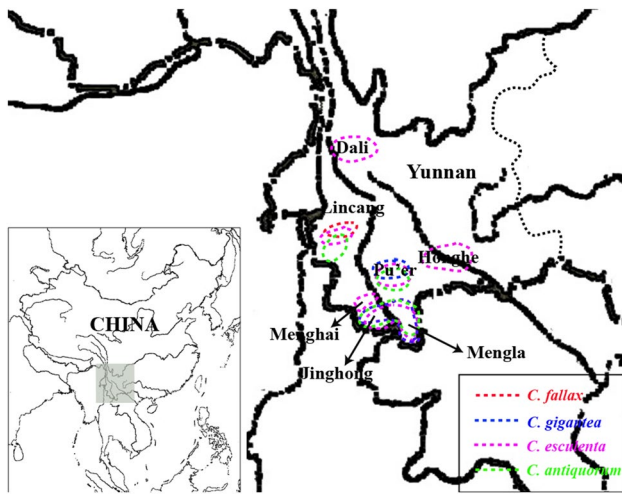


Fig. 1 Distribution of *Colocasia* in Yunnan based on field collections

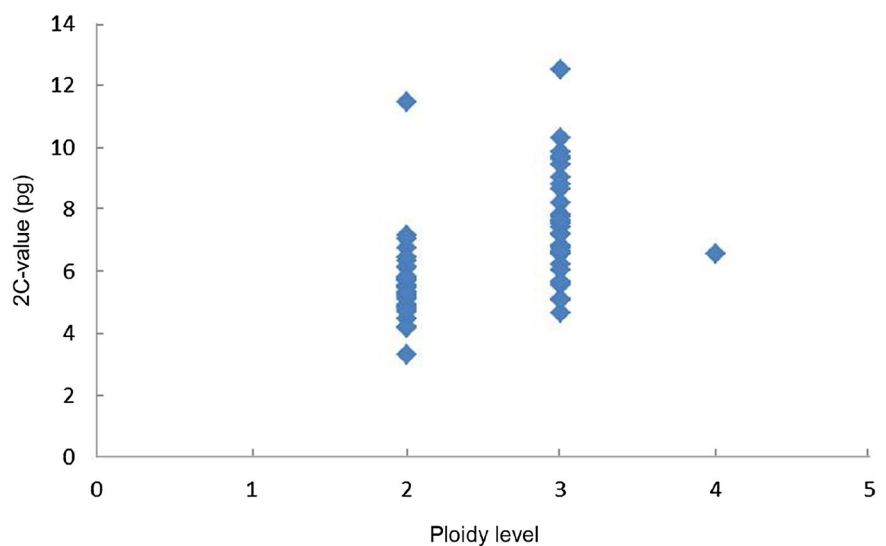
diploid species to have a larger variation, which also occurs in triploids.

Discussion

General karyotype characteristics

The study reveals a detailed picture of chromosome features and patterns of karyotype variation in *Colocasia*. Our karyological analyses reveal five different chromosome counts, $2n=26, 28, 38, 42,$ and 56 . The somatic numbers 28 and 42 predominate; other counts were encountered in one or two taxa only ($2n=26$ in *C. gigantea* and *C. esculenta*, $2n=38$ in *C. antiquorum*, and $2n=56$ in *C. esculenta*). The $2n$ value of 56 represents a new, and the largest

Fig. 2 Dispersion diagram representing the relationship between ploidy levels and DNA $2C$ values in *Colocasia* species analyzed



yet found, chromosome number for *Colocasia*. Inter- and intraspecific variation in chromosome number occurred in all studied accessions.

Karyotype formulae have a great uniformity among accessions of each species. At inter- and intraspecific levels, the most variable characters are type and number. The *Colocasia* species karyotypes we analyzed included exclusively M, m, and sm chromosomes, with st only being present in three accessions, which is inconsistent with previous studies (Cao and Long 2004; Yang et al. 2003).

Basic chromosome number and ploidy level variation

The basic *Colocasia* chromosome number has been a matter of continuous dispute since karyological surveys began in the genus. Several different chromosome numbers have been estimated for *Colocasia*, including $2n$ counts of $26, 28, 30, 36, 38, 42, 44, 46, 52, 58, 60, 65, 72, 84,$ and 116 (Cao and Long 2004; Ramachandran 1978; Subramanian 1979; Yang et al. 2003), and x values of 7 and 12 , while the basic chromosome number 14 has been repeatedly suggested (Coates et al. 1988; Darlington and Wylie 1955; Krishnan and Magoon 1977). Kawahara (1978) suggests that plants observed with a basic chromosome number of 12 have either been misidentified as *Colocasia* species, or that chromosome counts have been inaccurate. In our study *Colocasia esculenta* is the only *Colocasia* species with various intraspecific $2n$ chromosome and basic chromosome numbers. Populations with a chromosome number of $2n=42$ are triploid with a basic chromosome number of $x=14$, but are not hexaploid with a basic chromosome number of $x=7$ (Yang et al. 2003). Combining our results with previous studies, $x=14$ should still be considered the primary basic number. We also observed different chromosome basic numbers in another two species, *C. gigantea* and

C. esculenta ($2n=26$), and *C. antiquorum* ($2n=38$), with $x=13$ and 19. These two numbers are probably the result of secondary increases or reductions (Leong-Škorničková et al. 2007). This indicates that *Colocasia* is undergoing drastic differentiation. Following this logic, species investigated here correspond to $2x$, $3x$, and $4x$ cytotypes.

Chromosomes and evolution

The chromosome numbers observed in *Colocasia* species suggest that numerical changes may have been important in the evolution of the genus. Data from chromosome counts and $2C$ values confirm that ploidy/ $2C$ value levels have obvious differences in different species and in different accessions within a particular species. Our analyses show that about 51% accessions of *Colocasia* species are polyploidy in Yunnan Province. Therefore, our study corroborates Yunnan as the center of polyploid origin for *Colocasia*.

Chromosome size is also a feature subject to evolutionary change (Lavia et al. 2009). Chromosome evolution can either be directed toward an increase (Brandham and Doherty 1998) or a decrease in size (Martel et al. 2004). Furthermore, symmetrical karyotypes are widely accepted to be more primitive than asymmetrical ones (Stebbins 1971). Consider the chromosome length variation we observed, with a maximum L/S value of 3.27 in *C. antiquorum*, and a minimum value of 1.62 in *C. esculenta*, in relation to the AI values seen, ranging from 1.26 to 4.31: the type 2B *C. antiquorum* with AI=4.31 has the highest asymmetric tendency in our study, and type 1A *C. esculenta* with AI=1.26 has the smallest degree of asymmetry. Therefore, a possible evolutionary trend in *Colocasia* may be that species with smaller chromosomes and a more symmetric karyotype are more ancestral, while species with longer chromosome and more asymmetry are more derived. And, obviously, increases in chromosome arm length are accompanied by increases in karyotype asymmetry.

Difference in karyotype formula and interchromosomal asymmetries among species indicated that structure changes may lead to the diversity of the genus. Karyotype of *Colocasia* may be symmetrical and consisted almost exclusively of M, m, and sm chromosomes. These indicated that chromosome evolution in *Colocasia* may be constrained to nonrandom changes with particular restrictions for the occurrence or fixed of structural rearrangements (Seijo and Fernández 2003). The karyotype stability of *Colocasia* may be explained to orthoselection, which considers the occurrence of random chromosome mutation, but with the fixation of a restricted type of rearrangement (White 1978).

Large-scale analyses combining available genome size data for 3008 angiosperms have led to the proposal that

genome downsizing is a widespread biological response to polyploidization leading to diploidization of polyploid genomes (Leitch and Bennett 2004). In our study, *C. esculenta*, the only tetraploid accession endemic to Lancang county, Pu'er City, is also in the group with no highest DNA content. Difference in DNA content between and within species could be caused by the loss or gain of entire chromosomes or by change in chromosome size (Dart et al. 2004). Chromosomes are quite homogenous among the *Colocasia* species. We infer that the differences in DNA content among the *Colocasia* species we analyzed are caused by chromosomal gain under similar habitats. Polyploidization is obviously another possible contributor to $2C$ value variation. In this study, $2C$ values in some accessions were not proportional to ploidy levels. Reduction in DNA content as ploidy levels increase may be a necessary adaptation for the establishment and stabilization of polyploidy genomes (Ozkan et al. 2003; Tuna et al. 2001).

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