KARYOTYPE IN *PAEONIA INTERMEDIA* C.A. MEY

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Abstract

The chromosome number and karyotype of three populations in *Paeonia intermedia* C.A. Mey are presented in this paper. The populations, sampled from Altai Prefecture in Xinjiang, China, are diploids (2n = 10), with 2A type karyotype and the karyotypic formula of 2n = 2x = 10 = 6m + 2sm + 2st. There was no obvious differentiation among all the three sections of the genus *Paeonia* based on parameters of arm ratio and ratio of the longest/shortest chromosome (L_1/L_n). There is merely slight difference between the woody section (Sect. *Moutan*) and the herbaceous sections (Sects. *Onaepia* and *Paeonia*).

The genus *Paeonia* L. (Paeoniaceae) comprised of approximately 32 species of shrubs and perennial and are widely distributed in the Northern Hemisphere (Hong *et al*. 2001). The genus is divided into three sections (Sect.) namely, *Moutan* DC., *Onaeopia* Lindley and *Paeonia* L. (Stern 1946). Sect. *Paeonia* L., with their chromosomes large in size and low in number, has attracted considerable cytological and cytogenetical studies since 1930's. Among the species, so far cytologically studied from Sect. *Paeonia* are either diploid or tetraploid (2n = 20), only two species in this section were found intraspecifically both diploid and tetraploid (Dark 1936, Stern 1946, Hong *et al*. 1988), i.e., *Paeonia obovata* and *P. mairei*. However, because of the absence of a universal parameter to measure karyotype, only a few species reported have been sufficiently analysed karyotypically so far. The comparisons among the well analysed species were mostly made between diploids and tetraploids, or between two sections (Tzanoudakis 1983, Punina 1987, Hong *et al*. 1988, Zhang and Hou 1996).

In this species, the accessions from North Xinjiang were once misplaced under the name of *P. anomala* and *P. anomala* var. *intermedia* (Stern 1946). According to the previous study, *P. intermedia* was well isolated with and did not hybridize with a sympatric diploid species, *P. anomala* (Hong *et al*. 1994). However, the ploidy level and karyotype of Xinjiang's population in *P. intermedia* was not known previously. Therefore, the present research was undertaken to count the chromosomal number and to analyze the karyotype of the Aletai populations in *P. intermedia*.

Paeonia intermedia C.A. Mey., an endangered species with very few individuals in each population, was collected from Altai Prefecture, Xinjiang, China. Fifteen individuals of three populations with right cell division stages were successfully fixed through a wide survey that found totally over 150 individuals. The collection and fixation were performed for each individual separately. The plants of population-1 (individuals numbered from 131 - 150) grew at sunny hillside at an altitude of 1650 m, along with sparse grasses, in Hemu; population-2 and population -3 (numbered from 164 - 173 and from 175 - 186, respectively) grew at sunny hillside of an altitude of 1400 m, along with shrubs and grasses in Jiadengyu. The three populations are isolated from each other by mountains, rivers and roads. The voucher specimens from all populations are deposited in the herbarium (PE), Institute of Botany, the Chinese Academy of Sciences.

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Flower buds used for chromosome study were fixed in Carnoy's solution (3 absolute ethanol : 1 glacial acetic acid). After fixation for at least 24 hrs, the buds were transferred into 70% ethanol and stored at –20°C until further use.

The chromosome slides were made by conventional squashing method and stained with modified carbol-fuchsin (Darlington and La Cour 1975), and were mounted with Canada balsam. The photomicrographs were taken using a Leitz Orthoplan microscope under 100×. At least five cells were calculated and the results were shown in Table 1. The classification of karyotypes was followed after Stebbins (1971). The ratio of the longest/shortest chromosome (L_1/L_n) was calculated according to Zhang (1998).

The materials used for karyotypic analyses were individuals numbered from 131 - 186. The 15 individuals of three populations are all diploids, 2n = 10 (Fig. 1). The karyotype formula are shown in Table 1. The karyotype of *P. intermedia* was similar to all of the reported species in this genus, and all are 2A type. The chromosomes can be classified into three types. Type I: including chromosome 1, 2 and 3, was metacentric chromosome; Type II: only including cromosome 4, was submetacentric; Type III: only including cromosome 5, was subtelocentric (Fig. 1).
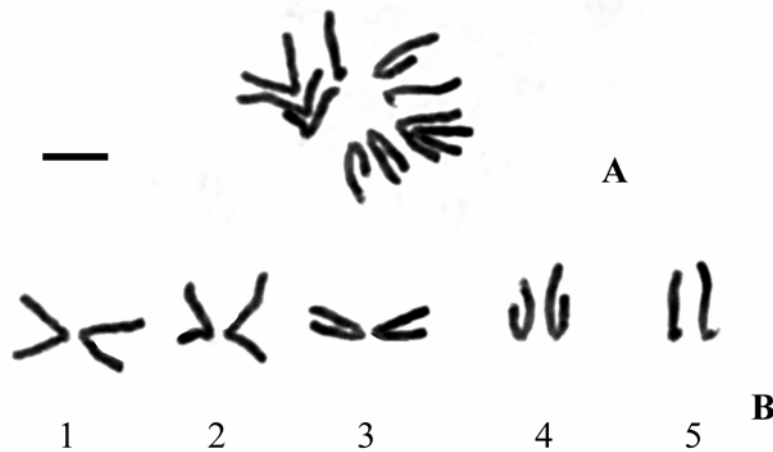

Fig. 1. Chromosomes of diploid *Paeonia intermedia* (A) well spread metaphase (2n = 2x =10).
    (B) Karyotype, chromosomes 1 - 5. (Bar = 10 µm).

The arm ratio of chromosome 1 in *P. intermedia* ranged from 1.12 to 1.30 (Table 1), which is consistent with that of the other species in Sect. *Paeonia* reported by Hong (1988). Furthermore, the arm ratio of chromosome 1 in Sect. *Paeonia* is similar (1.12 - 1.37), and does not overlap with that of chromosome 1 in Sect. *Moutan* (1.43 - 1.60) (Hong *et al*. 1988). At the same time, the arm-ratio of chromosome 1 in Sect. *Onaeopia* (1.13 - 1.2) (Stebbins and Ellerton 1939) is not overlap with that in Sect. *Moutan*, either. This result further confirms that the woody section (Sect. *Moutan*) apparently differentiated from the herbaceous one (Sects. *Paeonia* and *Onaeopia*).

The differentiation of karyotype in this genus also exists on the ratio of the longest chromosome/shortest one. The ratio of the longest/shortest chromosome (L_1/L_n) were calculated and the value ranged from 1.38 - 1.53 in *P. intermedia*, while the L_1/L_n value in *P. californica* is 1.30 and 1.80 in *P. brownii* in Sect. *Onaeopia* (Stebbins and Ellerton 1939), and 1.40 in Sect. *Moutan* (Hong *et al*. 1988).

Table 1. The parameters of chromosomes in *Paeonia intermedia*

Population	Individual	Chromosome number	Absolute length(μm)	Relative length	Arm ratio	Chr. Type*	L_l/L_n
1		1	12.07 ± 1.21	13.15+10.70=23.85	1.25 ± 0.21	m	1.49
		2	11.01 ± 1.31	11.95+9.77=21.72	1.24 ± 0.22	m	
	131	3	10.21 ± 1.13	11.54+8.60=20.14	1.36 ± 0.21	m	
		4	9.34 ±1.14	12.42+5.99=18.41	2.20 ± 0.24	sm	
		5	8.12 ±1.15	12.57+3.43=16.00	3.70 ± 0.39	st	
	134	1	13.41 ± 1.42	12.77+11.01=23.78	1.16 ± 0.08	m	1.48
		2	12.25 ± 1.10	11.59+10.15=21.74	1.15 ± 0.10	m	
		3	11.32 ± 1.21	10.8+9.25=20.05	1.18 ± 0.16	m	
		4	10.3 ± 0.94	12.09+6.19=18.28	1.97 ± 0.20	sm	
		5	9.06 ± 0.54	12.46+3.68=16.14	3.41 ± 0.32	st	
		1	13.15 ± 0.73	12.68+10.92=23.60	1.17 ± 0.16	m	1.51
		2	12.35 ± 1.15	12.19+9.95=22.14	1.23 ± 0.14	m	
	135	3	11.36 ± 0.66	11.31+9.07=20.38	1.26 ± 0.16	m	
		4	10.17 ± 0.82	11.93+6.33=18.26	1.89 ± 0.17	sm	
		5	8.70 ± 0.39	12.20+3.43=15.63	3.58 ± 0.39	st	
	148	1	12.55 ± 1.31	12.25+10.97=23.22	1.12 ± 0.08	m	1.44
		2	11.78 ± 1.01	11.83+10.01=21.84	1.19 ± 0.13	m	
		3	10.93 ± 0.91	11.07+9.18=20.25	1.22 ± 0.15	m	
		4	9.99 ± 0.82	12.56+5.95=18.51	2.15 ± 0.35	sm	
		5	8.70 ± 0.34	12.65+3.53=16.18	3.68 ± 0.71	st	
	150	1	11.08 ± 1.58	13.54+10.88=24.42	1.26 ± 0.18	m	1.53
		2	9.92 ± 1.33	11.83+10.04=21.87	1.19 ± 0.14	m	
		3	9.07 ± 1.28	11.25+8.73=19.98	1.30 ± 0.16	m	
		4	8.09 ± 1.30	11.87+5.92=17.79	2.02 ± 0.22	sm	
		5	7.22 ± 0.91	12.47+3.48=15.95	3.64 ± 0.55	st	
2	164	1	10.36 ± 1.02	13.17+10.18=23.34	1.30 ± 0.09	m	1.41
		2	9.53 ± 0.84	12.12+9.38=21.50	1.31 ± 0.15	m	
		3	8.93 ± 0.83	10.66+9.48=20.14	1.13 ± 0.11	m	
		4	8.25 ± 0.90	12.80+5.76=18.56	2.26 ± 0.37	sm	
		5	7.33 ± 0.97	13.02+3.44=16.46	3.82 ± 0.50	st	
	165	1	10.59 ± 0.80	12.87+11.07=23.94	1.17 ± 0.13	m	1.48
		2	9.68 ± 0.71	11.97+9.94=21.91	1.22 ± 0.17	m	
		3	8.86 0.63	11.30+8.74=20.04	1.31 ± 0.18	m	
		4	7.92 ± 0.48	11.98+5.97=17.95	2.03 ± 0.30	sm	
		5	7.14 ± 0.47	12.59+3.59=16.18	3.52 ± 0.32	st	
	167	1	11.17 ± 0.76	12.65+10.88=23.53	1.17 ± 0.11	m	1.45
		2	10.41 ± 0.62	12.17+9.76=21.93	1.26 ± 0.19	m	
		3	9.71 ± 0.75	11.42+9.06=20.48	1.28 ± 0.20	m	
		4	8.82 ± 0.58	12.32+6.26=18.58	2.00 ± 0.27	sm	
		5	7.69 ± 0.48	12.55+3.65=16.20	3.48 ± 0.47	st	
	170	1	12.00 ± 1.29	12.92+11.14=24.06	1.16 ± 0.12	m	1.44
		2	10.52 ± 0.79	11.79+9.35=21.14	1.28 ± 0.21	m	
		3	10.05 ± 0.79	11.53+8.66=20.19	1.34 ± 0.16	m	
		4	9.12 ± 0.89	12.36+5.95=18.31	2.08 ± 0.18	sm	
		5	8.34 ± 0.68	13.15+3.61=16.76	3.67 ± 0.39	st	
	173	1	11.54 ± 1.43	13.30+10.95=24.25	1.23 ± 0.17	m	1.51
		2	10.22 ± 0.90	11.76+9.79=21.55	1.22 ± 0.22	m	
		3	9.43 ± 0.79	10.62+9.27=19.89	1.15 ± 0.11	m	
		4	8.66 ± 0.95	12.09+6.15=18.24	2.03 ± 0.47	sm	
		5	7.62 ± 0.70	12.49+3.59=16.08	3.51 ± 0.43	st	

(Contd.)

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3	175	1	11.75 ± 0.70	13.04+10.81=23.85	1.18 ± 0.14	m	1.42
		2	10.86 ± 0.49	12.03+10.05=22.08	1.18 ± 0.18	m	
		3	10.10 ± 0.87	11.23+8.92=20.15	1.28 ± 0.22	m	
		4	9.09 ± 0.84	12.25+6.24=18.49	1.92 ± 0.17	sm	
		5	8.27 ± 0.55	13.32+3.57=16.89	3.73 ± 0.73	st	
	177	1	12.87 ± 0.43	12.50+10.69=23.19	1.18 ± 0.15	m	1.44
		2	12.05 ± 0.37	11.38+10.34=21.71	1.10 ± 0.06	m	
		3	11.57 ± 0.31	11.41+9.44=20.85	1.22 ± 0.13	m	
		4	10.06 ± 1.00	12.37+5.73=18.10	2.20 ± 0.38	sm	
		5	8.96 ± 0.37	12.53+3.61=16.14	3.50 ± 0.38	st	
	180	1	13.30 ± 1.14	13.03+11.11=24.14	1.18 ± 0.11	m	1.52
		2	12.14 ± 1.06	12.18+9.86=22.04	1.25 ± 0.17	m	
		3	11.00 ± 0.91	11.11+8.87=19.98	1.26 ± 0.14	m	
		4	9.88 ± 0.66	11.89+6.07=17.96	1.98 ± 0.28	sm	
		5	8.74 ± 0.58	12.46+3.42=15.88	3.74 ± 0.75	st	
	183	1	11.94 ± 1.37	12.73+10.26=22.99	1.25 ± 0.16	m	1.38
		2	10.67 ± 1.46	11.00+9.51=20.51	1.16 ± 0.08	m	
		3	10.18 ± 1.42	10.87+8.72=19.59	1.25 ± 0.13	m	
		4	9.85 ± 1.12	12.79+6.17=18.96	2.12 ± 0.39	sm	
		5	8.64 ± 0.51	12.96+3.75=16.71	3.49 ± 0.36	st	
	186	1	12.12 ± 0.57	13.02+11.41=24.43	1.15 ± 0.10	m	1.49
		2	10.87 ± 0.92	12.26+9.60=21.86	1.29 ± 0.16	m	
		3	9.88 ± 0.99	11.33+8.51=19.84	1.35 ± 0.18	m	
		4	8.73 ± 0.92	11.53+6.00=17.53	1.93 ± 0.23	sm	
		5	8.12 ± 0.62	12.80+3.54=16.34	3.65 ± 0.36	st	

*Chr. type: Chromosome type, classified according to Stebbins (1971).
L_l = The longest chromosome; L_n = The shortest chromosome.

This is the first time to report the chromosome number and karyotype in *Paeonia intermedia*. Due to historical taxonomic confusion of this species, especially with *P. anomala*, this research added valuable information to cytological data in genus *Paeonia*.

In all the three sections of this genus, karyotypic asymmetry varies slightly. However, from the meiotic studies (Sax 1937, Stebbins and Ellerton 1939, Walters 1942, Zhang *et al*. 1997, Wang and Zhang 2007, Wang *et al*. 2008), a great deal of abnormal meiotic configurations were found in all of the individuals observed, indicating that there existed many chromosome structural variations in this genus. These phenomena are contradictory to the karyotypic consistence recognized in *Paeonia*. In order to understand the contradiction better, further investigation and evidence are needed.

Acknowledgements

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