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STUDY ON CHROMOSOMAL STRUCTURAL HETEROZYGOSITY IN *PAEONIA EMODI*, AN ENDANGERED SPECIES

SHI-QUAN WANG

Ministry of Education Key Laboratory for Tropical Animal and Plant Ecology, College of Life Sciences, Hainan Normal University, Haikou 571158, Hainan Province, China
Corresponding author's e-mail: wsqmah@163.com

Abstract

Chromosomal meiotic behavior in pollen mother cells (PMCs) of *Paeonia emodi*, an endangered species, was investigated in natural population represented by nine individuals. The results showed that: (1) Mean chromosome configuration was 2n = 10 = 0.20 I + 4.90 II at metaphase. Most of the chromosomes were ring bivalents, but some were rod bivalents or univalents. The existence of rod bivalent and univalent indicated the differentiation between the homologous chromosomes, most probably due to chromosomal structural heterozygosity. (2) All individuals studied were chromosomal structural heterozygotes, i.e., inversion, as indicated by bridge and/or fragment. (3) There were some variations among individuals in size of fragments, which indicated that different inversions existed in this species, i.e., paracentric inversion polymorphisms. Chromosomal structural heterozygosity is a common phenomenon in *Paeonia*. Further research is necessary to probe into the reasons that structural heterozygosity exists widely, and whether there is relationship between heterozygosity and ecological adaptation in this species.

Introduction

Genus *Paeonia*, belonging to *Paeoniaceae*, is composed of 32 species, including shrubs and perennial herbs. There are three sections in *Paeonia*: Section *Onaepia*, Section *Moutan*, and Section *Paeonia* (Hong, 2010). Section *Paeonia* comprises approximately 25 herbaceous species, including diploids and tetraploids. *P. emodi* Wall ex Royle belongs to Section *Paeonia* and distributes in a single locality, Jiangcun, Gyirong County, Xizang (China), in N Pakistan, NW India, and W Nepal (Hong *et al*., 2001). *P. emodi* finds several applications in indigenous medical system. The roots, rhizomes and seeds are used as medicine to cure some disease (Khan *et al*., 2005). Owing to medicinal values and great ornamental, *P. emodi* is excessively excavated in China, and now becoming endangered with sharp decline in population size.

Chromosomal structural rearrangement plays an important part in evolution of plants, and meiotic pairing configuration is used as main proof to explain chromosomal structural rearrangement. Genus *Paeonia*, with a relatively small number (2n = 10 or 20) of chromosomes of large size, was studied extensively for understanding chromosomal structural mutations (Wang *et al*., 2008). In spite of the above variations, there is quite a uniform karyotype in *Paeonia* (Koeva & Sarkova, 1997).

An obviously high percentage of meiotic abnormalities has been observed in some species in recent years (Wang *et al*., 2008; Sadaf *et al*., 2012; Zaidi *et al*., 2012; Zaidi & Khatoon, 2012). The abnormal meiotic configurations, including univalents, bridges, fragments and lagging chromosomes, have been suggested to have resulted from chromosomal structural mutations (Wang *et al*., 2008). Now we do not know how the chromosomal mutations occurred and how they maintained in natural populations. In view of the above reasons, this study is focused on observation and analysis of meiotic behavior of pollen mother cells in *P. emodi*, in order to clarify whether the chromosomal structural abnormalities existed or not in this species, help to understand the genetic structure of populations, and deepen understanding of the evolutionary pattern.

Materials and Methods

Locality and date of material collection: *P. emodi* is an endangered species with very few individuals in population, composed of about 60 individuals in Jiangcun (2,350 m altitude), Gyirong county, South Xizang, China, which limited the scale of material collecting. Flower buds were collected from April 5-30, 2010. However, only nine individuals with right meiosis were harvested successfully.

Collection, storage and treatment of floral buds: Young floral buds were fixed separately in Carnoy's solution I (absolute ethanol: acetic acid = 3:1) at room temperature for 24 hours and then stored in 70% alcohol at -20ºC. Anthers were squashed in 45% acetic acid and stained with modified carbol-fuchsin (Li & Zhang, 1996).

Data Statistics and analysis: Meiotic abnormalities, for example, univalents, bridges, fragments and lagging chromosomes of pollen mother cells, were recorded and analyzed at different meiotic stages. Pairing index were calculated according to the following formula: Pairing index = (the number of ring bivalents × 2 + the number of rod bivalents) / 3000 × 100%. At the same time, Standard Error (mean ± S.E.) and frequency of meiotic abnormalities were calculated by using Excel. They were used descriptive statistics for the variables.

The length of fragments was measured by using Spot software (Diagnostic Instrument Incorporation) in order to distinguish different inverted segments in this species.

Results

Metaphase I: Five normal bivalents were observed in most PMCs at metaphase I of meiosis (Fig. 1A), though paring failure frequently occurred. The univalents moved to the same pole (Fig. 1B) or to two opposite pole (Fig. 1C). Mean frequency of univalents and bivalents (rod or ring) at metaphase I of the individuals studied are shown in Table 1.

In *P. emodi*, there were 4.90 bivalents (2.37 rod and 2.53 ring) and 0.20 univalent per cell on average, which figured out from 2700 metaphase I cells (Table 1). The frequency of PMCs including univalents ranged from 5.67 to 13%, on average 9.18%. The average meiotic configuration was 2n = 10 = 0.20 I + 4.90 II in this species. Pairing index ranged from 70.93 to 76.63% at metaphase I. Percent of three types of univalents (M, D and E) were 77.41, 17.78 and 4.81%, respectively.

Anaphase I and telophase I: Most of PMCs were normal at anaphase I (Fig. 1D) and at telophase I (Fig. 1I). There were several kinds of abnormalities as followed: bridges without fragments (Fig. 1E), bridges with fragments (Fig. 1F), fragments without bridges (Fig. 1G), lagging chromosomes (Fig. 1H) at anaphase I. At telophase I, abnormalities were found including bridges without fragments (Fig. 1J), bridges with fragments (Fig. 1K), fragments without bridges (Fig. 1L). The frequency of abnormalities at anaphase I and telophase I are shown in Table 2 and Table 3.

Bridge(s) and fragment(s) occurred by crossing-over within heterozygous paracentric inversions, appeared at anaphase and telophase in each of observed individuals. The frequency of bridges varied from 3.66 (in d08 genotype) to 21% (in d12 genotype) at anaphase I (Table 2), and from 1.33 (in d22 genotype) to 8% (in d12 genotype) at telophase I (Table 3), though the individuals studied were all inversion heterozygotes. The highest percentage of cells with fragments was 10.33% at anaphase I and 10.34% at telophase I (in d12 genotype), and the lowest was only 4% at anaphase I and 1.34% at telophase I (in d08 genotype) (Table 2 and Table 3). The average percentage of cells with bridges in this species was 7.63% at anaphase I and 3.3% at telophase I. The percent of cells with fragments was respectively 6.15 and 3.89% at anaphase I and telophase I (Table 2 and Table 3). In general, percent of cells with all sorts of abnormal configurations together was higher at anaphase I than that at telophase I.

Totally, 25 (0.93%) cells contain lagging chromosomes at anaphase I in nine individuals (Table 2; Fig. 1H). The occurrence frequency of such cells was quite low in most of individuals, except three individuals exceeded 1%. A total of nine cells (0.33%) were found with laggards at telophase I in this species (Table 3).

The fragments were considerably variable in size, ranging from less than 10 μm to more than 1.0 μm long, most between 2.0 and 7.0 μm. Fragment length range is shown in Table 4.

Discussion

Frequency of meiotic cells with univalents in *P. emodi* were considerably variable, 9.18% on average. Univalents resulted from asynapsis or pairing failure. It is well known that there are three M, one D and one E chromosomes in a cell, the occurrence ratio of the three types of chromosomes (M, D and E) should be 3:1:1, according to the univalent formation at random. In fact, the ratio between the three types of chromosomes, M:D:E, did not conform to the expected ratio, which showed univalent formation did not separate by chance alone.

The bridge/fragment at anaphase I and telophase I were the most obvious character of meiotic abnormalities observed on each individual in this species. Bridges and fragments, which resulted from crossing over in inversion regions, might be an indicator of paracentric inversion heterozygotes. Via crossing over or gene conversion, chromosomal location of rDNA loci affected the tempo of concerted evolution (Zhang & Sang, 1999). Obviously, all the individuals examined were heterozygotes of paracentric inversions.

Structural heterozytosity would make homologous chromosomes differentiated, which in turn will make homologues worse in pairing. High percentage of abnormal configurations might reflect structural differentiation between homologues in *P. emodi*.

It is interesting that all individuals of the species in this study are paracentric inversion heterozygotes. Because *Paeonia* is out-breedingly reproductive species, they often survived in small and limited populations whose seed and pollen dispersal distance was so short as to about 40 meters (Turpin & Schlising, 1971; Schlising, 1976), heterozygotes would easily become homozygotes by genetic segregation. Therefore, it is obvious that the structural heterozygosity would disappear quickly, unless it has a strong selective advantage.

Considerable *variations* in size of fragments were found in *P. emodi*, and the variation was regarded as different inversions, i.e. the chromosomal inversions presented in *P. emodi* were polymorphic. We suggest that the paracentric inversion polymorphism might play a role in adaptation to the varied ecological niches, as exemplified in *Drosophilia* (Hoffmann *et al.*, 2004). However, further research is necessary to probe into the reasons that structural heterozygosity exists widely, and the relationship between heterozygosity and ecological adaptation in this species.

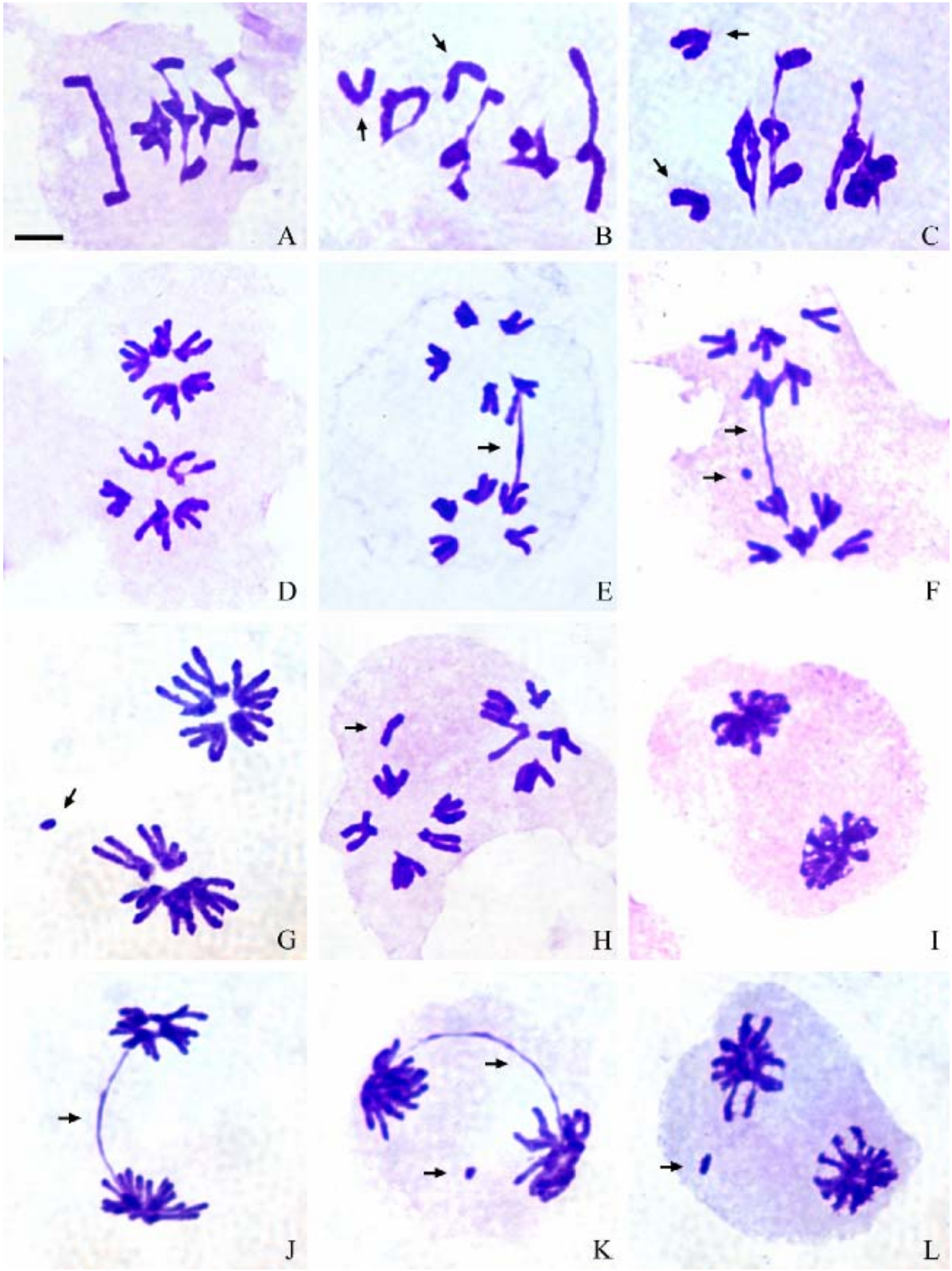

Fig. 1. First meiotic division of pollen mother cells in *Paeonia emodi*. (A-C) Metaphase I. (A) Five normal bivalents. (B) Four pairs of normal bivalents and two univalents (arrow). (C) Four pairs of normal bivalents and two univalents (arrow). (D-H) Anaphase I. (D) Normal. (E) Bridge (arrow) without fragment. (F) Single bridge with one fragment (arrow). (G) Fragment (arrow) without bridge. (H) Lagging chromosome (arrow). (I-L) Telophase I. (I) Normal. (J) Bridge (arrow) without fragment. (K) Single bridge with one fragment (arrow). (L) Fragment (arrow) without bridge. Bar = 10 μm.

Table 1. Chromosome pairing at metaphase I of PMCs in *Paeonia emodi*.

Individual	Nº cells observed	(univalents)							(bivalents)			Pairing index
		Nº cells with univalents	Percent of cells with univalents	Nº M	Nº D	NºE	Total	Nº univalents per cell	Total	Nº rod per cell	Nº ring per cell	
d05	300	39	13.00	80	8	2	90	0.30	4.85	2.36	2.49	73.40
d08	300	20	6.67	30	12	2	44	0.15	4.93	2.19	2.74	76.63
d12	300	28	9.33	44	8	4	56	0.19	4.91	2.42	2.49	73.90
d22	300	17	5.67	26	6	2	34	0.12	4.94	2.36	2.58	75.23
d32	300	29	9.67	46	16	4	66	0.22	4.89	2.69	2.20	70.93
d35	300	34	11.33	60	12	4	76	0.26	4.87	2.40	2.47	73.47
d38	300	25	8.33	34	14	2	50	0.17	4.91	2.23	2.68	76.00
d47	300	37	12.33	70	12	4	86	0.29	4.86	2.38	2.48	73.37
d49	300	19	6.33	28	8	2	38	0.13	4.94	2.34	2.60	75.33
Standard Error	300±0.00	27.56±2.67	9.18±0.89	46.44±6.51	10.67±1.11	2.89±0.35	60±6.87	0.20±0.02	4.90±0.01	2.37±0.05	2.53±0.05	74.25±0.58

M: median chromosomes; D: submedian chromosomes; E: subterminal chromosomes

Table 2. Frequency of meiotic abnormalities at anaphase I of PMCs in *Paeonia emodi*.

Individual	Nº cells observed	Nº normal cells observed	Percent of normal cells	Percent of cells with laggards	Percent of cells with bridges		Percent of cells with fragments (without bridges)
					With fragment	Without fragment	
d05	300	275	91.67	0.33	3.67	3.00	1.33
d08	300	278	92.67	1.00	1.33	2.33	2.67
d12	300	228	76.00	0.67	8.00	13.0	2.33
d22	300	275	91.67	0.33	2.67	3.67	1.67
d32	300	279	93.00	1.33	4.00	1.33	0.33
d35	300	271	90.33	1.33	1.00	3.33	4.00
d38	300	269	89.67	1.00	1.67	4.33	3.33
d47	300	267	89.00	0.67	4.67	2.00	3.67
d49	300	259	86.33	1.67	5.67	3.00	3.33
Standard Error	300±0.00	266.78±5.27	88.93±1.76	0.93±0.16	3.63±0.76	4.00±1.16	2.52±0.41

Table 3. Frequency of meiotic abnormalities at telophase I of PMCs in *Paeonia emodi*.

Individual	Nº cells observed	Nº normal cells observed	Percent of normal cells	Percent of cells with laggards	Percent of cells with bridges		Percent of cells with fragments (without bridges)
					With fragment	Without fragment	
d05	300	279	93.00	0.00	3.00	0.67	3.33
d08	300	291	97.00	0.00	0.67	1.67	0.67
d12	300	264	88.00	0.33	6.67	1.33	3.67
d22	300	294	98.00	0.00	1.33	0.00	0.67
d32	300	290	96.67	0.67	1.67	0.67	0.33
d35	300	283	94.33	0.67	0.33	2.00	2.67
d38	300	285	95.00	0.33	0.67	2.33	1.67
d47	300	287	95.67	0.00	1.67	0.67	2.00
d49	300	281	93.67	1.00	3.00	1.33	1.00
Standard Error	300±0.00	283.78±2.96	94.59±0.99	0.33±0.12	2.11±0.65	1.19±0.25	1.78±0.41

Table 4. Length (µm) range of fragments at meiotic anaphase I and telophase I in *Paeonia emodi*.

Meiotic stage	Nº cells observed	Length (µm)									Total fragments observed	Nº cells with fragments (%)
		1.1-2.0	2.1-3.0	3.1-4.0	4.1-5.0	5.1-6.0	6.1-7.0	7.1-8.0	8.1-9.0	9.1-10.0		
Anaphase I	2700	14	20	37	36	45	15	8	6	0	181	166 (6.15)
Telophase I	2700	3	20	30	20	20	7	7	2	1	110	105 (3.89)

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