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SHORT COMMUNICATION

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A cytological study on *Kelloggia* (Rubiaceae), an intercontinental disjunct genus between eastern Asia and western North America

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Abstract A cytological study was carried out for the first time on four populations of the only two species of *Kelloggia* (Rubiaceae), which occur disjunctly in eastern Asia (*K. chinensis* Franch.) and western North America (*K. galioides* Torr.). The consistent mitotic prophase chromosome condensation pattern and interphase nuclei type were determined for both species. The chromosome base number of the genus is suggested to be $x = 11$. The karyotype of $2n = 22 = 2x = 16\text{m} + 6\text{sm}$ was examined for both species. The karyotypical asymmetry of 1A and 2A was found in *K. chinensis* and *K. galioides*, respectively. According to the predominant evolutionary direction of karyotype asymmetry in angiosperms, *K. galioides* (2A) seems slightly more evolved than *K. chinensis* (1A). Our finding is consistent with the hypothesis of the Old World origin of *Kelloggia* based on molecular study.

Key words Cytology · *Kelloggia* · Disjunction · Eastern Asia · Western North America

Kelloggia Torr. ex Bentham (Rubiaceae), disjunct intercontinentally in eastern Asia and western North America, consists of only two species: *K. chinensis* Franch. from the Hengduan Mountains of southwestern China (Franchet 1892; Dempster 1975; Fang 2003) and *K. galioides* Torr. from western North America (Hooker 1873; Torrey 1874; Wilken 1993). *Kelloggia* was previously placed in the tribe Anthospermeae of Rubiaceae by Hooker (1873) and Schumann (1891). In light of its similarities on pollen morphology and pollination with taxa of Paederieae, Puff (1982) moved this genus to the tribe Paederieae. Bremer and Manen (2000) also held this opinion in their molecular phylogeny and classification of the subfamily Rubioideae although they did not sequence *Kelloggia*. The recent chloroplast DNA data (Nie et al. 2005) indicate the monophyly of *Kelloggia* with these two species and its sister relationship with the tribe Rubieae instead of inclusion in the tribe Paederieae, as suggested by Puff (1982).

Cytological data are essential to the study of plant evolution and diversification (Stebbins 1971; Stace 2000). To detect the taxonomic and evolutionary relationship within Rubiaceae, Kiehn (1995) presented a chromosome survey across the entire family. Karyological and cytotaxonomic investigations were also made on genus or tribe level (e.g. Hedyotideae, Lewis 1962, 1965; *Galium*, Kliphuis 1983, 1984, 1986; *Paederia*, Kiehn 1991). However, no reports regarding chromosome number and karyomorphology of *Kelloggia* have been available so far. In this paper, we report for the first time the chromosome numbers and karyotypes of both the two species of *Kelloggia* to investigate their evolution cytologically in their intercontinental disjunction.

For cytological observations, all slides were made from root-tip cells. The root tips were obtained from germinating seeds. The seeds of *K. galioides* and *K. chinensis* were collected from the mountains of western USA and southwestern China, respectively. Detailed collection data are shown in Table 1. The vouchers of all collections are deposited in the herbarium of the California Academy of Sciences (CAS) and the herbarium of Kunming Institute of Botany (KUN) (Table 1). Root tips were pretreated in a 0.002 mol/l 8-hydroxyquinoline solution at 22°C for 120 min, then fixed with Carnoy fluid (absolute alcohol: glacial acetic acid, 3:1, v/v) at 4°C for at least 30 min. The fixed roots were hydrolyzed in 1:1 solution of 1 N HCl: 45% acetic acid at 60°C for 25 s, stained with 1% aceto-orcein for 1 h at room temperature, and squashed for cytological observation. Permanent slides were made using the standard liquid nitrogen method (Li and Zhang 1991).

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The mitotic interphase nuclei (Fig. 1a) of both *K. galioides* and *K. chinensis* are lightly stained and exhibit no remarkable aggregated chromatin bodies. The chromatin shows almost homogeneous distribution in the whole regions of the nucleus. At mitotic prophase (Fig. 1b), the heterochromatin and euchromatin segments are obvious in both species. The heterochromatin segments are deeply stained and locate in the proximal regions while the euchromatin segments in the distal regions of chromosomes are lightly stained and extended. There is no difference between these two species on mitotic interphase nuclei and prophase chromosomes.

The designation of the centromere position as median (m) and submedian (sm) followed Levan et al. (1964), and the asymmetry of karyotype followed Stebbins (1971). The metaphase chromosomes of each species are shown in Fig. 1c–j. Their brief descriptions of the cytological features of each species are as follows.

Kelloggia chinensis

Plants of three populations of *K. chinensis* have the same karyotype formula $2n = 22 = 16m + 6sm$. The mean length of chromosomes of this species is 1.64 μm. The ratios of the longest to the shortest chromosome are 1.65, 1.67, and 1.75 in the populations of Xialang, Honglashan and Napahai, respectively, and no chromosomes have an arm ratio of more than 2.0 (Table 1; Fig. 1d–f, h–j). The asymmetry of the karyotype is categorized as 1A.

Kelloggia galioides

This species has the karyotype formula $2n = 22 = 16m + 6sm$. Two chromosomes have satellites-like bodies at the terminal regions of the short arms. The mean length of chromosomes of this species is 1.44 μm. The ratio of the longest to the shortest chromosome is 1.45, and 18.2% chromosomes have an arm ratio of more than 2.00 (Table 1; Fig. 1c, g). The asymmetry of the karyotype is categorized as 2A.

To identify the chromosome basic number of *Kelloggia*, chromosome counts from species of its close relatives, including the tribes Paederieae, Theligoneae, and Rubieae (Nie et al. 2005), were compared from the compilation works by Moore (1982), Goldblatt (1981, 1984), and the online database of Index of Plant Chromosome Numbers in Monographs in Systematic Botany (Missouri Botanical Garden). Two hundred and seventy-seven species from the three tribes have been counted. Most of the species (90.6%) present $x = 11$ while only 9.4% have other numbers ($x = 9$, 10 or 12). In the present study, *Kelloggia* is apparently diploidy with base number of $x = 11$.

The chromosome length and structure are stable characteristics for a certain group or taxon of Rubiaceae (Kiehn 1995). The length of chromosomes in Rubiaceae ranges from <0.4 to 8.0 μm (Bhattacharyya 1958; Selvaraj 1987; Kiehn 1991, 1995). The karyotype formulae of rubicious plants are often constituted by different proportion of median and submedian chromosomes (Selvaraj 1987). In *Kelloggia*, however, both species possess 16m and 6sm chromosomes. The mean lengths of chromosomes in four populations from the two species are also very similar.

Despite these similarities on cytology, small karyotypical variations between the two species, which can be used to indicate the possible evolutionary line in the genus, were detected in the study. The increasing of karyotype asymmetry in angiosperms seems to be a predominant evolutionary tendency (Stebbins 1971; Hong 1990) although some reversals of this trend have been reported (Jones 1978; Hong 1990). Two pairs of chromosomes have arm ratios more than 2.0 in *K. galioides* while no pair has an arm ratio more than 2.0 in three populations of *K. chinensis*. Here we speculate that the high asymmetry of karyotype in *K. galioides* (2A) might indicate it is more evolved than that of *K. chinensis*, of which the karyotype asymmetry is 1A. This hypothesis is also supported by the recent molecular and biogeographic studies (Nie et al. 2005), which suggested that *Kelloggia* had an Old World origin with long-distance dispersal from the Old World into western North America.

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Table 1. Samples and cytological features of four populations of *Kelloggia galioides* and *K. chinensis*

	K. galioides	*K. chinensis* (Napahai)	*K. chinensis* (Xialang)	*K. chinensis* (Honglashan)
Voucher	*Bartholomew 8817* (CAS, KUN)	*Sun 8175* (KUN)	*Sun 8178* (KUN)	*Yue 0063* (KUN)
Source	USA: California, Modoc County, Manzanita Mt.	China: Yunnan, Zhongdian County, Napahai	China: Yunnan, Zhongdian County, Xialang	China: Tibet, MangKang County, Honglashan
Altitude (m)	2,300	3,200	3,600	4,300
AR2	18.2%	0	0	0
RLSC	1.45	1.75	1.65	1.67
Karyotype formula	$2n = 2x = 16m + 6sm$	$2n = 2x = 16m + 6sm$	$2n = 2x = 16m + 6sm$	$2n = 2x = 16m + 6sm$
Karyotype asymmetry	2A	1A	1A	1A

AR2 percent of chromosomes with arm ratios more than 2.0, *RLSC* ratio of the longest to the shortest chromosome

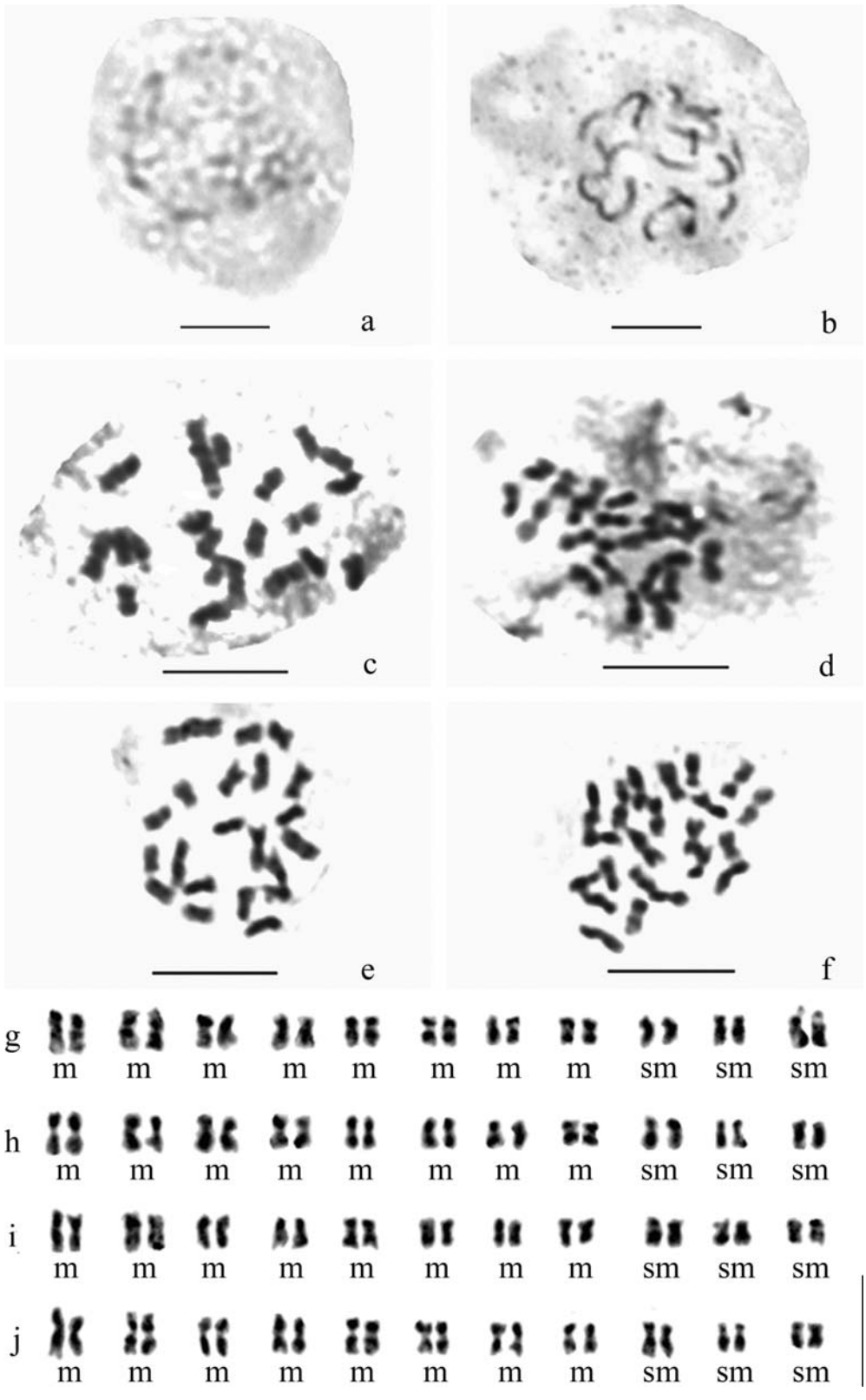

Fig. 1. Cytological features of *Kelloggia galioides* and *K. chinensis*. **a** *K. chinensis*, interphase nuclei. **b** *K. chinensis*, mitotic prophase chromosomes. **c–j** Mitotic metaphase chromosomes. **c, g** *K. galioides*, $2n = 22 = 2x = 16m + 6sm$. **d**, **h** *K. chinensis* (Xialang), $2n = 22 = 2x = 16m + 6sm$. **e, i** *K. chinensis* (Honglashan), $2n = 22 = 2x = 16m + 6sm$. **f**, **j** *K. chinensis* (Napahai), $2n = 22 = 2x = 16m + 6sm$. *m* median, *sm* submedian. *Scale bar* = 5 μm

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