

Karyotype studies on ten *Iris* species (Iridaceae) from Sichuan, China

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Abstract — Chromosome number and karyotype analysis of 10 taxa in the genus *Iris* (Iridaceae) from Sichuan, China were investigated in this study. The chromosome number and karyotype formula of 10 *Iris* taxa are as follows: *I. lactea*, $2n = 40 = 28m + 10sm + 2st$; *I. lactea* var. *chinensis*, $2n = 40 = 18m + 16sm + 6st$ (6SAT); *I. chrysographes*, $2n = 40 = 18m + 22sm$ (2SAT); *I. japonica*, $2n = 36 = 16m + 14sm + 6st$; *I. tectorum*, $2n = 28 = 16m$ (4SAT) + $12sm$ (2SAT); *I. confusa*, $2n = 32 = 8m + 18sm + 6st$; *I. germanica*, $2n = 40 = 16m + 20sm + 4st$; *I. sichuanensis*, $2n = 26 = 12m$ (4SAT) + $12sm$ (2SAT) + $2st$; *I. leptophylla*, $2n = 26 = 14m$ (2SAT) + $10sm$ (4SAT) + $2st$; *I. goniocarpa*, $2n = 26 = 12m + 12sm + 2st$ (2SAT). The karyotypes of *I. sichuanensis*, *I. leptophylla*, *I. goniocarpa*, *I. lactea*, *I. confusa* and *I. germanica* were firstly reported, and the chromosome number of *I. confusa* and *I. germanica* were newly observed. Based on the karyotype results and previous studies, we suggested that: (1) *I. wattii*, *I. confusa* and *I. japonica* are three independent species; (2) *I. sichuanensis*, *I. leptophylla* and *I. goniocarpa* are three independent species with close relationships; (3) it is reasonable to treat *I. lactea* var. *chinensis* as a variety of *I. lactea*.

Key Words: China, chromosome number, *Iris*, karyotype, taxonomy.

INTRODUCTION

Iris L. is a large genus of Iridaceae involving about 300 species in the world, which is distributed in the north temperate regions (ZHAO 1985). There are 60 species, 13 varieties, and 5 forms in China, which is a distribution center of *Iris* (WADDICK and ZHAO 1992). Twenty three species and 2 varieties of *Iris* were reported in Sichuan province, which accounts for 1/3 of *Iris* in China (GAO 1989). The flowers of *Iris* are large and colorful, and species of *Iris* are widely used in vegetative landscape. Because a few *Iris* species have great interspecific variations in morphology and some species are difficult to be distinguished morphologically, their taxonomic treatments and interspecific relationships are still controversial.

Iris sichuanensis Y. T. Zhao, *I. leptophylla* Lingelsh and *I. goniocarpa* Baker are three species in Subgen. *Iris* L. Their morphological characteristics, except the size of leaf, are similar to each other. The leaf of *I. sichuanensis* is bigger than that of *I. leptophylla* and *I. goniocarpa* (ZHAO 1980). Geographically, *I. sichuanensis* and *I. leptophylla* are distributed in Gansu and the north-west of Sichuan, while *I. goniocarpa* has a wide distribution in China, Bhutan, Sikkim, India and Nepal (ZHAO 1985). The taxonomic classification of these three species is always mixed up. Based on the size of leaf, ZHAO (1985) suggested that *I. sichuanensis* and *I. leptophylla* should be two independent species. However, according to the similar flower and geographic distribution of *I. sichuanensis* and *I. leptophylla*, GAO (1985) suggested that *I. sichuanensis* should be a homonym of *I. leptophylla*.

Iris japonica Thunb., *I. confusa* Sealy and *I. wattii* Baker are three widely distributed species in Subgen. *Crossiris* Spach. ZHAO (1980) reported that the flowers and fruits of *I. confu-*

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sa are similar to those of *I. japonica*, while the vegetative parts of *I. confusa* are similar to those of *I. wattii*. WADDICK (1994) suggested that *I. japonica* and *I. confusa* may be the variants of a same species. ZHOU *et al.* (2003) reported that *I. japonica* and *I. confusa* are two independent biological species on the base of the morphology, fertility and chromosome pairing studies. The relationship between *I. confusa* and *I. wattii* are still uncertain (WADDICK and ZHAO 1992).

Morphologically, *I. lactea* has white flowers and *I. lactea* var. *chinensis* has purple flowers. Geographically, *I. lactea* is restricted to northeast and southwest of China, whereas *I. lactea* var. *chinensis* is widely distributed in China, Korea, USSR and India (ZHAO 1985). Based on their geographical and morphological differences, ZHAO (1985) treated *I. lactea* var. *chinensis* as a variety of *I. lactea*. Because the purple flower population (*I. lactea* var. *chinensis*) always grows together with the white flower population (*I. lactea*) at the same place, GAO (1985) suggested that *I. lactea* var. *chinensis* should be more likely a homonym of *I. lactea*, rather than one of its varieties.

In our present study, chromosome number observation and karyotype analysis of *I. lactea*, *I. lactea* var. *chinensis*, *I. sichuanensis*, *I. leptophylla*, *I. goniocarpa*, *I. japonica* and *I. confusa* were carried out. Meanwhile, the karyotype analyses of other three taxa of *Iris* (*Iris chrysographes* Dykes, *Iris tectorum* Maxim, *Iris germanica* L.) from Sichuan were also reported here. The objectives are: (a) to report their chromosome numbers and the karyotypical characters of these Chinese *Iris* species; and (b) to investigate their interspecific relationships of the related species.

TABLE 1 — Materials used in this study.

Taxon	Locality	Altitude (m)	Accession No.
Subgen. <i>Limniris</i> (Tausch) Spach			
<i>Iris lactea</i> Pall.	Maoxian, Sichuan, China	1500	Iris041
<i>Iris lactea</i> var. <i>chinensis</i> (Fisch) Koidz.	Maoxian, Sichuan, China	1500	Iris042
<i>Iris chrysographes</i> Dykes	Kangding, Sichuan, China	3800	Iris003
Subgen. <i>Crossiris</i>			
<i>Iris japonica</i> Thunb.	Dujiangyan, Sichuan, China	550	Iris005
<i>Iris tectorum</i> Maxim	Ganzi, Sichuan, China	600	Iris006
<i>Iris confusa</i> Sealy	Dujiangyan, Sichuan, China	550	Iris004
Subgen. <i>Iris</i> L.			
<i>Iris germanica</i> L.	Yaan, Sichuan, China	591	Iris007
<i>Iris sichuanensis</i> Y. T. Zhao	Wenchuan, Sichuan, China	1000	Iris009
<i>Iris leptophylla</i> Lingelsh	Wenchuan, Sichuan, China	1000	Iris010
<i>Iris goniocarpa</i> Baker	Maoxian, Sichuan, China	1200	Iris001

MATERIALS AND METHODS

Ten *Iris* taxa from Sichuan, China were used in this study. Their names, geographic origins, and accession information of the taxa are listed in Table 1. All the materials were collected by the authors of this paper. Voucher specimens have been deposited at the Herbarium of Triticeae Research Institute, Sichuan Agricultural University, China (SAUTI).

For observation of somatic chromosomes, root tips were pretreated in saturated 1, 4-dichlorobenzene (C₆H₄CL₂) at room temperature for 3-4 h before being fixed in Carnoy's solution (95% ethanol : acetic acid = 3 : 1, vol/vol) at dark place for 2 h. They were then rinsed in distilled water and hydrolysed for 10 min in 0.05ml/L HCl at 42.5°C. The root tips were washed in distilled water again, stained and crushed in 1.5% carbollic acid-fuchsine solution for one night before observation. Photomicrographs of well-spread metaphase images were captured with a cooled CCD camera using a microscope (Olympus BX51). Idiograms were constructed based on the relative length and relative arm ratios of chromosome (LI and CHEN 1985). Chromosomes were arranged from the longest to the shortest, and were designated with the Arabic numerals (STEBBINS 1971).

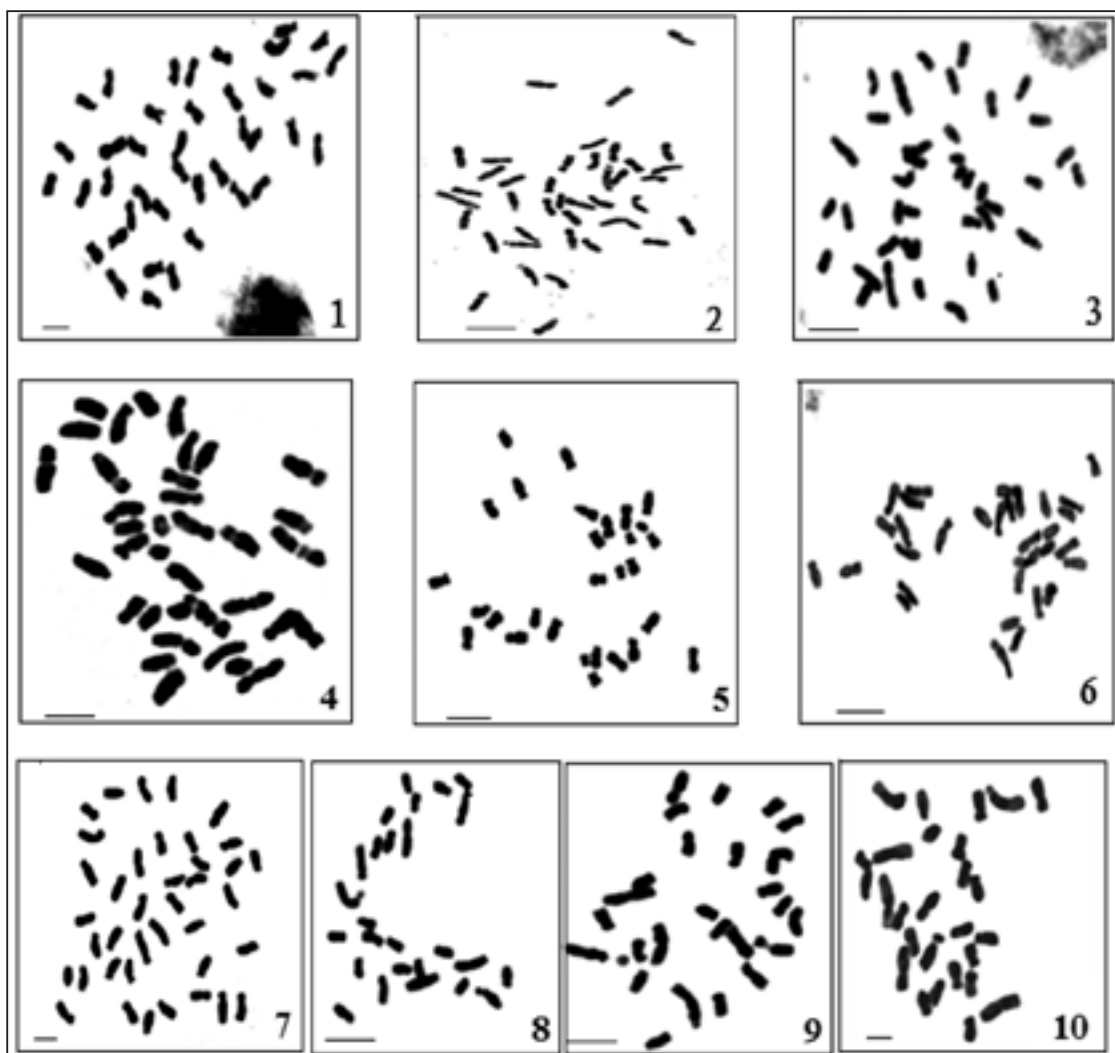
RESULTS

Metaphase chromosomes of 10 *Iris* taxa are shown in Figs. 1-10. Karyotypes and idiograms of 10 taxa in *Iris* are shown in Figs. 11-20 and Figs. 21-30, respectively. Chromosome parameters are listed in Table 2.

TABLE 2 — Parameters of chromosomes of 10 taxa in *Iris* from Sichuan, China.

Taxa	Karyotypic formula	A.A.R ¹	Lc ² /Sc ³	As.k ⁴ (%)	Type
<i>I. lactea</i>	2n = 40 = 28m + 10sm + 2st	1.63	2.28	43.84	2 B
<i>I. lactea</i> var. <i>chinensis</i>	2n = 40 = 18m + 16sm + 6st (6SAT)	2.12	2.58	38.72	2 B
<i>I. chrysographes</i>	2n = 40 = 18m + 22sm (2SAT)	1.79	3.71	26.92	2 B
<i>I. japonica</i>	2n = 36 = 16m + 14sm + 6st	2.07	2.57	38.22	2 B
<i>I. tectorum</i>	2n = 28 = 16m (4SAT) + 12sm (2SAT)	1.83	2.66	37.60	2 B
<i>I. confusa</i>	2n = 32 = 8m + 18sm + 6st	2.19	2.16	46.38	2 B
<i>I. germanica</i>	2n = 40 = 16m + 20sm + 4st	2.13	3.61	27.71	3 B
<i>I. sichuanensis</i>	2n = 26 = 12m (4SAT) + 12sm (2SAT) + 2st	1.97	2.82	35.40	3 B
<i>I. leptophylla</i>	2n = 26 = 14m (2SAT) + 10sm (4SAT) + 2st	1.90	2.90	34.51	2 B
<i>I. goniocarpa</i>	2n = 26 = 12m + 12sm + 2st (2SAT)	1.83	3.26	30.70	2 B

Note: ¹A.A.R, average arm ratio; ²Lc, longest chromosome; ³Sc, shortest chromosome; ⁴As.k (%), index of the karyotypic asymmetry; m, median; sm, submedian; st, subterminal; SAT, satellites. (LI, M. X., CHENG, R. Y. 1985)



Figs. 1-10 — Mitotic metaphase chromosomes of 10 *Iris* taxa. 1. *I. lactea*; 2. *I. lactea* var. *chinensis*; 3. *I. chrysographes*; 4. *I. japonica*; 5. *I. tectorum*; 6. *I. confusa*; 7. *I. germanica*; 8. *I. sichuanensis*; 9. *I. leptophylla*; 10. *I. goniocarpa*. Scale bars: 5 μ m.

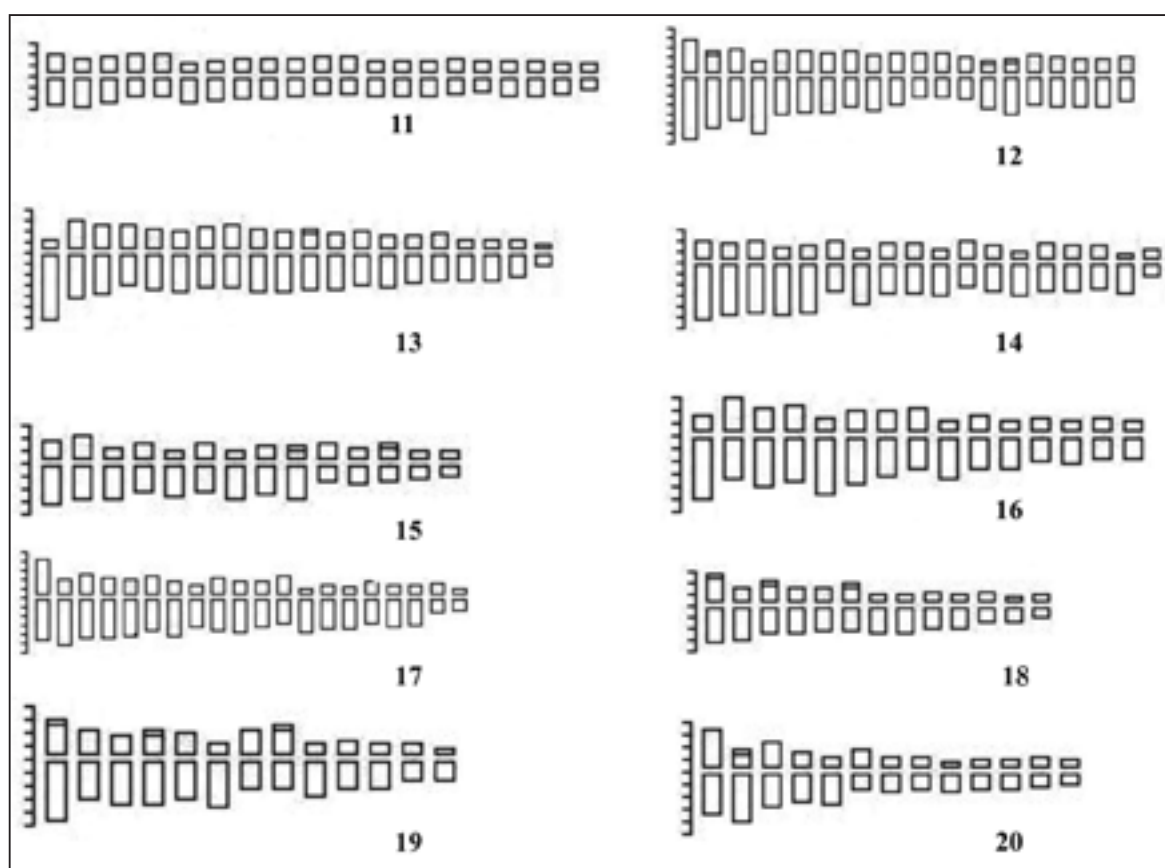
1. *I. lactea* - The chromosome number of *I. lactea* is $2n = 40$, and it consists of 28 median centromeric chromosomes, 10 submedian centromeric chromosomes and 2 subterminal centromeric chromosomes. The karyotype formula is $2n = 40 = 28m + 10sm + 2st$. No satellite is observed in this species. The chromosomes range from 1.78 to 4.06 in the relative length. The karyotype symmetry is type 2 B (Figs. 1, 11, 21). The karyotype is reported for the first time.

2. *I. lactea* var. *chinensis* - The chromosome number of *I. lactea* var. *chinensis* is $2n = 40$. It consists of 18 median centromeric chromosomes, 16 submedian centromeric chromosomes and 6 subterminal centromeric chromosomes. The karyotype formula is $2n = 40 = 18m + 16sm + 6st$ (6SAT). There are three pairs of satellites on the short arms of chromosome 2, 14 and 15. The chromosomes range from 3.76 to 9.71 in the relative length. The karyotype symmetry is type 2 B (Figs. 2, 12, 22).

3. *I. chrysographes* - The chromosome number of *I. chrysographes* is $2n = 40$. It consists of 18 median centromeric chromosomes and 22 submedian centromeric chromosomes. The karyotype formula is $2n = 40 = 18m + 22sm$ (2SAT). There is one pair of satellites on the short arm of chromosome 11. The chromosomes range from 1.89 to 7.02 in the relative length. The karyotype asymmetry is type 2 B (Figs. 3, 13, 23).

4. *I. japonica* - The chromosome number of *I. japonica* is $2n = 36$. It consists of 16 median centromeric chromosomes, 14 submedian centromeric chromosomes and 6 subterminal centromeric chromosomes. The karyotype formula is $2n = 36 = 16m + 14sm + 6st$. No satellite is observed in this species. The chromosomes range from 2.24 to 5.77 in the relative length. The karyotype symmetry is type 2 B (Figs. 4, 14, 24).

5. *I. tectorum* - The chromosome number of *I. tectorum* is $2n = 28$. It consists of 16 median centromeric chromosomes and 12 submedian cen-



Figs. 11-20 — Ideograms in 10 taxa of *Iris*. 11 *I. lactea*; 12 *I. lactea* var. *chinensis*; 13 *I. chrysographes*; 14 *I. japonica*; 15 *I. tectorum*; 16 *I. confusa*; 17 *I. germanica*; 18 *I. sichuanensis*; 19 *I. leptophylla*; 20 *I. goniocarpa*.



Figs. 21-30 — Karyograms of 10 *Iris* taxa. 21. *I. lactea*; 22. *I. lactea* var. *chinensis*; 23. *I. chrysographes*; 24. *I. japonica*; 25. *I. tectorum*; 26. *I. confusa*; 27. *I. germanica*; 28. *I. sichuanensis*; 29. *I. leptophylla*; 30. *I. goniocarpa*. Bars: 5 μ m.

trimeric chromosomes. The karyotype formula is $2n = 28 = 16m (4SAT) + 12sm (2SAT)$. There are two pairs of satellites on the short arms of chromosome 9 and 12. The chromosomes range from 1.76 to 4.68 in the relative length. The karyotype symmetry is type 2 B (Figs. 5, 15, 25).

6. *I. confusa* - The chromosome number of *I. confusa* is $2n = 32$. It consists of 8 median centromeric chromosomes, 18 submedian centromeric chromosomes and 6 subterminal centromeric. The karyotype formula is $2n = 32 = 8m + 18sm + 6st$. No satellite is observed in this species. The chromosomes range from 2.82 to 6.08 in the rela-

tive length. The karyotype asymmetry is type 2 B (Figs. 6, 16, 26). The chromosome number of *I. confusa* is newly observed and the karyotype of this species is firstly reported.

7. *I. germanica* - The chromosome number of *I. germanica* is $2n = 40$. It consists of 16 median centromeric chromosomes, 20 submedian centromeric chromosomes and 4 subterminal centromeric. The karyotype formula is $2n = 40 = 16m + 20sm + 4st$. No satellite is observed in this species. The chromosomes range from 2.30 to 8.30 in the relative length. The karyotype asymmetry is type 3 B (Figs. 7, 17, 27). The chromosome number of

I. germanica is newly observed and the karyotype of this species is firstly reported.

8. *I. sichuanensis* - The chromosome number of *I. sichuanensis* is $2n = 26$. It consists of 12 median centromeric chromosomes, 12 submedian centromeric chromosomes and 2 subterminal centromeric. There are three pairs of satellites on the short arms of chromosome 1, 3 and 6. The karyotype formula is $2n = 26 = 12m (4SAT) + 12sm (2SAT) + 2st$. The chromosomes range from 1.82 to 5.14 in the relative length. The karyotype asymmetry is type 3 B (Figs. 8, 18, 28). The karyotype and chromosome number of *I. sichuanensis* are reported for the first time.

9. *I. leptophylla* - The chromosome number of *I. leptophylla* is $2n = 26$. It consists of 14 median centromeric chromosomes, 10 submedian centromeric chromosomes and 2 subterminal centromeric. There are three pairs of satellites on the short arms of chromosome 1, 4 and 8. The karyotype formula is $2n = 26 = 14m (2SAT) + 10sm (4SAT) + 2st$. The chromosomes range from 2.34 to 6.78 in the relative length. The karyotype asymmetry is type 2 B (Figs. 9, 19, 29). The karyotype and chromosome number of *I. leptophylla* are reported for the first time.

10. *I. goniocarpa* - The chromosome number of *I. goniocarpa* is $2n = 26$. It consists of 12 median centromeric chromosomes, 12 submedian centromeric chromosomes and 2 subterminal centromeric. The karyotype formula is $2n = 26 = 12m + 12sm + 2st (2SAT)$. There is one pair of satellites on the short arms of chromosome 2. The chromosomes range from 1.87 to 6.09 in the relative length. The karyotype asymmetry is type 2 B (Figs. 10, 20, 30). The karyotype and chromosome number of *I. goniocarpa* are reported for the first time.

DISCUSSION

Relationships among I. sichuanensis, I. leptophylla and I. goniocarpa - Morphologically, *I. sichuanensis*, *I. leptophylla* and *I. goniocarpa* are very similar. ZHAO (1985) suggested that *I. sichuanensis* and *I. leptophylla* should be two independent species based on the size of leaf, while GAO (1985) suggested that *I. sichuanensis* should be a homonym of *I. leptophylla*.

Similar karyotypes of the three species are observed in this study. All of them have chromosome number of $2n = 26$. There are differences in the number of satellite chromosome among the three species. *I. goniocarpa* has two satellites,

while *I. leptophylla* and *I. sichuanensis* have six satellites. The number, size, and distribution of the satellite are remarkable characters to distinguish the related species or genera (STEBBINS 1971). Thus, data of karyotype analysis indicated that *I. sichuanensis*, *I. leptophylla* and *I. goniocarpa* may more likely constitute three independent species, and the relationship between *I. leptophylla* and *I. sichuanensis* should be closer than that with *I. goniocarpa*.

Karyotype symmetry of *I. leptophylla* and *I. goniocarpa* belongs to Stebbins's 2 B type, while that of *I. sichuanensis* is type 3 B. According to STEBBINS's theory (1971), the tendency of chromosomal evolution is from symmetry to asymmetry. So, karyotypes of *I. leptophylla* and *I. goniocarpa* show the ancestral character states, and they may have originated before than *I. sichuanensis*.

Relationships among I. japonica, I. confusa and I. wattii - Previous studies showed that *I. confusa* has chromosome number of $2n = 28, 30, 34, 36, 42$, *I. japonica* is of $2n = 28, 30, 31-33, 32, 34, 35, 36, 38, 42, 46, 52, 54, 55, 56, 60$, and *I. wattii* is of $2n = 30$ (DARLINGTON and WYLIE 1955; CHIMPHAMBA 1973; MAO *et al.* 1986; SHU *et al.* 1992; COLASANTE and SAUER 1993; LU *et al.* 1993; DONG *et al.* 1994; YEN *et al.* 1994; ELLIS 2000; SHEN *et al.* 2007). It is reported that cytotoxicity occurring in meiotic prophase of pollen mother cells (PMCs) might be the important reason for the origin of aneuploidy and multiploidy shown by different populations of *I. japonica* and *I. confusa* (YEN *et al.* 1994).

In this study, the chromosome number of *I. confusa* is $2n = 32$, while *I. japonica* is $2n = 36$. The karyotype of *I. japonica* is $2n = 36 = 16m + 14sm + 6st$ and *I. confusa* is $2n = 32 = 8m + 18sm + 6st$. SHEN *et al.* (2007) reported the karyotype of *I. japonica* is $2n = 34 = 16m + 18sm (2SAT)$. The variability in its chromosome number and karyotype occurred may be due to geographical difference.

SHEN *et al.* (2007) reported the karyotype of *Iris wattii* was $2n = 30 = 18m + 12sm (2SAT)$. The results showed that the karyotype of *I. wattii* is different from that of *I. japonica* and *I. confusa*. *Iris wattii* has two satellites, while no satellite was observed in *I. japonica* and *I. confusa*. Thus, karyotypes of the three *Iris* species are different from each other in chromosome size, chromosome number and satellite chromosomes. The present karyotype results suggest that it is reasonable to treat *I. japonica*, *I. confusa* and *I. wat-*

tii as three species.

Relationship between I. lactea and I. lactea var. chinensis - DORONKIN and KRASNIKOV (1984) reported that the chromosome number of *I. lactea* was $2n = 40$. However, various chromosome number of $2n = 40, 42, 44$ had been observed in *I. lactea* var. *chinensis* (MAO and XUE 1986; ZHANG 1994; YAN *et al.* 1995; HUANG *et al.* 1996; SHEN *et al.* 2007). In this study, the chromosome number of *I. lactea* and *I. lactea* var. *chinensis* are identical with $2n = 40$. Our results are consistent with DORONKIN and KRASNIKOV (1984), ZHANG (1994) and SHEN *et al.* (2007).

Most of the chromosomes of these two taxa are median centromeric or submedian centromeric chromosomes. Karyotype symmetries of both taxa belong to Stebbins's 2 B type. However, they are difference in the number of satellites. *Iris lactea* var. *chinensis* has three pairs of satellites, while no satellite is found in *I. lactea*. Furthermore, *I. lactea* var. *chinensis* has 6 sub-terminal centromeric chromosomes, but *I. lactea* just has two. The differences observed between the karyotypes of *I. lactea* and *I. lactea* var. *chinensis* give to our consideration of *I. lactea* var. *chinensis* as a variety of *I. lactea*.

Karyotypes of I. tectorum, I. germanica and I. chrysographes - Chromosome number of $2n = 28, 32$ and 36 had been observed in *I. tectorum* (MAO *et al.* 1986; DONG *et al.* 1994; LU 2000; QIN *et al.* 2002). In this study, *Iris tectorum* has chromosome number of $2n = 28$, which is consistent with LU (2000) and QIN *et al.* (2002). The karyotype of *I. tectorum* is $2n = 28 = 16m$ (4SAT) + $12sm$ (2SAT), while is different from the previous results from $2n = 4x = 28 = 16M + 4sm + 8st$ (LU 2000) and $2n = 28 = 10M + 18sm$ (QIN *et al.* 2002).

MAO and XUE (1986) reported that the chromosome number of *Iris germanica* was $2n = 28$. In the present study, the chromosome number of *I. germanica* is $2n = 40$, with the karyotype formula of $2n = 40 = 16m + 20sm + 4st$. *I. germanica* used in MAO and XUE (1986) was from Zhejiang, while the material of *I. germanica* in this study was collected from Sichuan. The materials from different places might result in the different chromosome number of *I. germanica*.

Previous studies showed that the chromosome number of *Iris chrysographes* is $2n = 40$ (DARLINGTON and WYLIE 1955; FEDOROV 1969; SHEN *et al.* 2007). In this study, the same chromosome number of $2n = 40$ in *I. chrysographes* was observed. The karyotype formula of *I. chrysographes* in this study is $2n = 40 = 18m +$

$22sm$ (2SAT) and the type is 2 B, which is different from the karyotype of $2n = 40 = 26m + 12sm + 2st$ and type of 2 A (SHEN *et al.* 2007). The reason might be attributed to the different geography distribution of *I. chrysographes*.

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