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KARYOTYPES OF *WHYTOCKIA* (GESNERIACEAE)WANG Yin-Zheng^{1,2} GU Zhi-Jian² HONG De-Yuan¹¹(Laboratory of Systematic and Evolutionary Botany, Institute of Botany, the Chinese Academy of Sciences, Beijing 100093)²(Kunming Institute of Botany, the Chinese Academy of Sciences, Kunming 650204)

Abstract The karyotypes of the genus *Whytockia* was investigated in order to reveal its systematic position in the tribe Klugieae and the evolution of karyotypes within this genus. The chromosome numbers are $2n = 18$ with their length over $2.0 \mu\text{m}$ in all the investigated species of *Whytockia*, which represents the most primitive characteristics in the tribe Klugieae. This paper suggests that $x = 9$ be the primitive basic number of chromosomes in the Klugieae. The morphology of the resting nuclei in *Whytockia* belongs to the complex chromocenter type. The chromosomes at mitotic prophase are classified into the gradient type. The evolution of karyotypes in *Whytockia* is toward increase of the chromosomes with submedian centromeres and especially those with subterminal centromeres. The karyotype of the variety *W. tsiangiana* (Hand.-Mazz.) A. Weber var. *wilsonii* A. Weber is distinctly different from that of *W. tsiangiana* var. *tsiangiana*, which conforms well with the gross morphology. This paper suggests that this variety be separated from *W. tsiangiana* as an independent species with affinity to *W. bijieensis*.

Key words Asymmetry; Basic number; Evolution; Karyotype; *Whytockia* W. W. Smith; Klugieae

The tribe Klugieae is characterized by a great diversity of morphological characters. Burtt(1977) stated that "it has all the appearance of a relict tribe comprising the surviving members of a numerous and highly diversified group". In respect to chromosomes, the Klugieae exhibits a remarkable diversity in chromosome number and a high proportion of polyploids and dysploids. Then, should it be interpreted as an increasing or decreasing series of dysploids? And what is its basic chromosome number?

The genus *Whytockia* endemic to China is considered as the most primitive representative in Klugieae. Six species and two varieties have been established since it was first described by Smith(1919) with only one species. However, there has been no information about chromosomes of *Whytockia* up to now. This paper aims to provide basic information on the karyotypes of *Whytockia*, a key genus in the phylogeny of the tribe Klugieae. Furthermore, it discusses the variation of chromosome numbers in the Klugieae and the karyotype evolution within *Whytockia*.

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1 Materials and Methods

The materials used in the present study are shown in Table 1. Root tips were used in cytological preparations. They were pretreated with a mixture of 0.002 mol/L 8-hydroxyquinoline and 0.1% colchicine (1:1) for 4 hrs at about 20 °C, and fixed in Carnoy's I (100% ethanol and glacial acetic acid, 3:1) for 1 hr at about 5 °C. The fixed materials were macerated in 1 mol/L HCl for about 1 min at 60 °C, stained with Carbol fuchsin and then squashed.

Table 1 The origin of materials

Species	Locality	Vouchers
<i>W. bijieensis</i> Y. Z. Wang	Bijie, Guizhou	Wang Yin-Zheng 94002(PE), 95001(KUN)
<i>W. hekouensis</i> Y. Z. Wang	Hekou, Yunnan	Wang Yin-Zheng 93008(PE)
<i>W. purpurascens</i> Y. Z. Wang	Hekou, Yunnan	Wang Yin-Zheng 93082(PE)
<i>W. tsiangiana</i> (Hand.-Mazz.) A. Weber		
var. <i>tsiangiana</i> A. Weber	Jiangkou, Guizhou	Wang Yin-Zheng 94001(PE)
var. <i>wilsoni</i> A. Weber	Leibo, Sichuan	Wang Yin-Zheng 93026(PE)

Karyotypes were analyzed following Li(1991). The chromosome numbers were counted based on 15 cells. The terminology and treatment describing the resting nucleus and chromosomes at prophase referred to Tanaka(1980, 1971). The parameters of karyotypes came from the measurement of chromosomes in 5 cells. The asymmetry of karyotypes were classified according to Stebbins(1971).

2 Results

2.1 *Whytockia bijieensis* Y. Z. Wang et Z. Y. Li(Wang and Li, 1997)

At the resting stage (Fig. 1: 1), the nucleus contains 15~20 rod-shaped or ellipsoidal heteropycnotic bodies with the diameter of 1~2 µm. The other chromatin of the nucleus consists of chromomeres and chromonemata which are scattered almost homogeneously within the entire region of the nucleus. This type of nucleus is referred to as the complex chromo-center type of Tanaka(1971).

At the mitotic prophase(Fig. 1: 2), all chromosomes have early condensed segments at proximal region of one or both arms. Some of them have early condensed segments not only at the proximal regions of one or both arms but also at the interstitial and terminal regions of one arm. Early and late condensed segments of chromosomes show a gradual transition between them. This type of prophase chromosomes is referred to as the gradient type of Tanaka (1980)

At the mitotic metaphase (Fig. 1: 3, 10), 18 chromosomes are 2.08~3.65(\bar{x} =2.90) µm long(Table 2), and vary gradually in length. Of the 18 chromosomes, there are one pair of M-chromosomes (Fig. 1: 10, No. 2), four pairs of m-chromosomes (Fig. 1: 10, Nos. 4, 5, 6, 9) and four pairs of sm-chromosomes (Fig. 1: 10, Nos. 1, 3, 7, 8). The karyotype is formulated as $2n=2M+8m+8sm(1sat)$ (Table 2). One chromosome of the third pair has a satellite at the terminal region of the short arm (Fig. 1: 10). The asymmetry of karyotype is classified as 2A.

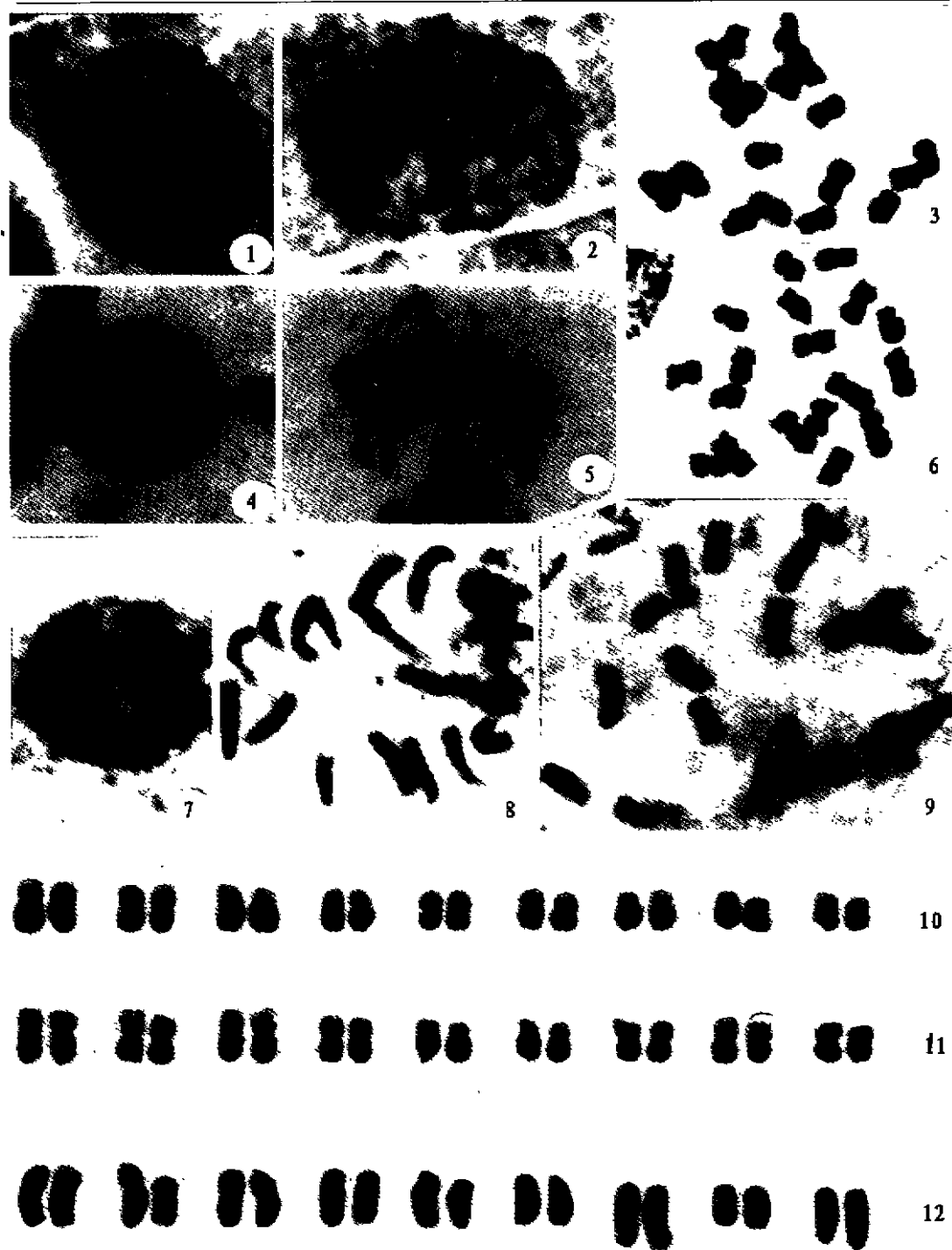


Fig. 1 Microphotographs of karyotypes of *W. bijeensis*, *W. tsiangiana* var. *wilsonii* and *W. hekouensis* ($\times 2000$)
 1~3 *W. bijeensis* 1. Resting nucleus; 2. Mitotic prophase chromosomes; 3. Mitotic metaphase chromosomes 4~6. *W. tsiangiana* var. *wilsonii* 4. Resting nucleus; 5. Mitotic prophase chromosomes; 6. Mitotic metaphase chromosomes. 7~9. *W. hekouensis* 7. Resting nucleus; 8. Mitotic prophase chromosomes; 9. Mitotic metaphase chromosomes. 10~12. Karyograms of *W. bijeensis*(10), *W. tsiangiana* var. *wilsonii*(11) and *W. hekouensis*(12).

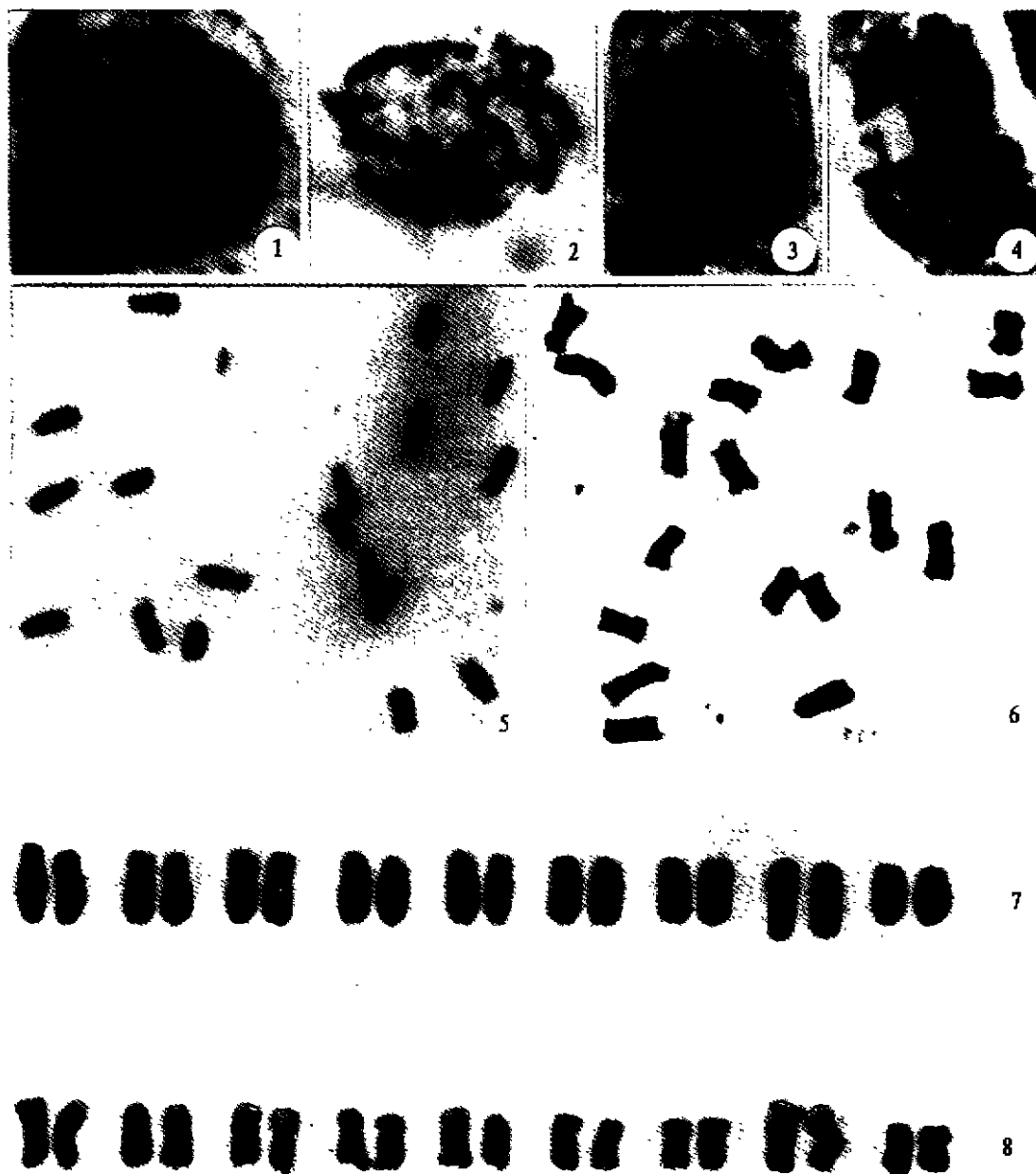


Fig. 2 Microphotographs of karyotypes of *W. purpurascens* and *W. tsiangiana* var. *tsiangiana*
 1~2. *W. purpurascens* ($\times 2000$) 1. Resting nucleus; 2. Mitotic prophase chromosomes. 3~4. *W. tsiangiana* var. *tsiangiana* ($\times 2000$) 3. Resting nucleus; 4. Mitotic prophase chromosomes. 5. Mitotic metaphase chromosomes of *W. purpurascens* ($\times 1500$). 6. Mitotic metaphase chromosomes of *W. tsiangiana* var. *tsiangiana* ($\times 2000$). 7~8. Karyograms of *W. purpurascens* (7, $\times 2500$) and *W. tsiangiana* var. *tsiangiana* (8, $\times 2000$).

2.2 *Whytockia hekouensis* Y. Z. Wang (Wang, 1995)

At the resting stage (Fig. 1: 7), many rod-shaped heteropycnotic bodies are distributed almost throughout the entire region of the nucleus. This type of nucleus is referred to as the complex chromocenter type of Tanaka (1971).

At the mitotic prophase (Fig. 1: 8), all chromosomes have early condensed segments at proximal region of one or both arms. Some of them have early condensed segments at the terminal region of one arm. This prophase chromosomes is referred to as the gradient type of Tanaka (1980).

At the mitotic metaphase (Fig. 1: 9, 12), 18 chromosomes are $2.28 \sim 4.55 (\bar{x} = 3.63)$ μm long (Table 2), and vary gradually in length from the longest to the shortest. Of the 18 chromosomes, two pairs are median (Fig. 1: 12, Nos. 3, 9), five pairs submedian (Fig. 1: 12, Nos. 1, 2, 6, 7, 8) and two pairs subterminal (Fig. 1: 12, Nos. 4, 5). The karyotype is formulated as $2n = 4m + 10sm(1sat) + 4st$ (Table 2). One chromosome of the eighth pair has a satellite at the terminal region of the short arm (not shown). Seven chromosomes (Fig. 1: 12, Nos. 1, 3, 4, 8, 9) have secondary constriction at the interstitial region of long arms. The asymmetry of karyotype is classified as 3A.

Table 2 The parameters of chromosomes in *Whytockia*

Chr. No.	<i>W. bijieensis</i> $2n = 18 = 2M + 8m + 8sm(1sat)$			<i>W. hekouensis</i> $2n = 18 = 4m + 10sm(1sat) + 4st$			<i>W. purpurascens</i> $2n = 18 = 4m + 6sm + 8st(1sat)$		
	RL	AR	Type	RL	AR	Type	RL	AR	Type
1	14.86	1.74	sm	13.88	1.72	sm	12.23	2.90	sm
2	12.52	1.00	M	12.95	2.12	sm	11.98	1.92	sm
3	11.99	1.80	sm(1sat)	12.75	1.16	m	11.76	1.63	m
4	11.14	1.20	m	11.74	3.14	st	11.58	4.34	st
5	10.78	1.06	m	11.37	3.72	st	11.04	2.52	sm
6	9.91	1.49	m	10.83	2.39	sm	10.66	4.33	st
7	9.87	2.67	sm	9.10	2.76	sm	10.62	1.15	m
8	9.56	2.51	sm	8.76	2.88	sm(1sat)	10.31	3.74	st(1sat)
9	9.36	1.07	m	8.61	1.32	m	9.86	5.32	st

Chr. No.	<i>W. tsiangiana</i> var. <i>tsiangiana</i> $2n = 18 = 4m + 8sm(2sat) + 6st$			<i>W. tsiangiana</i> var. <i>wilsoni</i> $2n = 18 = 2M + 8m(1sat) + 8sm(2sat)$		
	RL	AR	Type	RL	AR	Type
1	13.15	3.74	st	13.69	1.99	sm
2	12.31	1.82	sm	12.50	1.00	M
3	12.00	1.29	m	12.27	2.09	sm(2sat)
4	11.59	2.78	sm	11.47	1.29	m
5	10.71	4.71	st	10.51	2.93	sm
6	10.56	3.21	st	10.38	2.42	sm
7	10.13	2.11	sm	10.01	1.64	m
8	9.84	1.77	sm(2sat)	9.85	1.61	m(1sat)
9	9.71	1.20	m	9.32	1.05	m

Note: RL = relative length; AR = arm ratio; sat = satellite

2.3 *Whytockia purpurascens* Y. Z. Wang (Wang, 1995)

This species has the complex type of the resting nucleus (Fig. 2: 1) and the gradient type of the prophase chromosomes (Fig. 2: 2). At the mitotic metaphase (Fig. 2: 5, 7), 18 chromosomes are $2.84 \sim 4.08 (\bar{x} = 3.55)$ μm long (Table 2), and vary gradually in length. Of the 18 chromosomes, two pairs are median in centromeric position (Fig. 2: 7, Nos. 3, 7), three pairs submedian (Fig. 2: 7, Nos. 1, 2, 5) and four pairs subterminal (Fig. 2: 7, Nos. 4, 6, 8, 9). The karyotype is formulated as $2n = 4m + 6sm + 8st(1sat)$ (Table 2). One chromosome of the eighth pair has a satellite at the terminal region of the short arm (not

shown). Ten chromosomes (Fig. 2: 7, Nos. 1, 3, 4, 5, 6, 7, 8) have a secondary constriction at the interstitial region of long arm. The asymmetry of karyotype is classified as 3A.

2.4 *Whytockia tsiangiana* var. *tsiangiana* A. Weber

The resting nucleus (Fig. 2: 3) and the prophase chromosome morphology (Fig. 2: 4) are similar to that of *W. purpurascens* described above.

At the mitotic metaphase (Fig. 2: 6, 8), 18 chromosomes are $2.93 \sim 4.56$ ($\bar{x} = 3.44$) μm long (Table 2), and vary gradually in length from the longest to the shortest. Of the 18 chromosomes, two pairs are median (Fig. 2: 8, Nos. 3, 9), four pairs submedian (Fig. 2: 8, Nos. 2, 4, 7, 8) and three pairs subterminal (Fig. 2: 8, Nos. 1, 5, 6). The karyotype is formulated as $2n = 4m + 8sm(2sat) + 6st$ (Table 2; Fig. 2: 8). Eleven chromosomes (Fig. 2: 8, Nos. 1, 2, 3, 4, 5, 7, 8) have a secondary constriction at the interstitial region of long arm. The eighth pair chromosomes have a satellite respectively at the terminal region of the short arm (Fig. 2: 6, 8; Table 2). The karyotype is classified as 3A.

2.5 *W. tsiangiana* var. *wilsonii* A. Weber

At the resting stage (Fig. 1: 4), the nucleus contains many small ellipsoidal to rod-shaped or spherical chromocenters with $0.7 \sim 1.6 \mu\text{m}$ in diameters. The other chromatin of the nucleus consists of chromomeres and chromonemata which are distributed almost homogeneously throughout the entire region of the nucleus. This resting nucleus is referred to as the complex chromocenter type of Tanaka (1971).

The morphology of chromosomes at the mitotic prophase (Fig. 1: 5) is similar to that of *W. bijieensis* described above.

At the mitotic metaphase (Fig. 1: 6, 11), 18 chromosomes are $2.36 \sim 3.73$ ($\bar{x} = 2.61$) μm long (Table 2). They vary gradually in length. The karyotype is formulated as $2n = 2M + 8m(1sat) + 8sm(2sat)$ (Table 2; Fig. 1: 6, 11). Of the 18 chromosomes, there are one pair of M-chromosomes (Fig. 1: 11, No. 2), four pairs of m-chromosomes (Fig. 1: 11, Nos. 4, 7, 8, 9) and four pairs of sm-chromosomes (Fig. 1: 11, Nos. 1, 3, 5, 6). Three chromosomes (Fig. 1: 11, Nos. 3, 4) have a secondary constriction respectively at the interstitial region of long arm. Three chromosomes have a satellite respectively at the terminal region of the short arm (Fig. 1: 11, Nos. 3, 8). The asymmetry of karyotype is classified as 2A.

3 Discussion

On the basis of morphological data, the genus *Whytockia* is considered as the most primitive group in the tribe Klugieae from which the other genera have evolved (Wang *et al.*, 1996; Weber, 1982, 1978, 1977). Most genera in the Klugieae have a high proportion of polyploids and dysploids (Skog, 1984; Ratter, 1975). Among them, *Rhynchoglossum* has $2n = 20, 22, 36, 42, 54$, *Epithema* $2n = 16, 18, 24$, *Monophyllaea* $2n = 20, 22, 24, 32$, *Stauranthera* $n = 18, 20$, and one examined species of *Loronia* $n = 22$. $2n = 16$ in *Epithema* was examined only from one population of *E. saxatile*, which has $2n = 18$ chromosomes in most of other localities (A. Weber, pers. comm.). $2n = 16$ is probably a secondary reduction of chromosome numbers within the same species. The basic chromosome numbers of the above genera in the Klugieae form a continuous series $x = 8, 9, 10, 11$ and 12. On the contrary, the species examined in *Whytockia* show a constant chromosome number, $2n = 18$. Furthermore, chromosomes of all the genera in the Klugieae are generally less than $2.0 \mu\text{m}$ long except that of *Whytockia*. The longest chromosome examined in *Monophyllaea* is only $3 \mu\text{m}$ (A. Weber, pers. comm.), while the chromosomes in *Whytockia* is between $2 \sim 5 \mu\text{m}$ long, and the longest reaches $6 \mu\text{m}$. Apparently, *Whytockia* represents the most primitive

characteristics in chromosome number and size in the tribe Klugieae. In addition, the frequencies of $x=8, 9, 10, 11$ and 12 investigated up to date in the Klugieae are 8%, 40%, 28%, 12% and 12% respectively. $X=10$ occurs much more frequently in *Monophyllaea* than in the other genera. This genus has a close affinity to *Whytockia*, but is apparently highly advanced in morphology and anatomy. Thus, we suggest that $x=9$ be the primitive basic chromosome number of the tribe Klugieae.

Differences among the species within the genus *Whytockia* in karyotype mainly lie in frequency of median, submedian and subterminal chromosomes. *W. bijieensis* and *W. tsiangiana* var. *wilsonii* are characterized by having median and submedian chromosomes, and lacking subterminal ones. On the contrary, the other three taxa have the subterminal chromosomes but lack M-chromosomes. The proportion of the chromosomes over 2.0 of arm ratio is 22.2% in *W. bijieensis* and 33.3% in *W. tsiangiana* var. *wilsonii*. The asymmetry of karyotypes of these two taxa is classified as 2A. The proportion of the chromosomes over 2.0 of arm ratio is 55.6% in *W. hekouensis* and *W. tsiangiana* var. *tsiangiana*, and 66.7% in *W. purpurascens*. The asymmetry of karyotypes of these three taxa is classified as 3A. Even though the former two taxa have the similar karyotype, the asymmetry of karyotypes of *W. tsiangiana* var. *wilsonii* increases compared to *W. bijieensis*. The differences among the latter three taxa mainly lie in frequency of submedian and subterminal chromosomes. The frequencies of submedian chromosomes are 55.6% in *W. hekouensis*, 44.4% in *W. tsiangiana* var. *tsiangiana* and 33.3% in *W. purpurascens*. The frequencies of subterminal chromosomes are 22.2% in *W. hekouensis*, 33.3% in *W. tsiangiana* var. *tsiangiana* and 44.4% in *W. purpurascens*. Moreover, satellites have been observed respectively in the third and eighth pair chromosomes in the five investigated species and variety.

Whytockia bijieensis and *W. tsiangiana* var. *wilsonii* gather almost all the primitive characters of the genus *Whytockia*, that is, two free or almost free stigmata, erect stem, large corolla, lacking true compitum as a primitive state of syncarpy and so on. On the contrary, *W. purpurascens* and *W. tsiangiana* var. *tsiangiana* demonstrate a highly phylogenetical derivation in morphology and anatomy, as evidenced by two subconnated or wholly connated stigmata, a creeping stem, and the unilocular state in the upper part of ovary. However, *W. hekouensis* is characterized by a series of transitional features in *Whytockia*, such as erect stems, subconnated stigmata, a compital pore rather than unilocular state in the upper part of ovary. Taking the morphological and karyotypical information together into consideration, the evolution of karyotype in *Whytockia* seems toward increasing asymmetry. That is to say, the frequencies of submedian chromosomes, especially those of subterminal ones, increase in karyotypical evolution within the genus *Whytockia*.

In addition, A. Weber(1982) treated several local populations of this genus in Sichuan of China as a new variety—*W. tsiangiana* (Hand.-Mazz.) A. Weber var. *wilsonii* A. Weber. The karyotype of this variety is distinctly different from that of *W. tsiangiana* var. *tsiangiana* as demonstrated above. The difference in karyotype is well consistent with the gross morphology. The former possesses erect stems, two almost free stigmata, large and purplish red corolla 1.8~2.4 cm long, while the latter possesses creeping stems, capitate stigmata(two stigmata wholly connated), and small and white corolla 0.8~1.2 cm long. This variety is close to *W. bijieensis* in stigma and karyotype, but different from it in other distinctive characters of this variety. So, we suggest that this variety be separated from *W. tsiangiana* as an independent species in *Whytockia* with affinity to *W. bijieensis*.

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摘要 本文首次报道了中国特有异叶苣苔属的染色体数目及核型。该属所研究种类的染色体数目均为 $2n=18$, 染色体长度在 $2.0\ \mu\text{m}$ 以上, 在尖舌苣苔族所报道的染色体中显示出较原始的性状。尖舌苣苔族的染色体基数可能是 $x=9$ 。异叶苣苔属的间期核均为复杂型; 前期染色体呈渐变型。核型从对称型向不对称型的演化主要表现在近中部着丝粒, 尤其是近端部着丝粒染色体比例的增大。毕节异叶苣苔 *W. bijieensis* 和峨眉异叶苣苔 *W. tsiangiana* var. *wilsonii* 的核型分别为 $2n=2M+8m+8sm(1sat)$ 和 $2n=2M+8m(1sat)+8sm(2sat)$, 较为对称。紫红异叶苣苔 *W. purpurascens* 和白花异叶苣苔 *W. tsiangiana* var. *tsiangiana* 的核型分别为 $2n=4m+6sm+8st(1sat)$ 和 $2n=4m+8sm(2sat)+6st$, 比较特化。河口异叶苣苔 *W. hekouensis* 的核型是 $2n=4m+10sm(1sat)+4st$, 处于二者之间。峨眉异叶苣苔和原变种白花异叶苣苔的核型差异较大, 在外部形态方面二者之间的性状变异也间断较大。本文建议将该变种从白花异叶苣苔 *W. tsiangiana* 中移出自成一种, 并和毕节异叶苣苔近缘。

关键词 非对称; 染色体基数; 进化; 核型; 异叶苣苔属; 尖舌苣苔族