ORIGINAL ARTICLE

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Karyomorphology of *Maianthemum* sensu lato (Polygonatae, Ruscaceae)

Abstract We report results of karyotype analyses using nine species of *Maianthemum* from China. The species, except *M. atropurpureum* (with $2n = 72$), had $2n = 36$, and the results support the earlier suggestion that *Maianthemum* has $x = 18$ with $2n = 36$ in most species. The species examined, however, showed marked differences in karyotype, particularly in the numbers of metacentric, submetacentric, and acrocentric chromosomes as well as in the number of satellites. In addition, we distinguished three different modes based on the number of clear gaps in chromosome length variation: unimodal, bimodal, and trimodal. The unimodal variation (with no gap) was found in *M. dahuricum* and *M. atropurpureum*, the bimodal variation (with one gap) in *M. tatsienense*, and the trimodal variation (with two gaps) in *M. bifolium*, *M. forrestii*, *M. japonicum*, *M. henryi*, *M. purpureum*, and *M. lichiangense*. In the trimodal variation, the positions of the two gaps may differ from species to species. In addition, the frequency of acrocentric chromosomes per complement was generally higher in the trimodal variation than in the unimodal and bimodal variations. Results of our analyses, which had not been clearly presented prior to this, may provide a better understanding of species evolution in the tribe Polygonatae.

Key words Chromosome · Karyotype · *Maianthemum* · Polygonatae · Ruscaceae

Introduction

Maianthemum Wiggers sensu lato (including *Smilacina*), a genus of the tribe Polygonatae of Ruscaceae (Rudall et al.

2000; APGII 2003), comprises about 35 species distributed in eastern Asia and North America, with one species *M. bifolium* extending its distribution range from Asia to Europe (Wu and Raven 2000). In the Old World, the genus is represented by 19 species, which occur mainly in the Hengduan Mountains, southwest China (Li 1990). Recent morphological and molecular studies do not clearly show relationships of *Maianthemum* with other genera in Polygonatae (Rudall et al. 2000; Yamashita and Tamura 2000; Yamashita and Tamura 2004). Like other genera of Ruscaceae (including Convallariaceae), *Maianthemum* has been relatively well studied cytologically. Till now, 18 of the 35 species have been studied with respect to chromosome features (Table 1). Those cytological data show that there is a variation in karyotype of somatic chromosomes in *Maianthemum*, and that evaluation is needed as to which karyotype is basic or derived in the genus.

This paper provides results of our study on karyotype analyses of somatic chromosomes in nine Old World species of *Maianthemum*, and taken together with results of earlier studies, discusses how the genus is likely to be characterized according to chromosome characters. We will also discuss how we can use chromosome data in a future extensive study of *Maianthemum* and Polygonatae.

Materials and methods

Nine species of *Maianthemum* that were collected from China were examined in this study (Table 2). Voucher specimens are deposited in the Herbarium of the Kunming Institute of Botany, the Chinese Academy of Sciences (KUN). Individuals of those species were collected from their native habitats, transplanted in clay pots, and cultivated in the Botanical Garden of the Kunming Institute of Botany.

Root tips were collected from each individual and pretreated with a solution of 0.1% aqueous colchicines for 4–5 h at 20–21°C. After fixation by Carnoy solution (ethanol:acetic acid = 3:1) at 4°C, the root tips were disso-

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Table 1. Chromosome numbers of *Maianthemum* reported in earlier publications

Species	2n	References
M. atropurpureum	36	Wang et al. (1993)
M. bifolium	36, 42, and 54	Therman (1956); Kawano et al. (1967); Sokolovskaya and Probatova (1985); Laane and Lie (1985); Hong and Zu (1987)
M. canadense	36	Therman (1956); Kawano et al. (1967)
M. dahuricum	36	Hong and Zu (1987)
M. dilatatum	36	Therman (1956); Kawano et al. (1967)
M. fuscum	36, 54, and 72	Mehra and Sachdeva (1979); Gu et al. (1992); Wang et al. (1993); Gu and Sun (1998)
M. henryi	36	Hong and Zu (1987); Wang et al. (1993)
M. hondoensis	36	Hara and Kurosawa (1964)
M. japonicum	36	Hong and Zu (1987); Hong and Wilhelm (1990)
M. oleraceum	36	Mehra and Sachdeva (1979)
M. paniculatum	36	Kawano and Iltis (1963)
M. purpureum	36	Mehra and Sachdeva (1976)
M. racemosum	36, 72, and 144	Therman (1956); Kawano and Iltis (1963, 1966)
M. stellatum	36	Kawano and Iltis (1963)
M. tatsienense	36	Wang et al. (1990, 1993)
M. formosanum	36	Chuang et al. (1963)
M. trifolium	36	Therman (1956); Kawano and Iltis (1963)
M. yezoense	36	Hara and Kurosawa (1963)

Table 2. Species of *Maianthemum* examined in this study, and their respective collection data, chromosome number, karyotype formula, and mode of length variation

Species	Collection	2n	Karyotype formula	Mode of length variation
M. bifolium (L.) F.W. Schmidt	People's Republic of China. Yichun, Heilongjiang. *Meng Y 221*	36	12m + 10sm + 14st(2sat)	Trimodal
M. dahuricum (Turcz. ex Fisch. and C.A. Mey.) LaFrankie	People's Republic of China. Yichun, Heilongjiang. *Meng Y 214*	36	14m + 16sm + 6st(2sat)	Unimodal
M. forrestii (W.W. Sm.) LaFrankie	People's Republic of China. Lushui, Yunnan. *Meng Y 245*	36	14m + 16sm(1sat) + 6st	Trimodal
M. tatsienense (Franchet) LaFrankie	People's Republic of China. Lushui, Yunnan. *Meng Y 246*	36	18m + 10sm + 8st	Bimodal
M. japonicum (A. Gray) LaFrankie	People's Republic of China. Yichun, Heilongjiang. *Meng Y 223*	36	12m(2sat) + 14sm + 10st	Trimodal
M. henryi (Baker) LaFrankie	People's Republic of China. Lushui, Yunnan. *Meng Y 244*	36	10m + 14sm + 12st(2sat)	Trimodal
M. purpureum (Wall.) LaFrankie	People's Republic of China. Gonshan, Yunnan. *Meng Y 231*	36	12m + 10sm + 14st(2sat)	Trimodal
M. atropurpureum (Franchet) LaFrankie	People's Republic of China. Zhongdian, Yunnan. *Meng Y 251*	72	24m + 30sm + 18st	Unimodal
M. lichiangense (W.W. Sm.) LaFrankie	People's Republic of China. Zhongdian, Yunnan. *Meng Y 247*	36	14m + 12sm + 10st	Trimodal

ciated in a mixture of 1 N HCl and 45% acetic acid (1:1) for 30 s at 60°C, stained by 1% acetic orcein for 2–3 h, and squashed on a glass slide. Observations were made using nuclei at interphase, prophase and metaphase, and measurements of chromosome arms were based on at least five cells per individual.

Terminology of chromosome morphology based on the position of a centromere followed Levan et al. (1964). For comparison among different karyotypes at mitotic metaphase, a karyotype formula like $2n = 36 = 12m + 10sm + 14st(2sat)$ was used following Kim et al. (2003). In this example, the chromosomes comprise 12 metacentric chromosomes, 10 submetacentric chromosomes, and 14 acrocentric chromosomes (including two chromosomes with a satellite).

Results

In all nine species examined, the nucleus had many chromatin threads and chromomeres at interphase (Fig. 1), and they were darkly, densely stained throughout the whole chromosome at prophase (Fig. 2). Chromosomes at metaphase were counted $2n = 36$ or 72 (Fig 1, *3–11*). They were diverse in shape, having a centromere at the median, submedian, subterminal, or terminal position. Their sizes also varied and ranged from 21.5–2.4 μm. A satellite may be present on one, two or more chromosomes. Based on their shape and size, the chromosomes at metaphase showed a unimodal, bimodal, or trimodal arrangement. Descriptions of the metaphase chromosomes for individual species are given below.
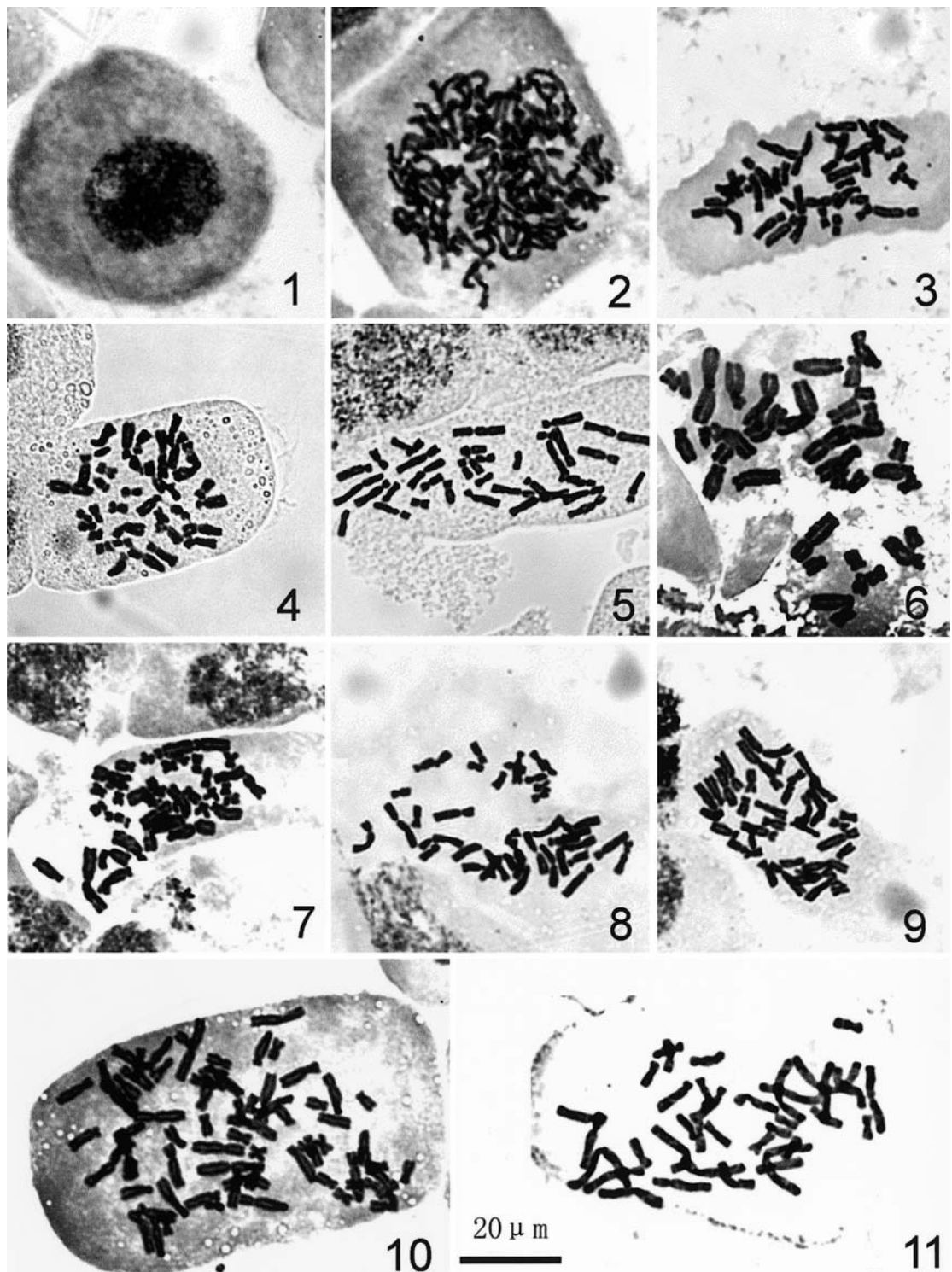
Fig. 1. Somatic chromosomes of *Maianthemum* in the resting nucleus, and at prophase and metaphase. *1* Resting nucleus. *2* Prophase. *3 M. bifolium*. *4 M. duhricum*. *5 M. forrestii*. *6 M. tatsienense*. *7 M. japonicum*. *8 M. henryi*. *9 M. purpureum*. *10 M. atropurpureum*. *11 M. lichiangense*. *Scale bars* equal 20 μm

Maianthemum bifolium

The chromosome number of *Maianthemum bifolium* was $2n = 36$, with the karyotype formula of 12m + 10sm + 14st(2sat). Satellites were observed in the proximal regions of short arms in the 11th pair of chromosomes. The chromosome length ranged from about 11.6 μm (the 1st and longest pair) to 3.2 μm (the 18th and shortest pair). However, there were clear gaps in chromosome length between the 1st pair and the subsequent 2nd to 10th pairs (about 9.2–

Fig. 2. Ideograms of somatic metaphase chromosomes of *Maianthemum*. *12 M. bifolium* (2n = 36). *13 M. duhricum* (2n = 36). *14 M. forrestii* (2n = 36). *15 M. tatsienense* (2n = 36). *16 M. japonicum* (2n = 36). *Scale bar* equals 10 μm

6.3 μm long) and further between the 10th pair and the subsequent 11th to 18th pairs (about 4.2–3.2 μm long) (Fig. 2). Thus the chromosomes showed a trimodal variation with respect to length. The chromosome features were similar to those reported by Kawano et al. (1967). In addition to 2n = 36, 42, and 54 have also been reported for the species (Table 1). These numbers may be erroneous.

Maianthemum dahuricum

The chromosome number of *Maianthemum dahuricum* was 2n = 36, with the karyotype formula of 14m + 16sm + 6st(2sat). Satellites were observed in the proximal regions of short arms in the 9th pair of chromosomes. The chromosome length gradually changed from about 9.7 to 2.9 μm

(Fig. 2, *13*). Since there was no clear gap in length between the chromosomes, the chromosomes were unimodal in variation. The karyotype differed from the one ($2n = 36 = 20m + 12sm + 2st + 2t[2sat]$) reported by Hong and Zu (1987). More individuals must be examined for reconfirmation.

Maianthemum forrestii

Maianthemum forrestii was investigated for the first time. The chromosome number was $2n = 36$, with the karyotype formula of $14m + 16sm(1sat) + 6st$. A satellite was observed in the interstitial region of the short arm of one of the 10th pair of chromosomes. The chromosomes showed a trimodal variation in length (Fig. 2, *14*). The chromosome length ranged from about 20.5 (to 14.8) µm (the 1st and longest pair) to 4.2 µm (the 18th and shortest pair). There were clear gaps in chromosome length between the 1st pair and the subsequent 2nd to 10th pairs (about 13.2–7.2 µm long) and further between the 10th pair and the subsequent 11th to 18th pairs (about 6.2–4.2 µm long).

Maianthemum tatsienense

The chromosome number of *Maianthemum tatsienense* was $2n = 36$, with the karyotype formula of $18m + 10sm + 8st$. Satellites were not observed. The chromosome length ranged from about 15.8 (to 14.5) µm long (the 1st and longest pair) to 3.7 µm (18th and shortest pair), showing a bimodal variation. The first pair was much longer than the others. The chromosomes from the 2nd pair to the 18th shortest pair gradually varied in length from about 12.1 to 3.7 µm (Fig. 2, *15*). The karyotype of this species differed from those reported earlier (i.e., $2n = 36 = 16m + 10sm + 10st[2sat]$ [Wang et al. 1990], and $2n = 36 = 22m + 2sm + 12st[2sat]$ [Wang et al. 1993]).

Maianthemum japonicum

The chromosome number of *Maianthemum japonicum* was $2n = 36$, with the karyotype formula of $12m(2sat) + 14sm + 10st$. Satellites were observed in the interstitial regions of short arms in the 10th pair of chromosomes. The chromosomes showed a trimodal variation in length. The chromosome length ranged from about 13.2 µm (the 1st and longest pair) to 2.4 µm (the 18th and shortest pair). There were clear gaps in chromosome length between the 1st pair and the subsequent 2nd through 8th pairs (about 8.4–5.3 µm long) and further between the 8th pair and the subsequent 9th through 18th pairs (about 4.0–2.4 µm long) (Fig. 2, *16*). The karyotype we reported here differed from those reported earlier (i.e., $2n = 36 = 20m + 6sm + 10st[2sat]$ [Hong and Zu 1987] and $2n = 36 = 20m[2sat] + 6sm + 10st$ [Hong and Wilhelm 1990]).

Maianthemum henryi

The chromosome number of *Maianthemum henryi* was $2n = 36$, with the karyotype formula of $10m + 14sm + 12st[2sat]$. Satellites were observed in the interstitial regions of short arms in 10th pair of chromosomes. The chromosomes showed a trimodal variation in length. The chromosome length ranged from about 13.2 µm (the 1st and longest pair) to 3.2 µm (the 18th and shortest pair). There were clear gaps in chromosome length between the 1st pair and the subsequent 2nd to 10th pairs (about 10.3–6.3 µm long) and further between the 10th pair and the subsequent 11th to 18th pairs (about 4.2–3.2 µm long) (Fig. 3, *17*). The karyotype we reported here differed from those reported earlier (i.e., $2n = 36 = 16m + 10sm + 10st[2sat]$ [Hong and Zu 1987] and $2n = 36 = 12m + 16sm + 6st + 2t[2sat]$ [Wang et al. 1993]).

Maianthemum purpureum

The chromosome number of *Maianthemum purpureum* was $2n = 36$, with the karyotype formula of $12m + 10sm + 14st[2sat]$. Satellites were observed in the interstitial regions of short arms in the ninth pair of chromosomes. The chromosomes showed a trimodal variation in length. The chromosome length ranged from about 12.6 µm (the 1st and longest pair) to 3.4 µm (the 18th and shortest pair). There were clear gaps in chromosome length between the 1st pair and the subsequent 2nd to 10th pairs (about 8.9–5.8 µm long) and further between the 10th pair and the subsequent 11th to 18th pairs (about 4.7–3.4 µm long) (Fig. 3, *18*). The karyotype we reported here agrees with the one reported previously by Mehra and Sachdeva (1976).
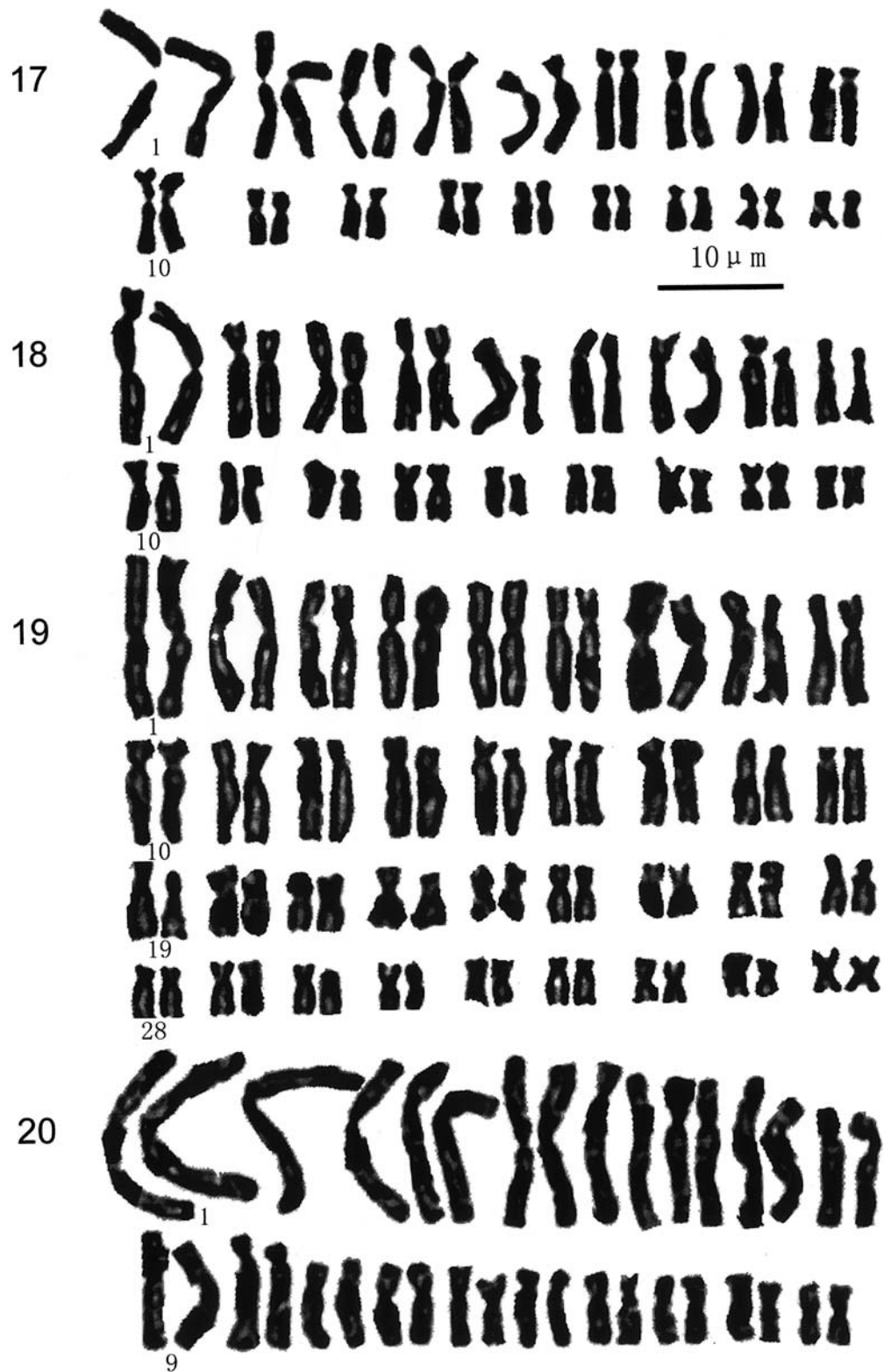
Maianthemum atropurpureum

The chromosome number of *Maianthemum atropurpureum* was $2n = 72$, which is the first record for the species. The karyotype formula is presented as $24m + 30sm + 18st$. Satellites were not observed. The chromosome length gradually changed from about 13.2 to 3.2 µm (Fig. 3, *19*). Since there was no clear gap in length among the chromosomes, the chromosomes were unimodal in variation.

Maianthemum lichiangense

The species *Maianthemum lichiangense* was investigated for the first time. The chromosome number was $2n = 36$, with the karyotype formula of $14m + 12sm + 10st$. Satellites were not observed. The chromosomes showed a trimodal variation in length. The chromosome length ranged from about 21.5 µm (the 1st and longest pair) to 4.7 µm (the 18th and shortest pair). There were clear gaps in chromosome length between the 1st to 7th pairs (about 21.5–12.4 µm long) and the subsequent 8th to 10th pairs (about 9.2–8.4 µm long), and further between the 8th to 10th pairs and the subsequent 11th to 18th pairs (about 7.1–4.7 µm long) (Fig. 3, *20*).

Fig. 3. Ideograms of somatic metaphase chromosome of *Maianthemum*. *17 M. henryi* (2n = 36). *18 M. purpureum* (2n = 36). *19 M. atropurpureum* (2n = 72). *20 M. lichiangense* (2n = 36). *Scale bar* equals 10 μm

Discussion

As presented above, eight of the nine species of *Maianthemum* investigated had 2n = 36. Only *M. atropurpureum* had 2n = 72, but the species is known to have 2n = 36 also (Wang et al. 1993). By putting these data together with information reported in earlier studies (see Table 1), we now have chromosome data from a total of about 20 species in *Maianthemum*. There is no doubt that *Maianthemum* has x = 18 with 2n = 36 in most species as already suggested (Tamura 1995). The species examined, however, showed marked differ-

ences in karyotype, particularly in the numbers of metacentric, submetacentric, and acrocentric chromosomes as well as in the number of satellites (Table 2). In addition, our karyotype data were often different from those reported in the earlier studies. There is a possibility that variation exists in karyotype among different individuals of the same species. This must be confirmed in future studies using more individuals from the same population as well as from different populations. Until it is confirmed whether a species has a consistent karyotype or not, the available karyotype data do not allow us to critically compare between species.

Nevertheless, our study also showed that there are three different modes in chromosome length variation: unimodal, bimodal, and trimodal. A few authors had also already noticed the presence of the gaps in chromosome length when chromosmes were serially arranged from long to short ones (Therman 1956; Kawano and Iltis 1966; Kawano et al. 1967; Sen 1974; LaFrankie 1986), but they did not distinguish the three variation modes as we did. The unimodal variation was found in *Maianthemum dahuricum* and *M. atropurpureum*, the bimodal variation in *M. tatsienense*, and the trimodal variation in *M. bifolium*, *M. forrestii*, *M. japonicum*, *M. henryi*, *M. purpureum*, and *M. lichiangense*. In both the bimodal and trimodal variations, the first pair of chromosomes was longer than the remainder, except in *M. lichiangense*, where the first gap existed between the 7th and 8th pairs. In the case of trimodal variation, another gap existed between the 10th and 11th pairs in *M. bifolium*, *M. forrestii*, *M. henryi*, *M. purpureum*, and *M. lichiangense*, but between the 8th and 9th pairs in *M. japonicum*. In addition, there was a general tendency that the frequency of acrocentric chromosomes per complement was higher in the trimodal variation (27–39% except in *M. forrestii* [with 22%]) than in the unimodal (11 and 25%) and bimodal variation (22%). Differences in the position of the first and second gaps in the trimodal variation as well as in the frequency of acrocentric chromosomes per complement may suggest the presence of reciprocal translocation among the chromosomes throughout the past species evolution.

Recently, Yamashita and Tamura (2004) discussed the discrimination of unimodal and trimodal variation in chromosome length in the tribe Convallarieae (with $x = 19$ and 18). In the unimodal variation, as in *Maianthemum*, there was no clear gap in chromosome length when chromosmes were arranged serially from long to short ones. On the other hand, the trimodal variation was composed of one pair of long chromosomes, seven pairs of medium-sized chromosomes, and 11 pairs of short chromosomes. In light of the phylogenetic tree generated by molecular evidence, Yamashita and Tamura (2004) showed that unimodal variation was plesiomorphic in the tribe, from which the trimodal variation was derived. In the case of *Maianthemum*, tribe Polygonatae, or even all other Ruscaceae as well, it is very likely that species sharing the same mode of chromosome length variation have close affinities.

Thus, while more species of *Maianthemum*, based on exact determination associated with voucher specimens, should be investigated with respect to their respective karyotypes, molecular analyses are required to clarify species and generic relationships. Resultant phylogenetic relationships would clarify trends of chromosome evolution. Recognition of the three modes in chromosome length variation as well as of additional differences within the trimodal variation we reported in this study will provide us new insight into a better understanding of species relationships and evolution.

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