Chromosome morphology of 12 species and one variety of *Lachenalia* and five species of closely related, allied genera (Liliaceae)

Shuichi Hamatani^{1,5}, Katsuhiko Kondo², Eiichi Kodaira^{3,4}, and Hisao Ogawa³

¹The Hiroshima Botanical Garden, 495 Kurashige 3, Saeki-ku, Hiroshima 731-5156, Japan;

²Laboratory of Plant Chromosome and Gene Stock, Graduate School of Science, Hiroshima University, 1-4-3 Kagamiyama, Higashi-Hiroshima 739-8526, Japan;

³The Kyoto Botanical Garden, Shimogamo-nakaragi-cho, Sakyo-ku, Kyoto 606-0823, Japan;

⁴Kyoto Herbal Garden, Takeda Pharmaceutical Company Limited, 11, Ichijoji Takenouchi-Cho, Sakyo-Ku, Kyoto 606-8134, Japan

⁵Author for correspondence (hamasyu@ma6.seikyoku.ne.jp)

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ABSTRACT. Somatic chromosomes in 12 species and one variety of *Lachenalia* and their closely related, two species of *Massonia* and three species of *Polyxena*, all in the Liliaceae, were studied. The resting nuclei of all of the taxa studied were commonly classified as the round prochromosome type. The mitotic prophase chromosomes of the taxa studied were commonly classified as the proximal type. However, their chromosome numbers varied from $2n=14$, 16, 18, 22, 24, 26 to 28. The chromosome numbers of $2n=14$ in *Lachenalia algoensis*, *L. aloides* ‘Lutea’, and *L. longibracteata*; $2n=18$ in *L. latimeriae*; and $2n=28$ in *Massonia pustulata* and *Polyxena longituba* were reported here for the first time. The 13 taxa of *Lachenalia* studied were classified karyotypically into six groups. The two species of *Massonia* studied showed commonly the bimodal karyotype with four large chromosomes and the three species of *Polyxena* studied showed commonly the bimodal karyotype with three large chromosomes. The species of *Massonia* displayed much larger chromosomes at mitotic metaphase than those of the other two genera.

KEYWORDS: *Lachenalia*, *Massonia*, *Polyxena*, Liliaceae, Karyotypes

Lachenalia is an ornamentally valuable genus in the Liliaceae or sometimes placed in the Hyacinthaceae. The genus is endemic to the west of southern Africa and consists of approximately 115 species (Duncan 1998). Cladistic analysis uses detailed data on variations in the characteristics of flowers and other organs (Duncan 1988; Duncan *et al.* 2005).

Fifty-eight species and eight varieties in the genus have indicated the aneuploid series with chromosome numbers of $2n=10$, 12, 14, 16, 17, 18, 21, 22, 23, 24, 26, 27, 28, 29, 30, 32, 40, 42, and 56 (*e.g.*, Moffett 1936; Fernandes and Neves 1962; Gows 1964; Ornduff and Watters 1978; Nordenstam 1982; Johnson and Brandham 1997; Hamatani *et al.* 1998, 2004). However, a few taxa have reported karyotypes; *e.g.*, 12 species of the genus were grouped by their karyotypes using microtome sectioning by Moffett (1936); correlationship between variation in chromosome number and presence of B-chromosomes was studied by Francis and Liebenberg (1990); 25 species and three varieties of the genus were classified into eight groups by their karyotypes (Hamatani *et al.* 1998, 2004). Those results were not consistent with the cladistic analysis by Duncan *et al.* (2005), which uses detailed data on variations in the characteristics of flowers and other organs. Moreover, it is hoped that the chromosomes of more species can be studied to validate the result of the cladistic analysis.

Massonia and *Polyxena* are close relatives of *Lachenalia*. Molecular studies using *atpB* and *trnL-F*

DNA sequences and the shape of the seeds among those three genera were compared (emphasized) by Pfosser *et al.* (2003). However, chromosomes of *Massonia* and *Polyxena* have been scarcely studied; *e.g.*, only a few species of the two genera have documented their chromosome numbers by Johnson and Brandham (1997).

The present study compared the karyotypes of the 12 species and one variety of *Lachenalia*, two species of *Massonia*, and three species of *Polyxena*.

MATERIALS AND METHODS

The plants used were listed in Table 1. They were cultivated in the Hiroshima Botanical Garden and the Kyoto Botanical Garden. Root tips were harvested, pretreated in 2 mM 8-hydroxyquinoline at 20°C for 2 h, and fixed and stored in 3:1 ethanol:acetic acid below 0°C for a few days. They were macerated in a 1:1 mixture of 45% acetic acid and 1 N hydrochloric acid at about 60°C for 1.5 min and then stained and squashed with 2% aceto-orcin. Data on the resting nuclei and mitotic prophase chromosomes were classified according to Tanaka (1980), and mitotic metaphase chromosomes were classified according to Levan *et al.* (1964) and Hamatani *et al.* (2004).

RESULTS AND DISCUSSION

The somatic chromosome numbers of the 12 species and

Table 1. Chromosome numbers of 17 species and one variety of *Lachenalia* and allied genera studied

Species and variety	Chromosome number (2n)		Reference
	Present count	Previous count	
<i>Lachenalia algoensis</i> Schonl.	14	-	
<i>L. aloides</i> (L.f.)Engl. 'Lutea'	14	-	
<i>L. juncifolia</i> Bak.	22	22	Johnson and Brandham 1997
<i>L. latifolia</i> Tratt.	24	24	Johnson and Brandham 1997
<i>L. latimerae</i> W.F.Barker	18	-	
<i>L. longibracteata</i> Phillips	14	-	
<i>L. muirii</i> W.F.Barker	14	14	Johnson and Brandham 1997
<i>L. namaquensis</i> Schltr. ex W.F.Barker	16	16	Johnson and Brandham 1997
<i>L. orchioides</i> (L.)Ait.	28	28	Johnson and Brandham 1997
var. <i>glaucina</i> (Jacq.)W.F.Barker			
<i>L. orchioides</i> (L.)Ait. var. <i>orchioides</i>	28	16	de Wet 1957
		16,17	Moffett 1936
		28,29	Johnson and Brandham 1997
<i>L. pusilla</i> Jacq.	14	14	Johnson and Brandham 1997
			Hamatani, Hashimoto and Kondo 1998
		16	Nordenstam 1982
<i>L. rosea</i> Andrews	14	14	Johnson and Brandham 1997
<i>L. viridiflora</i> W.F.Barker	14	14	Nordenstam 1982
			Hancke and Liebenberg 1990
			Johnson and Brandham 1997
<i>Massonia depressa</i> Houtt.	26	26	Johnson and Brandham 1997
<i>M. pustulata</i> Jacq.	28	22	Johnson and Brandham 1997
<i>Polyxena ensifolia</i> (Thunb.)Schönland	26	24,26	Johnson and Brandham 1997
<i>P. longituba</i> A.M. van der Merwe	28	-	
<i>P. paucifolia</i> (W.F.Barker)	26	24, 26	Johnson and Brandham 1997
A.M.van der Merwe et Manning.			

Table 2. Karyotype of 17 species and one variety of *Lachenalia* and allied genera studied

Species and variety	Chromosome number (2n)	Description by chromosome length*	Classification advocated by
			Hamatani et al. (2004)
<i>Lachenalia algoensis</i>	14	6L + 8S	Group 2
<i>L. aloides</i> 'Lutea'	14	2L + 4M + 8S	Group 1
<i>L. juncifolia</i>	22	gradual decrease	Group 5
<i>L. latifolia</i>	24	gradual decrease	Group 5
<i>L. latimerae</i>	18	3L + 15S	New Group 9
<i>L. longibracteata</i>	14	gradual decrease	Group 3
<i>L. muiri</i>	14	2L + 12S	Group 1
<i>L. namaquensis</i>	16	4L + 12S	Group 4
<i>L. orchioides</i> var. <i>glaucina</i>	28	gradual decrease	Group 3
<i>L. orchioides</i> var. <i>orchioides</i>	28	gradual decrease	Group 3
<i>L. pusilla</i>	14	gradual decrease	Group 3
<i>L. rosea</i>	14	6L + 8S	Group 2
<i>L. viridiflora</i>	14	6L + 8S	Group 2
<i>Massonia depressa</i>	26	4L + 22S	-
<i>M. pustulata</i>	28	4L + 24S	-
<i>Polyxena ensifolia</i>	26	3L + 23S	-
<i>P. longituba</i>	28	3L + 25S	-
<i>P. paucifolia</i>	26	3L + 23S	-

* "L" means "large", "M" means "medium" and "S" means "small", relatively.

Fig. 1. Somatic chromosomes at resting stage in *Lachenalia*, *Massonia*, and *Polyxena* studied. A. *Lachenalia algoensis*, B. *L. aloides* 'Lutea', C. *L. juncifolia*, D. *L. latifolia*, E. *L. latimeriae*, F. *L. longibracteata*, G. *L. muirii*, H. *L. namaquensis*, I. *L. orchioides* var. *glaucina*, J. *L. orchioides* var. *orchioides*, K. *L. pusilla*, L. *L. rosea*, M. *L. viridiflora*, N. *Massonia depressa*, O. *M. pustulata*, P. *Polyxena ensifolia*, Q. *P. odorata*, R. *P. paucifolia*. Bar = 5 μ m.

Fig. 2. Somatic chromosomes at mitotic prophase in *Lachenalia*, *Massonia*, and *Polyxena* studied. A. *Lachenalia algoensis*, B. *L. aloides* 'Lutea', C. *L. juncifolia*, D. *L. latifolia*, E. *L. latimeriae*, F. *L. longibracteata*, G. *L. muirii*, H. *L. namaquensis*, I. *L. orchioides* var. *glaucina*, J. *L. orchioides* var. *orchioides*, K. *L. pusilla*, L. *L. rosea*, M. *L. viridiflora*, N. *Massonia depressa*, O. *M. pustulata*, P. *Polyxena ensifolia*, Q. *P. odorata*, R. *P. paucifolia*. Bar = 5 μ m.

Fig. 3. Somatic chromosomes at mitotic metaphase in *Lachenalia*, *Massonia*, and *Polyxena* studied.

A. *Lachenalia algoensis*, B. *L. aloides* 'Lutea', C. *L. juncifolia*, D. *L. latifolia*, E. *L. latimerae*, F. *L. longibracteata*, G. *L. muiirii*, H. *L. namaquensis*, I. *L. orchiodes* var. *glaucina*, J. *L. orchiodes* var. *orchiodes*, K. *L. pusilla*, L. *L. rosea*, M. *L. viridiflora*, N. *Massonia depressa*, O. *M. pustulata*, P. *Polyxena ensifolia*, Q. *P. odorata*, R. *P. paucifolia*. Bar = 5 μ m.

Fig. 4. Karyotypes in *Lachenalia*, *Massonia*, and *Polyxena* studied.

A. *Lachenalia algoensis*, B. *L. aloides* 'Lutea', C. *L. juncifolia*, D. *L. latifolia*, E. *L. latimerae*, F. *L. longibracteata*, G. *L. muirii*, H. *L. namaquensis*, I. *L. orchoides* var. *glaucina*, J. *L. orchoides* var. *orchoides*, K. *L. pusilla*, L. *L. rosea*, M. *L. viridiflora*, N. *Massonia depressa*, O. *M. pustulata*, P. *Polyxena ensifolia*, Q. *P. odorata*, R. *P. paucifolia*. Bars = 5 μ m.

one variety of *Lachenalia*, two species of *Massonia* and three species of *Polyxena* studied were shown in Table 1. Resting and mitotic prophase and metaphase chromosomes were shown in Figs. 1-3. Their karyotypes are shown in Fig. 4.

The resting nuclei of the 18 taxa studied were of the round prochromosome type (Fig. 1). The mitotic prophase chromosomes of the taxa were of the proximal type (Fig. 2).

The chromosome number of $2n=14$ was found in *Lachenalia algoensis*, *L. aloides* 'Lutea' and *L. longibracteata*; the chromosome number of $2n=18$ was found in *L. latimerae*; the chromosome number of $2n=26$ was found in *Polyxena paucifolia*; and the chromosome number of $2n=28$ was found in *P. longituba*. They were all reported here for the first time. The chromosome number of $2n=28$ in *Massonia pustulata* given here was different from the previous count by Johnson and

Fig. 4. Continued.

Brandham (1997). The chromosome numbers of those of the other 11 taxa studied verified the previous counts. The chromosome numbers of *L. orchioides* var. *orchioides* was previously reported to be $2n=16$ by de Wet (1957), $2n=16$ and 17 by Moffett (1936), $2n=28$ and 29 by Johnson and Brandham (1997). Thus, the chromosome count obtained in the present study supported those reported by Johnson and Brandham (1997).

The karyotypes of the taxa studied were described in Table 2. The 12 species and one variety of *Lachenalia* studied were classified into six groups according to the karyotype system of Hamatani *et al.* (2004). *Lachenalia aloides* 'Lutea' and *L. muiirii* ($2n=14$) showed a bimodal karyotype with two large-size chromosomes and 12 small-size chromosomes, and thus, were classified as the Group 1. It was suggested that the 12 small-size chromosomes of *L. aloides* 'Lutea' might be divided from four medium-size chromosomes and eight small-size chromosomes. *Lachenalia algoensis*, *L. rosea*, and *L. viridiflora* ($2n=14$) showed a bimodal karyotype with six large-size chromosomes and eight small-size chromosomes, and were classified as the Group 2. In the karyotypes of *L. longibracteata* and *L. pusilla* ($2n=14$), the chromosomes showed a gradual decrease in size from the largest to the smallest chromosomes. *Lachenalia pusilla* was previously described to have the bimodal karyotype which was classified as the Group 2 of the

karyotype system of Hamatani *et al.* (1998). However, the present strain of the species showed a monomodal karyotype of a gradual decrease in size from the largest to the smallest chromosomes. Additionally, the karyotypes of *L. longibracteata* and *L. pusilla* had commonly smaller chromosomes than the other species did. They were classified as the Group 3 (gradual decrease, $2n = 14$ and 42 with $x=7$). Since the karyotypes of *L. orchioides* var. *glaucina* ($2n=28$) and *L. orchioides* var. *orchioides* ($2n=28$) showed a gradual decrease in size from the largest to the smallest chromosomes (monomodal) and had the basic chromosome number of $x = 7$, these two taxa could also be classified in the Group 3. *Lachenalia namaquensis* ($2n=16$) could be classified in the Group 4, since the species showed a bimodal karyotype with four large chromosomes and the other 12 small chromosomes. The karyotypes of *L. juncifolia* ($2n = 22$) and *L. latifolia* ($2n = 24$) shown commonly gradual decrease in size from the largest to the smallest chromosomes were classified in the Group 5. The karyotype system of Hamatani *et al.* (2004) suggested to include another three groups in addition to the above five groups mentioned: The Group 6 was characterized by a bi-modal karyotype with two large chromosomes and the chromosome number of $2n=22$, or that with four large chromosomes and the chromosome number of $2n=44$; the Group 7 was characterized by a bimodal karyotype

with three large chromosomes ($2n=22$); and the Group 8 was characterized by a bimodal karyotype with two large chromosomes ($2n=12$). Thus, the monomodal karyotype of *L. latimerae* ($2n=18$) shown a gradual decrease in size from the largest to the smallest chromosomes was regarded to have the basic chromosome number of $x=9$ and did not belong to any of the above eight groups. Thus, the karyotype of *L. latimerae* might organize another new Group 9.

Johnson and Brandham (1997) stated that the basic chromosome numbers of *Lachenaria* would be $x=5, 6, 7, 8, 9, 10, 11, 13$, and 15 and the most frequently occurred, basic chromosome numbers of $x=7$ and 8 . Our present and past papers (Hamatani *et al.* 1998, 2004) also suggested that the most frequent, basic chromosome numbers were $x=7$ and 8 . The basic chromosome numbers of $x=7$ and 8 in *Lachenaria* might contribute satisfactory speciation in the genus.

Lachenaria aloides 'Lutea' which had the bimodal karyotype with two large chromosomes could be placed in the Group 1. However, another strain of the taxon showed the trimodal karyotype of four medium-size chromosomes, two large-size chromosomes and eight small-size chromosomes. It might be a complex type of tri-modality with a gradual decrease in size from the largest to the smallest chromosomes of Group 3. *Lachenaria longibracteata* was also placed in the Group 3 that showed the karyotype of a gradual decrease in chromosome size from the largest to the smallest chromosomes but another strain of the species may also be placed in the Group 2 that showed bimodal karyotype with six large chromosomes. Many more study may deposit more diversity of karyotypes to make complex groupings of the Groups 1-3.

Lachenaria latimerae had three large chromosomes; two could form a pair but were different in arm ratio from the other one which had a satellite. Thus, this species might be originated from hybridization.

It is difficult to speculate the mutual relationships among the species that have the basic chromosome number of $x=7$, those that have the basic chromosome number of $x=8$, and those that have the basic chromosome numbers other than $x=7$ and 8 .

Massonia depressa showed the chromosome number of $2n=26$, and *M. pustulata* showed the chromosome number of $2n=28$. The strains studied in the two species had commonly bimodal karyotype with four large chromosomes. The four large chromosomes in the karyotype of *M. depressa* were submedian-centromeric, while those of *M. pustulata* were median-centromeric. In contrast, small chromosomes of the karyotypes studied in the two species were commonly either median-centromeric or submedian-centromeric.

Polyxena ensifolia and *P. paucifolia* showed the chromosome number of $2n=26$, and *P. longituba* showed the chromosome number of $2n=28$. The strains studied in

the three species had commonly bimodal karyotype with three large chromosomes. The karyotypes in each of the three species in *Polyxena* studied were different from each other and those in *Massonia*. Differences in size between the large and the small chromosomes in *Polyxena* were less than those in *Massonia*. One of the three large chromosomes common to the strains studied in the three species in *Polyxena* displayed particularly large arm ratios. Thus, the strains studied in the three species of *Polyxena* might be originated from hybridization.

The karyotypes in the strains studied in *Polyxena* were much more similar to those in *Lachenalia* than those in *Massonia* with respect to sizes of large and small chromosomes. This conclusion supports the molecular phylogenetic relationships using *atpB* and *trnL-F* DNA sequences by Pfosser *et al.* (2003).

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