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Chromosome numbers in *Orchidantha* (Lowiaceae) and their biogeographic and systematic implications

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The somatic chromosome numbers of *Orchidantha maxillarioides*, *O. insularis*, *O. chinensis* var. *chinensis,* and *O. chinensis* var. *longisepala* were counted, of which *O. insularis* and both varieties of *O. chinensis* are reported for the first time. The chromosome numbers of *O. maxillarioides* and *O. insularis* are 2n = 18, apparently diploid, while that of *O. chinensis* is 2n = 54, and therefore hexaploid (x = 9). We discuss the biogeographic and systematic implications of the different chromosome numbers and ploidy in *Orchidantha*.

Keywords: biogeography, chromosome number, cytotaxonomy, Lowiaceae, *Orchidantha*

Introduction

Orchidantha (Lowiaceae) comprises 16 species, one with two varieties (Larsen 1961, 1973, 1993, Wu 1964, Holttum 1970, Nagam & Sakai 1999, Wu & Kress 2000, Pedersen 2001, Jenjittikul & Larsen 2002). Few cytological data are available for the genus. Larsen (1966) reported 2n = 18 as the chromosome number of *O. maxillarioides* and recognized 9 as the basic chromosome number (x) and described most of the chromosomes as V-shaped and remarkably large, with a median–submedian kinetochore. Mahanty (1970) counted the same chromosome numbers and briefly described the karyotypes of *O. longiflora* and *O. maxillarioides*, which he formulated as 2n = 18 = 6m + 10sm + 2st, ranging in length from 4.3 μm to 6.6 μm and thus being the largest chromosomes in the Zingiberales. Larsen (1993) reported a new species, and its metaphase chromosomes were also determined to be 2n = 18. However, there has been no cytological study of the Chinese species of *Ochidantha* since Wu (1964) described them. The purposes of this paper are to report the chromosome numbers of *Orchidantha* species endemic to southern China, to redetermine those of *O. maxillarioides*, and to analyze the geographic relationships and systematic implications and ploidy of *Orchidantha* based on the cytological data.

Material and methods

The examined specimens are cited at the end of this paper. All the materials were collected from the ginger garden of the South China Botanic Garden, the Chinese Academy of Sciences. Voucher specimens are preserved in the herbarium of the South China Botanic Garden (BGSC).

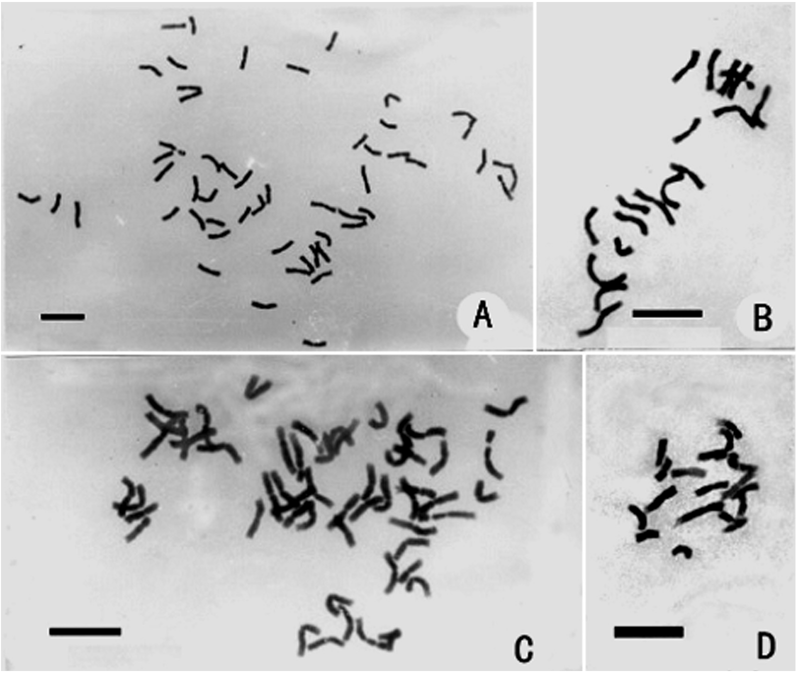

Fig. 1. Metaphase chromosomes of species of *Orchidantha*. — **A**: *O. chinensis* var. *chinensis*. — **B**: *O. maxillarioides*. — **C**: *O. chinensis* var. *longisepala*. — **D**: *O. insularis* (scale bars = 10 μm).

Vigorously growing root-tips were collected and pretreated with 8-hydroxy quinoline (0.002 mol l^{-1}) for two hours at 4 °C, then fixed in acetic-methyl alcohol (1:3) for more than two hours. After that, the material was treated according to Zhuang's (1990) method. Cells with well-dispersed chromosomes were observed and photographed with an Olympus Vanox Microscope.

Results

The somatic metaphase chromosome numbers of both *O. chinensis* var. *chinensis* and *O. chinensis* var. *longisepala* were determined to be 2n = 54 (Fig. 1A and C). Those of *O. insularis* and *O. maxillarioides* were determined to be 2n = 18 (Fig. 1B and D).

Discussion

The first report of the chromosome number of a species of *Orchidantha* was made for *O. maxillarioides* by Larsen (1966). Subsequently, Mahanty (1970) confirmed Larsen's count and also reported the chromosome number of *O. longiflora*. Larsen (1993) reported the chromosome number of *O. holttumii*; all of these have 2n = 18.

Our results confirm previous reports that the chromosome number of *O. maxillarioides* is 2n = 18. The number of *O. insularis* is also 2n = 18, but *O. chinensis* var. *chinensis* and *O. chinensis* var. *longisepala* are 2n = 54. According to Larsen (1966) x = 9 is the basic chromosome number of *Orchidantha*, so *O. insularis* is diploid, while *O. chinesis* var. *chinensis* and *O. chinensis* var. *longisepala* are hexaploid. *Orchidantha longisepala* was reported as a new species by Fang (1996), but considered to be a variety of *O. chinensis* by Wu (2000), because there is almost no morphological difference between *O. chinensis* and *O. longisepala* except in their labellum shape. The present cytological evidence supports Wu's notion. It is intriguing that there are only two ploidy levels, diploid and hexaploid, represented among the species studied in *Orchidantha*, and neither triploids nor tetraploids have been found yet. However, it is almost impossible for a hexaploid to derive directly from a diploid ancestor. A triploid or a tetraploid is likely to be an intermediate state between diploid and hexaploid.

The monogeneric family Lowiaceae, native to Asia, including Cambodia, Indonesia (Borneo), Malaysia, Laos, Vietnam and China (Guangdong, Guangxi and Hainan), is the only family in Zingiberales restricted to a single continent. The cytological data for five species hitherto studied indicate that only *O. chinensis* var. *chinensis* and *O. chinensis* var. *longisepala* are polyploid (hexaploid), while the other four species are all diploid. The hexaploid species occur in the northernmost area of the distribution of *Orchidantha*. Larsen (1966) regarded this genus as a relict group on the basis of its distribution, but he did not elaborate his views any further, nor suggest the initial area of origin and migratory paths of *Orchidantha*. Actually, it is premature to build a case for the initial area of origin and sequence of geographic dispersal for *Orchidantha* based only on the distribution of extant *Orchidantha*. Nevertheless, considered along with evidence for a phylogenetic position of the Lowiaceae related to other extant Zingiberales, especially the most basal position of the Musaceae and Strelitziaceae (Kress 2001), in combination with cytological data and evidence of musaceous seed fossils from the southern hemisphere (Manchester & Kress 1993), it is possible to introduce an alternative phytogeographic scenario: *Orchidantha* probably originated in South Africa, perhaps predating the breakup of Africa and India, with subsequent migration into Asia, and the common ancestor is extinct now.

Larsen (1966) discussed the basic chromosome number of Zingiberales, based on the limited cytological data and the classification at that time and recognized that the most frequent basic chromosome number was 9. Therefore, he suggested that the original basic number of Zingiberales should be nine. Mahanty (1970) suggested that 12 be the original basic number for the Zingiberaceae, probably derived from 11, which he considered to be the basic number for the extant Zingiberales as a whole. Currently, with the increased number of chromosome counts, the basic number of Zingiberales is probably 12, not 9 (Appendix). Nevertheless, it is quite inadequate to depend only on the cytological data to resolve the basic number problem of Zingiberales. Taking evidence from other subjects into consideration, especially morphological, anatomical and molecular evidence, the results should be more reliable. According to Kress's system (2001) of Zingiberales based on morphological and molecular data, the Lowiaceae, Strelitziaceae, Musaceae and Heliconiaceae constitute a basal paraphyletic group ancestral to the sister groups of Zingiberaceae + Costaceae and Cannaceae + Marantaceae, which together form a monophyletic terminal lineage. The relationship between the Lowiaceae and Strelitziaceae is a sister group with 99% bootstrap support. Moreover, the recent anatomical data of flowers and seeds further confirm that Lowiaceae is closely related to Strelitziaceae (Liao *et al*. 1998, 2001, Wen *et al*. 1999, Tang *et al*. 2000). On the other hand, it is notable that there are two kinds of basic number in Strelitziaceae: 11, the basic number of *Ravenala madagascariensis*, and 7, of *Strelitzia reginae* (Chen *et al*. 1990). While the habit of *R. madagascariensis* is a very tall, "woody herb", *S. reginae* is herbaceous. Generally, a woody habit is considered primitive and herbaceous advanced. Therefore, our conclusion is that it is more reasonable to regard 11 as the original basic number and nine as a derived basic number of Zingiberales. As to 12, we agree with Mahanty's (1970) view that 12 is the original basic number for the Zingiberaceae and probably derived from 11, which is the basic number for the extant Zingiberales as a whole.

GEOGRAPHIC ORIGIN OF EXAMINED SPECIMENS (all grown at Ginger garden of BGSC and vouchers deposited at that herbarium). — *Orchidantha chinensis* T.L. Wu: **China**. Guangdong, Xinyi, VI.2002 *Y. J. Tang & J. J. Song 02001*. — *Orchidantha chinensis* var. *longisepala* (D. Fang) T.L. Wu: **China**. Guangxi, Shiwandash, VI.2002 *Y. J. Tang & J. J. Song 02002*. — *Orchidantha insularis* T.L. Wu: **China**. Hainan, Diaoluo Mountain, VI.2002 *Y. J. Tang & J. J. Song 02003*. — *Orchidantha maxillarioides* (Ridl.) Schum.: **Singapore**. Botanical Garden of Singapore, VI.2002 *Y. J. Tang & J. J. Song 02004*.

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Appendix. The basic chromosome numbers of the genera examined in Zingiberales.

Family	Genus	Basic number (x)	Author
Musaceae	*Ensete*	9	Cheesman & Larter (1935)
	Musa	9,10,11	Simmonds (1954), Sharma *et al.* (1959)
	Musella	9	Isobe & Hashimoto (1994)
Strelitziaceae	*Phenakospermum*	11	Goldblatt & Manning (1989)
	Ravenala	11	Mahanty (1970), Cheesman & Larter (1935)
	Strelitzia	7,11	Goldblatt (1980), Chen & Huang (1990)
Lowiaceae	*Orchidantha*	9	Larsen (1966), present report
Heliconiaceae	*Heliconia*	11,12	Andersson (1984), Cheesman & Larter (1935)
Cannaceae	*Canna*	9	Bisson *et al.* (1968)
Marantaceae	*Calathea*	9,11	Mahanty (1970)
	Ctenanthe	10	Mahanty (1970)
	Ischnosiphon	21	Sato (1948)
	Maranta	12,13	Sato (1948)
	Marantochloa	13	Mahanty (1970)
	Monostagma	9	Sato (1948, 1960)
	Phrynium	9	Goldblatt (1980)
	Stromanthe	9,11	Sato (1960)
	Thalia	13	Mahanty (1970)
Costaceae	*Costus*	9	Chen *et al.* (1988), Mahanty (1970)
	Tapeinochilus	9	Mahanty (1970)
Zingiberaceae	*Aframomum*	12	Poulsen & Lock (1997)
	Alpinia	12	Chen & Huang (1989)
	Amomum	12	Sharma *et al.* (1959), Chen & Huang (1989)
	Boesenbergia	9,10,12	Chen & Huang (1989), Beltran & Kiew (1984), Saensouk & Larsen (2001)
	Burbidgea	12	Bisson *et al.* (1968)
	Caulokaempferia	10,12	Chen (1988)
	Cautleya	13,17	Sharma *et al.* (1959)
	Curcuma	21	Ramachandran (1969)
	Curcumorpha	25	Ramachandran (1969)
	Distichochlamys	13	Newman (1995)
	Elettariopsis	12	Beltran & Kiew (1984)
	Etlingera	12	Beltran & Kiew (1984)
	Globba	8,11,12	Lim (1972)
	Hedychium	17	Mahanty (1970)
	Hornstedia	12	Beltran & Kiew (1984), Chen *et al.* (1989)
	Kaempferia	11	Sharma *et al.* (1959)
	Plagiostachys	12	Chen *et al.* (1989)
	Pommereschea	11	Larsen (1973)
	Pyrgophyllum	21	Chen (1989)
	Roscoea	12	Sharma *et al.* (1959)
	Rhyncanthus	11	Chen *et al.* (1987)
	Scaphochlamys	14	Beltran & Kiew (1984)
	Siliquamomum	12	Chen (1989)
	Stahilianthus	11	Bisson *et al.* (1968)
	Zingiber	11	Sharma *et al.* (1959)