

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

Juan-Juan Song, Jing-Ping Liao, Yuan-Jiang Tang* & Zhong-Yi Chen

South China Botanic Garden, The Chinese Academy of Sciences, Guangzhou 510650, China
(*corresponding author's e-mail: yjtang@scib.ac.cn)

Received 20 Feb. 2004, revised version received 16 Apr. 2004, accepted 31 May 2004

Song, J. J., Liao, J. P., Tang, Y. J. & Chen, Z. Y. 2004: Chromosome numbers in *Orchidantha* (Lowiaceae) and their biogeographic and systematic implications. — *Ann. Bot. Fennici* 41: 429–433.

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 $4.3 \mu\text{m}$ to $6.6 \mu\text{m}$ 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 *Orchidantha* 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).

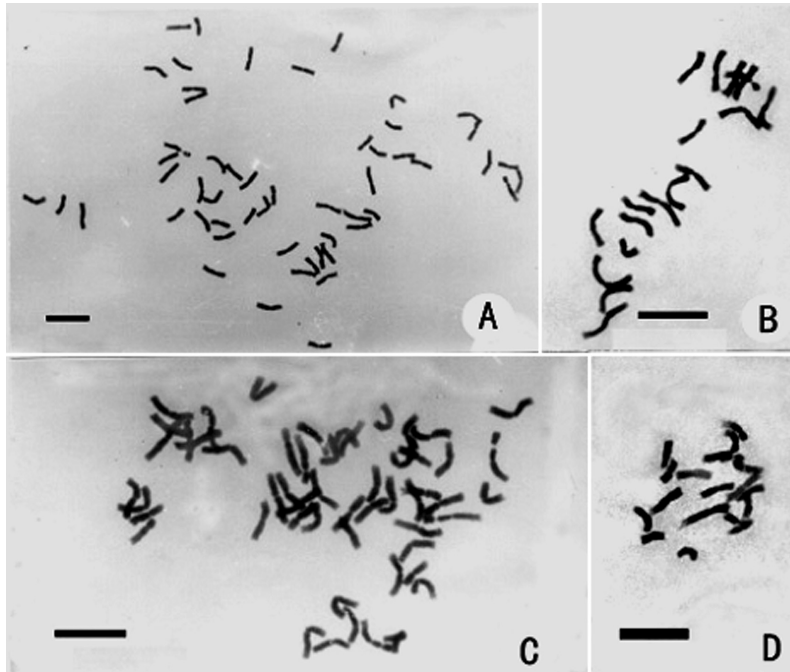


Fig. 1. Metaphase chromosomes of species of *Orchidantha*. — A: *O. chinensis* var. *chinensis*. — B: *O. maxillarioides*. — C: *O. chinensis* var. *longisepala*. — D: *O. insularis* (scale bars = 10 μ m).

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. chinensis* 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 phylogeographic 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.

Acknowledgements

The authors are very grateful to Prof. Qi-Gen Wu, Mr. Shen-jen Chen and Mr. Jian-Ping Fang for their comments on the manuscript and kind help in material collection. This research was supported by the National Natural Science Fund of China (No: 40332021), the National Natural Science Fund of China (No: 39470059) and the Technological Development Plan of Guangdong Environment Protection Bureau (No. 970165), China.

References

- Andersson, L. 1984: The chromosome number of Heliconia (Musaceae). — *Nordic J. Bot.* 4: 191–193.
- Beltran, I. C. & Kiew, K. 1984: Cytotaxonomic studies in the Zingiberaceae. — *Notes Roy. Bot. Garden Edinburgh* 41: 541–559.
- Bisson, S., Guillemet, S. & Hamel, J. L. 1968: Contribution à l'étude caryo-taxonomique des Scitamineées. — *Mem. Mus. Nat. d'Hist. Nat.* 13: 59–145.
- Cheesman, E. E. & Larter, L. N. H. 1935: Genetical and cytological studies of *Musa*. III. Chromosome numbers in the Musaceae. — *J. Genet.* 30: 31–52.
- Chen, Z. Y. 1989: Evolutionary patterns in cytology and pollen structure of Asian Zingiberaceae. — In: Holm-Nielsen, L. B., Nielsen, I. C. & Balslev, H. (eds.), *Tropical forests*: 185–191. Academic Press Ltd., London.
- Chen, Z. Y. & Huang, X. X. 1990: Cytological observation on *Strelitzia nicolai* Regel et Koern. — *Acta Bot. austro-Sin.* 6: 47–49.
- Chen, Z. Y., Chen, S. Z. & Huang, X. X. 1988: A report on chromosome numbers on Chinese Zingiberaceae (5). — *Guihaia* 8: 143–147.
- Chen, Z. Y., Chen, S. Z., Huang, X. X. & Huang, S. F. 1987: Reports on the chromosome numbers on Chinese species of Zingiberaceae (IV). — *Guihaia* 7: 39–44.
- Fang, D. & Qin, D. H. 1996: Five new species of monocotyledoneae from Guangxi. — *Guihaia* 16: 3–8.
- Goldblatt, P. 1980: Polyploidy in angiosperms: Monocotyledons. — In: Lewis, W. H. (ed.), *Polyploidy: biological relevance*: 219–239. Plenum Press, New York.
- Goldblatt, P. & Manning, G. C. 1989: Chromosome number in *Phenakospermum* and *Strelitzia* and the basic chromosome number in Strelitziaceae (Zingiberales). — *Ann. Missouri Bot. Garden* 76: 932–933.
- Holtum, R. E. 1970: The genus *Orchidantha* (Lowiaceae). — *Garden Bull. Sing.* 25: 239–246.
- Jenjittikul, T. & Larsen, K. 2002: *Orchidantha foetida* (Lowiaceae) a new species from Thailand. — *Nordic J. Bot.* 22: 405–408.
- Kress, W. J., Prince, L. M., Hahn, W. J. & Zimmer, E. A. 2001: Unraveling the evolutionary radiation of the families of the Zingiberales using morphological and molecular evidence. — *Syst. Biol.* 50: 926–944.
- Larsen, K. 1961: New species of *Veratrum* and *Orchidantha* from Thailand and Laos. — *Bot. Tidsskr.* 56: 345–350.
- Larsen, K. 1966: Chromosome cytology and relationship of the Lowiaceae. — *Nat. Hist. Bull. Siam Soc.* 21: 21–24.
- Larsen, K. 1973: A new species of *Orchidantha* (Lowiaceae) from Vietnam. — *Adansonia* 2: 481–482.
- Larsen, K. 1993: A new species of *Orchidantha* (Lowiaceae) from Borneo. — *Nordic J. Bot.* 13: 285–288.
- Liao, J. P., Wen, Y. Q. & Wu, Q. G. 1998: Studies on vascular system anatomy of *Orchidantha chinesis* flower. — *J. Trop. Subtrop. Bot.* 6: 275–282.
- Liao, J. P., Tang, Y. J., Wen, Y. Q. & Wu, Q. G. 2001: Studies on vascular system anatomy of the flower of *Musa coccinea* Andr. — *Guihaia* 21: 321–325.
- Lim, S. N. 1972: Cytogenetics and taxonomy of the genus *Globba* L. (Zingiberaceae) in Malaya IV Distribution in relation to polyploidy. — *Garden Bull. Sing.* 26: 115–126.
- Mahanty, H. K. 1970: A cytological study of the Zingiberales with special reference to their taxonomy. — *Cytologia* 35: 13–49.
- Manchester, S. R. & Kress, W. J. 1993: Fossil bananas (Musaceae): *Ensete oregonense* sp. nov. from the Eocene of western North America and its phylogeographic significance. — *Amer. J. Bot.* 80: 1264–1272.
- Minoru, I. & Hashimoto, K. 1994: The chromosome count of nine taxa in *Musa* and its allied genus *Musella*. — *Bull. Hiroshima Bot. Garden* 15: 7–11.
- Nagamasu, H. & Sakai, S. 1999: *Orchidantha inouei* (Lowiaceae), a new species from Borneo. — *Nordic J. Bot.* 19: 149–152.
- Newman, M. 1995: *Distichochlamys*, a new genus from Vietnam. — *Edinburgh J. Bot.* 52: 65–67.
- Pedersen, L. B. 2001: Four new species of *Orchidantha* (Lowiaceae) from Sabah. — *Nord. J. Bot.* 21: 121–128.
- Poulsen, A. D. & Lock, J. M. 1997: New species and new records of Zingiberaceae and Costaceae from tropical East Africa. — *Kew Bull.* 52: 601–616.
- Ramachandran, K. 1969: Chromosome number in Zingiberaceae. — *Cytologia* 34: 213–221.
- Saensouk, S. & Larsen, K. 2002: *Boesenbergia baimaii*, a new species of Zingiberaceae from Thailand. — *Nordic J. Bot.* 21: 595–597.
- Sato, D. 1948: The karyotype and phylogeny of Zingiberaceae. — *Jap. J. Genet.* 23: 44–45.
- Sato, D. 1960: The karyotype analysis in Zingiberales with special reference to the protokaryotype and stable karyotype. — *Sci. Pap. College General Educ. Univ. Tokyo* 10: 225–243.
- Sharma, A. K. & Bhattacharyya, N. K. 1959: Cytology of several members of Zingiberaceae and a study of the inconstancy of their chromosome complement. — *Cel-lule* 59: 299–345.
- Simmonds, N. W. 1954: Chromosome behaviour in some tropical plants. — *Heredity* 8: 139–146.
- Tang, Y. J., Liao, J. P., Wen, Y. Q. & Wu, Q. G. 2000: Studies on vascular system anatomy of *Strelitzia nicolai* flower. — *Acta Bot. Yunnanica* 22: 291–297.
- Wen, Y. Q. & Liao, J. P. 1999: Floral morphology and anatomy of *Orchidantha chinensis* (Lowiaceae). — *J. Trop. Subtrop. Bot.* 7: 329–336.
- Wu, T. L. 1964: Lowiaceae, a family new to the flora of China. — *Acta Phytotax. Sin.* 9: 335–343.
- Wu, T. L. & Kress, W. H. 2000: Lowiaceae. — In: Wu, Z. Y. & Raven, P. H. (eds.), *Flora of China* 24: 319. Sci. Press & Missouri Bot. Garden Press, Beijing & St. Louis.
- Zhuang, D. H., Kitajima, A., Ishida, M. & Sobajima, Y. 1990: Chromosome numbers of *Diospyros kaki* cultivars. — *J. Japan. Soc. Hort. Sci.* 59: 289–297.

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

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