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Facultative Apomixis in an Endangered Dioecious Species, *Woonyoungia septentrionalis* (Magnoliaceae)

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Abstract: The breeding system of an endangered dioecious species, *Woonyoungia septentrionalis* (Dandy) Law, was studied in two natural populations, the Mulun population and the Dahuangni population, in Guangxi Province, China from June 2001 to early September 2002. The female flowers from previously bagged buds were treated respectively with paper bags, net bags and hand pollination by comparing with natural pollination. The results showed that female flowers treated with paper bags, net bags, hand pollination, and natural pollination all set fruits and seeds, but there was great difference in their fruit set, seed set, pre-emergent reproductive success (PERS) and seed germination rate for two populations. In both populations, PERS of hand pollination and natural pollination were higher than those of paper bagged and net bagged treatments. Among them, PERS of hand pollination was the highest, and PERS of net bagged treatment was the lowest. Both sexual reproduction and apomixis occurred simultaneously in *W. septentrionalis* and seeds from both sexual and apomixis could germinate into seedlings. Therefore, the breeding system of *W. septentrionalis* belonged to facultative apomixis. The apomixis in Magnoliaceae was reported for the first time.

Key words: *Woonyoungia septentrionalis*; breeding systems; facultative apomixis; apospory

Dandy (1931) described a new species of the genus *Kmeria* based on a specimen with male flower buds (*R. C. Ching 5247*) collected by R. C. CHING from Tangjiapu, Southeast Luocheng County, Guangxi, China. The vegetation of its type locality has been destroyed, and it had not been found since then and has been regarded to be extinct. The species was eventually found again in Mulun, Huangjiang, Guangxi, China and two more populations were found later in Maolan, Libo, Guizhou and Gulinqing, Maguan, Yunnan, China (Law *et al.*, 1987; Law,1997). Law (1997) described it as a new monotypic genus, *Woonyoungia* Law. Its sole species, *W. septentrionalis* (Dandy) Law was listed as a critically endangered species (under state protection category Ⅰ) in 1999. Therefore, it is compelling to carry out studies on its conservation biology.

W. septentrionalis is a dioecious species. We carried out a preliminary study on its breeding system for the first time in Mulun, Huangjiang, Guangxi and Dahuangni, Luocheng, Guangxi from 2001 to 2002, aiming at providing data on the breeding system of *W. septentrionalis*, and shed light on the mechanisms that caused its endangered status.

1 Materials and Methods

1.1 Study site

This study was conducted outdoors in Hechi District, Guangxi Province, China from June 2001 to early September 2002. Its average annual temperature is 18.7 ℃ and its average annual rainfall is 1 638 mm, with most of the rainfall happens from April to August. Two study sites were chosen from Hechi District, about 100 km apart. The first is in Bannantun, Huangjiang County, located in 25°03' N and 107°58' E at altitude from 490 to 550 m, where *Woonyoungia septentrionalis* (Dandy) Law grows with *Styrax suberifolia*, *Loropetalum chinense*, *Schima superba*, *Micromelon integerrimum*, *Elaeocarpus japonicus*, *Xylosma longifolium*, *Cinnamomum parthenoxylon*, *Radermachera sinica*, *Cinnamomum camphora*, *Ormosia apiculata*, *Adenanthera pavonina*, *Cyclobalanopsis glauca*, *Ficus cyrtophylla*, *Sloanea sinensis*, and *Caryota monostachya* in the evergreen broad-leaved forest. The second is in Dahuangnitun, Luocheng County, located in 24°52' N and 108°49' E at altitude from 320 to 400 m, where the accompanying species of *W. septentrionalis* include *Castanopsis*

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hickelii, Castanopsis fabric, Cyclobalanopsis patelliformis, Celtis cinnamomea, Choerospondias axillaries, Engelhardtia roxburghiana, Aphananthe aspera, Ilex memecylifolia, Beilschmiedia tsangii, Photinia prunifolia, Loropetalum chinense, Radermachera sinica, Diospyros strigosa and *Tarenna mollissima*.

1.2 Breeding system

We marked the female flower buds from five individuals prior to anthesis with plastic tags and bagged them with water-proof paper bags. When the female flower buds began to bloom, we treated them respectively with paper bags (to check the possibility of apomixis), net bags (to check the possibility of anemophily and apomixis), hand pollination and natural pollination. The pollen used for hand pollination was collected from three male trees about 800 m apart from the pollinated female trees. We removed all bags 15 d later.

1.3 Fruit production

We collected ripe fruits in early September, recorded the total number of ripe fruit, full seeds and ovules, and calculated fruit set, seed set, weight of per 1 000 seeds, and PERS (pre-emergent reproductive success, Ashworth and Galetto, 2001). Calculation formulae are as follows: fruit set (%) = number of ripe fruits / number of treated flowers × 100%; seed set (%) = number of full seeds / number of ovules; PERS = fruit set × seed set (%).

1.4 Seed germination

We sowed the seeds in the sands in late September. Three months later we recorded the total number of seedlings germinated and calculated the seed germination rate. Calculation formula is as follows: seed germination rate (%) = number of seedlings germinated / number of full seeds.

2 Results

In the Mulun population and the Dahuangni population, some of the female flowers were treated with paper bags, net bags, hand pollination, and natural pollination set fruits and seeds, but there was great difference in their fruit set, seed set, PERS and seed germination rate for two populations, and there was no anemophily in *W. septentrionalis* (Tables 1, 2). In the Mulun population, fruit set of hand pollination and natural pollination were higher than 80%, and fruit set of hand pollination was slightly higher than that of natural pollination; fruit set of paper bagged treatment and net bagged treatment were lower than 50%, but seed set of the four treatments were similar. Seed set of net bagged treatment was slightly higher than that of paper bagged treatment; PERS was the same as fruit set; seeds from flowers of both paper bagged and net bagged treatment could germinate successfully; their seed germination rate were above 25.66%, and seed germination rate of net bagged treatment was up to 55.34%, near to that of hand pollination. In the Dahuangni population, its fruit set, seed set and PERS of four treatments were lower than those of the Mulun population, but its seed germination rate of four treatments were similar to that of the Mulun population. In both populations, PERS of hand pollination and natural pollination were higher than those of paper bagged and net bagged treatment. Among them, PERS of hand pollination was the highest, PERS of net bagged treatment was the

Table 1 Fruit set and seed set of *Woonyoungia septentrionalis* under different treatments in Mulun population (2001)

Treatments	No. of flowers treated	No. of aggregate fruits	No. of ovules	No. of full seeds	No. of seedlings germinated	Fruit set (%)	Seed set (%)	PERS (%)	Seed germination rate (%)
Flowers netted	30	10	126	103	57	33.33	81.75	27.25	55.34
Flowers bagged	30	15	176	113	29	50.00	64.20	32.10	25.66
Hand pollinated	20	19	248	179	101	95.00	72.18	68.57	56.42
Naturally pollinated	120	96	1 292	917	424	80.00	71.00	56.80	46.24

Fruit set (%) = number of ripe fruits / number of treated flowers × 100%; seed set (%) = number of full seeds / number of ovules; PERS, pre-emergent reproductive success = fruit set × seed set (%); seed germination rate (%) = number of seedlings germinated / number of full seeds.

Table 2 Fruit set and seed set of *Woonyoungia septentrionalis* under different treatments in Dahuangni population (2002)

Treatments	No. of flowers treated	No. of aggregate fruits	No. of ovules	No. of full seeds	No. of seedlings germinated	Fruit set (%)	Seed set (%)	PERS (%)	Seed germination rate (%)
Flowers netted	30	1	16	4	2	3.33	6.25	0.20	50.00
Flowers bagged	30	7	108	26	7	23.33	24.07	5.62	26.92
Hand pollinated	30	26	394	242	146	86.67	61.42	53.23	60.33
Naturally pollinated	140	91	1 274	670	342	65.00	52.59	34.18	51.04

The illustration is the same as in Table 1.

lowest. Our results revealed that both sexual reproduction and apomixis occurred simultaneously in *W. septentrionalis* and seeds from both sexual and apomixis could germinate into seedlings. Therefore the breeding system of *W. septentrionalis* belonged to facultative apomixis.

3 Discussion

Most plants reproduce sexually through the union of chromosomally reduced female (egg) and male (sperm) gametes derived from meiosis. Plants of some species form their seeds by an asexual process called apomixis (*sensu stricto*), which mainly includes apospory, diplospory and apogamy (Battaglia, 1963). Apomixis has been reported in over 35 families of angiosperms including over 300 species (Nygren, 1967; Hanna and Bashaw, 1987). No investigation on apomixis of Magnoliaceae has been carried out, although some studies on embryology and reproductive biology of Magnoliaceae were reported (Maneval, 1914; Padmenabhan, 1960; Kaeiser and Boyce, 1962; Hayashi, 1964; Kapil and Bhandari, 1964; Ly-To-Ba *et al.*, 1970; Yamazaki, 1982; Hayashi, 1984; Fan *et al.*, 1992; Liao *et al.*, 2000). The data presented in this paper show that sexual reproduction and apomixis occur simultaneously in *W. septentrionalis* and both kinds of seeds can germinate into seedlings. Therefore the breeding system of *W. septentrionalis* belonged to facultative apomixis. The apomixis in Magnoliaceae was reported for the first time. The embryological evidence also supports our observation and affirms the apomixis of *W. septentrionalis* to be apospory (to be published separately).

The fruit set and seed set differ significantly in the two populations studied. It may be caused by thew limitation of sampling, or the differences in the living conditions, physical, physiological, or other unknown factors in the two localities.

The evolutionary trends of the sexual systems in Magnoliaceae are believed to be as follows (Zeng *et al.*, 2001): bisexual → polygamous (*Parakmeria*) → unisexual and monoecious (*Kmeria*) → dioecious (genus *Woonyoungia*). The habitats of *W. septentrionalis* have been significantly fragmented. Recently only four populations were found (Mulun, Huangjiang, Guangxi; Dahuangni, Luocheng, Guangxi; Maolan, Libo, Guizhou and Gulinqing, Maguan, Yunnan). The population number became very finite, and the size of populations became very small and the distances between them were very long. Therefore, *W. septentrionalis* was listed as a critically endangered species (under state protection category Ⅰ). Richards (1997) listed five advantages of apomixis, among them are assured reproduction, to fix and disseminate an extremely fit genotype seems most likely to be relevant to the case in *Woonyoungia*. Under the most conditions, there is a long distance between the male and female individuals and its natural pollination is difficult to achieve. Therefore, apomixis is likely to be the result that *W. septentrionalis* adapts itself gradually to the diminishing and fragmented habitats.

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References:

Ashworth L, Galetto L. 2001. Pollinators and reproductive success of the wild cucurbit *Cucurbita maxima* ssp. *andreana* (Cucurbitaceae). *Plant Biol*, **39**:398-404.

Battaglia E. 1963. Apomixis. Maheshwari P. Recent Advance in the Embryology of Angiosperm. Delhi, India: University of Delhi. 221-411.

Dandy J E. 1931. Four new Magnolieae from Kwangsi. *J Bot*, **69**: 231-233.

Fan R-W (**樊汝汶**), Ye J-G (**叶建国**), Yin Z-F (**尹增芳**), Gao H-D (**高捍东**), You L-X (**尤录祥**). 1992. Studies on seed development and embryogenesis in *Liriodendron chinense* (Hemsl.) Sarg. *Acta Bot Sin* (**植物学报**), **34**:437-442. (in Chinese with English abstract)

Hanna W W, Bashaw E C. 1987. A pomixis: its identification and use in plant breeding. *Crop Sci*, **27**:1136-1139.

Hayashi Y. 1964. Megasporogenesis, female gametophyte and embryogeny of *Magnolia liliflora* and *Michelia fuscata*. *Sci Rep Tohoku Univ Ser Biol*, **30**:89-98.

Hayashi Y. 1984. Embryology of *Magnolia salicifolia* Maxim. (Magnoliaceae). *JPN J Bot*, **59**:298-307.

Kaeiser M, Boyce S G. 1962. Embryology of *Liriodendron tulipifera* L. *Phytomorphology*, **12**:103-109.

Kapil R N, Bhandari N N. 1964. Morphology and embryology of *Magnolia* Dill. ex Linn. *Proc Nat Inst Sci India*, **30**:245-262.

Law Y-H (**刘玉壶**), Pan C-F (**庞成发**), Chen Z-Y (**陈忠毅**), Long R-G (**龙日光**). 1987. Rediscovering of *Kmeria septentrionalis*. *Plants* (**植物杂志**), (5):17. (in Chinese)

Law Y-H (**刘玉壶**). 1997. *Woonyoungia* Law: a new genus of Magnoliaceae from China. *Bull Bot Res* (**植物研究**), **17**:353-356. (in Chinese with English abstract)

Liao J P, Chen Z L, Cai X Z, Wu Q G. 2000. Embryology of *Manglietia glauca* var. *sumatrana* and *Michelia guangxiensis* and the abnormal development. Liu Y H, Fan H M, Chen Z Y, Wu Q G, Zeng Q W. Proceedings of International Symposium of Family Magnoliaceae. Beijing: Science Press. 177-187.

Ly-To-Ba, Guignard J L, Mestre J C. 1970. Embryogénie des Magroliacées. Development de l'embryon chez *Magnolia*

grandiflora L. *C R Acd Sci Paris*, **271**:1272-1275.

Maneval W E. 1914. The development of *Magnolia* and *Liriodendron*, including a discussion of the primitiveness of the Magnoliaceae. *Bot Gaz*, **57**:1-31.

Nygren A. 1967. Apomixis in the angiosperms. *Handb der Pflanzenphys*, **18**:551-556.

Padmenabhan D. 1960. A contribution to the embryology of *Michelia champaca*. *J Madras Unv B*, **30**:155-165.

Yamazaki T. 1982. Recognized type in early development of the embryo and the phylogenetic significance in the Dicotyledons. *Acta Phytotax Geobot*, **33**:400-409. (in Japanese with English summary)

Richards A J. 1997. Plant Breeding Systems. 2nd ed. London: Chapman and Hall. 339-399.

Zeng Q-W (**曾庆文**), Gao Z-Z (**高泽正**), Zhang D-X (**张奠湘**). 2001. Numerical variation of flower parts and the axillary flowers in *Woonyoungia septentrionalis* (Dandy) Law. *J Trop Subtrop Bot* (**热带亚热带植物学报**), **9**:306-310. (in Chinese with English abstract)

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濒危植物焕镛木的兼性无融合生殖

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摘要：　2001~2002年连续两年在广西环江木论和广西罗城大黄泥的2个焕镛木(*Woonyoungia septentrionalis* (Dandy) Law)自然种群中，对单性异株的濒危植物焕镛木进行繁育系统测定，对即将开花的雌花花蕾分别进行套袋、套网、人工授粉处理，并用自然授粉雌花作对照，其座果率和结实率统计结果表明：自然授粉、人工授粉、套袋和套网处理的花均能结实，但它们的座果率和结实率存在较大的差异。在两个种群中，人工授粉和自然授粉的总结实率(PERS)均比套袋和套网处理的高，其中人工授粉的最高，套网处理的最低。由此可见，焕镛木既能通过有性生殖方式结实，又能通过无融合生殖方式结实，而且这两种生殖方式获得的种子均能萌发成幼苗，由此断定，焕镛木的繁育系统为兼性无融合生殖。这是首次报道木兰科植物存在无融合生殖现象。

关键词：　焕镛木；繁育系统；兼性无融合生殖；无孢子生殖

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