

Research Paper

Differentiation in fructification percentage between two morphs of *Amomum tsaoko* (Zingiberaceae)

Yao-Wen Yang^{1,2}, Zi-Gang Qian¹, Ai-Rong Li², Chun-Xia Pu^{1,2}, Xiao-Li Liu¹ and Kai-Yun Guan^{*2}

¹ The Center for Reproducing Fine Varieties of Chinese Medicinal Plants, Yunnan College of TCM, Kunming 650500, China

² Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, China

Amomum tsaoko is a flexistylous ginger. Flexistily is a unique floral mechanism promoting outcrossing, which is known only in some species of Zingiberaceae till date. This is a pioneer report on flexistily in *A. tsaoko* from the aspect of fructification percentage to clarify its influence on reproduction. We observed in 2007 and 2008 that the fructification percentage of the anaflexistyled and the cataflexistyled inflorescence were $14.89 \pm 10.35\%$ and $11.31 \pm 7.91\%$ respectively, with significant difference (d.f. = 141.920, $t = 2.518$, $P = 0.013 < 0.05$). The greatly significant difference between 2007 and 2008 were present in both the flower number (d.f. = 93, $t = -2.819$, $P = 0.006 < 0.01$) and the fructification percentage (d.f. = 93, $t = -2.894$, $P = 0.005 < 0.01$) of the cataflexistylous inflorescence. Although the two morphs were similar in morphological characteristics, there was some gender differentiation between them, showing a possibility that the anaflexistylous morph might function more as females and the cataflexistylous morph more as males. Reproduction of the cataflexistylous morph was significantly sensitive to change of environmental factors, in contrast to the anaflexistylous morph, thus the yield varied between the abundant year (2008) and the off year (2007).

Key Words: *Amomum tsaoko*, flexistily, fructification percentage, gender differentiation, tradeoff.

Introduction

Plants are famously diverse in reproductive strategies (Barrett 2002), displaying a broad range of floral forms across species and presenting numerous examples of floral or reproductive polymorphisms maintained within a population (Pannell *et al.* 2005). The sexual diversity in plants has been a main concern for evolutionary biologists (Barrett 2002).

Amomum tsaoko Crevost et Lemaire is a ginger with a flexistylous mechanism that is a unique and motile sexual dimorphism to promote outcrossing (Cui *et al.* 1995a, 1995b, Li *et al.* 2001a, 2001b, 2002, Takano *et al.* 2005, Wang *et al.* 2005a, 2005b, Zhang *et al.* 2003). There are two floral morphs in a flexistylous species, termed as anaflexistylous (protogynous) and cataflexistylous (protandrous) morphs according to the direction of stigma movement and time of pollen release during anthesis. Through active stylar curvature, each morph changes from one sexual phase to the other in the middle of the 1-d flowering period, and the two

morphs form a reciprocal cooperation between stigmas and anthers to promote outcrossing (Li *et al.* 2001a, Zhang and Li 2002). The sexual organs are separated temporarily and spatially by active stylar curvature, thus interference of male and female functions may reduce to small extent (Barrett 2002, Li *et al.* 2001a, 2001b, Zhang 2004, Zhang *et al.* 2003, Zhang and Li 2002).

Fruit maturation indicates reproductive success of plants, which is the most basic purpose of flowering plants (Barrett and Harder 1996). In this context, fructification percentage is an important index of the reproductive success of plants, which also reflects the fitness of a plant. Other flexistylous species *Alpinia nieuwenhuizii* Val., a Bornean endemic, is known to be different in fruit set between the two morphs, and indicating some gender differentiation (Takano *et al.* 2005). In this regard, it has not been reported in *A. tsaoko* yet (to distinguish *Amomum* from *Alpinia*, we abbreviate them as *A.* and *Al.*, respectively thereafter).

With both flexistylous and non-flexistylous species in *Amomum*, which is an ideal model for discussing flexistily phylogeny, flexistily in this genus is worth to be studied in details (Ren *et al.* 2007). To fully understand the evolution and maintenance of the sexual system in angiosperms, it is essential to investigate flexistily in *Amomum* from various aspects, especially clarifying influence of this unique floral

Communicated by L. Guarino

Received July 9, 2013. Accepted February 3, 2016.

First Published Online in J-STAGE on May 21, 2016.

*Corresponding author (e-mail: guanky@mail.kib.ac.cn)

mechanism on reproduction and difference of reproductive trait between two morphs. Therefore, we reported the differentiation between the two morphs of *A. tsaoko* in the paper. The main goal of this study is to address the following questions: (1) is fructification percentage different between the two morphs of *A. tsaoko*? (2) Is there the same gender differentiation between the two morphs of *A. tsaoko* as those of *Al. nieuwenhuiizii*? (3) Why does the yield of *A. tsaoko* vary between the abundant year and the off year?

Materials and Methods

Research species

A. tsaoko is a perennial herb of Zingiberaceae, usually 2–3 m in height. Inflorescence consists of a densely flowered spike that arises from rhizomes. Distinct characters of the plant from the other *Amomum* species are a showy yellow labellum with two red nectar guides, anther appendage, crimson and leathery ligule. Flowering occurs from April to June, and by September–December capsules are ripe (Wu and Raven 2000).

Study site

A. tsaoko distributes in Yunnan province, Southwest China (Wu and Raven 2000), but now its wild (or natural) population is almost extinct in Yunnan. Since its fruit is used as common materia medica (Chinese Pharmacopoeia Commission 2010), and a food condiment in China, it is cultivated as an economic plant in Yunnan.

The study site was at Biaopo (N 24°50.289', E 98°46.501', 2070 m in altitude), Gaoligong Mountain, Yunnan province, Southwest China. These plant individuals had grown for about 20 years in the humid valley covered by evergreen broadleaf forest, around which primary forest was kept as a part of natural reserve. These plant individuals grew naturally there, without artificial manipulation, and the villagers only entered for collection of the fruits in September–December.

Field observation and fructification percentage

The study was conducted from May 2007 to October 2008. Our preliminary observations suggested that the longevity of a single flower was only 1 d, both the non-fertilized and fertilized flowers withering at dusk. There were average 89.6 flowers (range from 48 to 129) and average 3–4 flowers opening for each day per inflorescence. Cui *et al.* (1996) reported that the fertilized ovaries enlarged over the next five days, and grew rapidly from the next 20 d to 30 d, while some fertilized ovaries fell off under low humidity. On May 25, 2007, we selected at random 28 individuals in the population, including 12 cataflexistyled individuals and 16 anaflexistyled individuals for 2-year observation. In October 2007, six individuals of the 28 plants just had one infructescence respectively, so 1–4 infructescences were picked at random from each individual. Thus 26 anaflexistyled infructescences (352 fruits in total) and 31 cataflexi-

styled infructescences (201 fruits in total) were collected. In October 2008, 3–8 infructescences were picked at random from each individual, so 52 anaflexistyled infructescences (582 fruits in total) and 64 cataflexistyled infructescences (756 fruits in total) were collected. The infructescence of each morph that randomly selected was subjected to observations, and got both the number of mature fruits (excluding immature ones which fell off after anthesis) and scars of flowers in an infructescence, and the number of seeds in each fruit.

Statistical analyses

Fructification percentage was calculated by the formula (each infructescence: mature fruit number ÷ total flower number × 100%). Date on the fructification percentage, the flower number per inflorescence and the seed number per fruit were checked by Levene test, then compared between two morphs and between the two years by T Test in SPSS (13.0 version). Correlations were tested by Bivariate Correlation in SPSS (13.0 versions).

Results

Quantitative character of the inflorescence and fruit

As far as the population was concerned, 2008 was the abundant year and 2007 was the off year. The quantitative characters of the inflorescence also partly reflected the variation (Table 1).

Difference between the two morphs

Significant differences between the two morphs were only observed in the fructification percentage per inflorescence in 2007, and the average of the two years respectively (Table 2).

Table 1. Quantitative character of the inflorescence and fruit of *A. tsaoko* in the study site

Quantitative Character	Year	Anaflexistylous morph	Cataflexistylous morph
Flower number per inflorescence	2007	81.00 ± 16.19 (46–106)*	80.77 ± 18.55 (52–129)
	2008	86.67 ± 21.24 (33–125)	94.23 ± 23.22 (21–135)
Fructification percentage per inflorescence (%)	2007	17.19 ± 8.31 (3.09–33.85)	8.05 ± 6.88 (0.78–30.12)
	2008	13.75 ± 11.12 (1.03–54.00)	12.88 ± 7.95 (1.57–37.78)
	Average**	14.89 ± 10.35 (1.03–54.00)	11.31 ± 7.91 (0.78–37.78)
Seed number per fruit	2007	27.13 ± 11.22 (5–67)	27.26 ± 11.40 (3–59)
	2008	29.31 ± 11.11 (5–64)	29.00 ± 11.05 (3–58)

* Mean ± SD (range from 46 to 106).

** The average of the two years.

Table 2. Difference between the two morphs of *A. tsaoko* in the study site

Quantitative character	Year	d.f.	t	p
Flower number per inflorescence	2007	55	0.48	0.962
	2008	114	-1.812	0.073
Fructification percentage per inflorescence (%)	2007	55	4.545	0.000**
	2008	116	0.488	0.627
	Average***	141.920	2.518	0.013*
Seed number per fruit	2007	1711	-0.243	0.808
	2008	1289	0.506	0.613

* The mean difference is significant at the 0.05 level.

** The mean difference is greatly significant at the 0.01 level.

*** The average of the two years.

Table 3. Difference between the two years in the study site

Quantitative character	Morph	d.f.	t	p
Flower number per inflorescence	Anaflexistylous	76	-1.198	0.235
	Cataflexistylous	93	-2.819	0.006**
Fructification percentage per inflorescence (%)	Anaflexistylous	76	1.396	0.167
	Cataflexistylous	93	-2.894	0.005**
Seed number per fruit	Anaflexistylous	1353	-3.517	0.000**
	Cataflexistylous	1647	-3.123	0.002**

** The mean difference is greatly significant at the 0.01 level.

Table 4. Correlations between flower number and fructification percentage per inflorescence in the study site

D.f.	Pearson correlation (R)	Sig. (2-tailed) P	Note
172	-0.195	0.010*	In 2007 and 2008
115	-0.245	0.008**	In 2008
77	-0.324	0.004**	Among anaflexistylous morph

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is greatly significant at the 0.01 level (2-tailed).

Difference between the two years

Of the anaflexistylous morph, there was greatly significant difference between the two years just in the seed number per fruit; of the cataflexistylous morph, greatly significant differences between the two years were detected in the flower number per inflorescence, the fructification percentage per inflorescence, and the seed number per fruit (Table 3).

Correlation between flower number and fructification percentage

There were significantly negative correlations between flower number and fructification percentage per inflorescence (Table 4).

Discussion

Gender (maleness or femaleness) was a quantitative phenomenon in plants; phenotypic gender was usually measured by the morphological feature of flowering, while functional gender was usually estimated by the relative success of

female function (seed production) or male function (making female gametes to fertilize) (Lloyd 1979). Although the two morphs of *A. tsaoko* were similar in morphological characteristics, our study suggested that there was some gender differentiation between them. There was a possibility that the anaflexistylous morph might function more as females and the cataflexistylous morph more as males since higher fructification percentage and lower flower numbers in the anaflexistylous inflorescence in comparison to the cataflexistylous inflorescence supported this view, as reported previously flexistylous *Al. nieuwenhuzii* (Takano *et al.* 2005). In addition, both the flower number and the fructification percentage in the cataflexistylous inflorescence were significantly different between the two years (2007 and 2008), in contrast to the anaflexistylous inflorescence. Our results indicated that both the flower number and the fructification percentage of the anaflexistylous morph was steady between the two years, while those of the cataflexistylous morph was inconstant. The result not only demonstrated that reproduction of the cataflexistylous morph could be easily influenced by environmental factors than the anaflexistylous morph, but also explained the main reason that the yield varied between the abundant year and the off year.

Because reproductive resources of a plant were limited, there would be tradeoff of two or more organs on account of competition in the same resource (Campbell 2000, Lloyd 1987). On the one hand, the tradeoff would show between male function and female function, such as between stamen and pistil, pollen and ovule, flower and fruit (Campbell 2000); on the other hand, the tradeoff would exhibit between number and size of organ, e.g. flower number and size, pollen number per flower and size, or seed number per fruit and size, etc. (Lloyd 1987, Thomson 1989). A significant negative relation was present between the flower number per inflorescence and the fructification percentage, indicating a tradeoff, which was more obvious or strong in the anaflexistylous inflorescences and in the abundant year (2008). The same tradeoff didn't show in the cataflexistylous inflorescences with the lower fructification percentage, indicating a certain differentiation between the two morphs, and also presenting that the cataflexistylous flower would mainly provide pollen and function more as male. These averages of the flower number, the fructification percentage per inflorescence, the seed number per fruit were higher in 2008 than 2007, with exception of the fructification percentage of the anaflexistylous inflorescence. Likewise, the tradeoff was stronger in the abundant year (2008), thus the more abundant the yield, and the stronger the tradeoff.

The seed number per fruit was not significantly different between the two morphs, but that of each morph was greatly significant different between the two years, indicating that environmental factors would easily influence on the seed number per fruit of both morphs. It might also indicate that the condition of pollination was different between the two years.

A. tsaoko is a flexistylous ginger, its stylar curvature

might be related to these differentiations. Firstly, style movement appeared to have asymmetrical effects, in contrast to male function, female function appeared to be affected weakly by stigma movement (Sun *et al.* 2007, 2011). Secondly, the anaflexistyled flower was proterogynous, which style curved upwards at around 15:00 in the afternoon to accomplish the gender transition from the female phase to the male phase (Cui *et al.* 1995a), so the female stage was longer than the male stage in one-day anthesis; the cataflexistyled flower was protandrous, which gender transition from the male phase to the female phase was accomplished at about 16:00 in the afternoon by the stylar downward curvature (Cui *et al.* 1995a), therefore the female stage was shorter than the male stage in one-day anthesis. Thirdly, the gender in the afternoon might be less efficient than the one in the morning (Takano *et al.* 2005); the similar phenomenon might occur in *A. tsaoko*, and more observations would be needed. Further insight into reproductive trait would reveal better gender differentiation between the two morphs, such as an examination of the selfing rate in the two morphs using molecular markers.

Flexistily was a special form of heterodichogamy promoting outcrossing (Ren *et al.* 2007), and heterodichogamy was understood to come from synchronous dichogamy and was heading towards dioecy (Renner 2001). Since Darwin, most adaptive interpretations of sexual diversity had focused on mechanisms that functioned to promote outcrossing and reduced the likelihood of inbreeding depression (Takano *et al.* 2005). However, reproductive assurance was a self-protecting mechanism or strategy (Baker 1955). Sex expression enhanced sexual reproduction through an optimal model of bisexual resource allocation (Charnov 1979), and plants invested more resources in male phasing than in female phasing (Zhang and Jiang 2001). Thus, the gender differentiation between the two morphs of *A. tsaoko* might exhibit its resource allocation strategy for enhancement of sexual reproduction or accomplishment of reproductive assurance. It might not only indicate that the influence of stylar movement on reproduction was different between the two morphs of *A. tsaoko*, and also the maintenance and evolutionary direction were different between the two morphs. As far as flexistily in *Amomum* was concerned, further insight into which would be worth studying in details to fully understand its origin and evolution.

Acknowledgements

The research was supported by a grant from Science and Technology Projects of Yunnan Province (U0120150187) and the Science Foundation of Yunnan Education Bureau (2010Z097).

Literature Cited

- Baker, H.G. (1955) Self-compatibility and establishment after "long-distance" dispersal. *Evol.* 9: 347–349.
- Barrett, S.C.H. and I.D. Harder (1996) Ecology and evolution of plant mating. *Trends Ecol. Evol. (Amst.)* 11: 73–79.
- Barrett, S.C.H. (2002) The evolution of plant sexual diversity. *Nat. Rev. Genet.* 3: 274–284.
- Campbell, D.R. (2000) Experimental tests of sex-allocation theory in plants. *Trends Ecol. Evol. (Amst.)* 15: 227–232.
- Charnov, E.L. (1979) Simultaneous hermaphroditism and sexual selection. *Proc. Natl. Acad. Sci. USA* 76: 2480–2484.
- Chinese Pharmacopoeia Commission (eds.) (2010) *Pharmacopoeia of the People's Republic of China*. Vol. 1. Chinese Medicine Science and Technology Press, Beijing, pp. 222–223.
- Cui, X.L., R.C. Wei and R.F. Huang (1995a) A preliminary study on the genetic system of *Amomum tsaoko*. *J. Yunnan Univ. (Nat. Sci.)* 17: 290–297.
- Cui, X.L., R.C. Wei and R.F. Huang (1995b) Study on the artificial population structure of *Amomum tsaoko*. *SW China J. Agri. Sci.* 8: 114–118.
- Cui, X.L., R.C. Wei and R.F. Huang (1996) Biological characters of flowering and fruiting habit in *Amomum tsaoko*. *SW China J. Agri. Sci.* 9: 109–113.
- Li, Q.J., Z.F. Xu, W.J. Kress, Y.M. Xia, L. Zhang, X.B. Deng, J.Y. Gao and Z.L. Bai (2001a) Flexible style that encourage outcrossing. *Nature* 410: 432.
- Li, Q.J., Z.F. Xu, W.J. Kress, Y.M. Xia, L. Zhang, X.B. Deng and J.Y. Gao (2001b) Study on the flexistily pollination mechanism in *Alpinia* plants (Zingiberaceae). *Acta Bot. Sin.* 43: 346–369.
- Li, Q.J., W.J. Kress, Z.F. Xu, Y.M. Xia, L. Zhang, X.B. Deng and J.Y. Gao (2002) Mating system and stigmatic behaviour during flowering of *Alpinia kwangsiensis* (Zingiberaceae). *Plant Syst. Evol.* 232: 123–132.
- Lloyd, D.G. (1979) Parental strategies in angiosperms. *In: International Symposium on Reproduction in Flowering Plants*. New Zeal. J. Bot. 17: 595–605.
- Lloyd, D.G. (1987) Selection of offspring size at independence and other size-versus-number strategies. *Am. Nat.* 129: 800–817.
- Pannell, J.R., M.E. Dorken and S.M. Eppley (2005) 'Haldane's Sieve' in a metapopulation: sifting through plant reproductive polymorphisms. *Trends Ecol. Evol. (Amst.)* 20: 374–379.
- Ren, P.Y., M. Liu and Q.J. Li (2007) An example of flexistily in a wild cardamom species (*Amomum maximum* (Zingiberaceae)). *Plant Syst. Evol.* 267: 147–154.
- Renner, S.S. (2001) Heterodichogamy, how common is it? *Trends Ecol. Evol. (Amst.)* 16: 595–597.
- Sun, S., J.Y. Gao, W.J. Liao, Q.J. Li and D.Y. Zhang (2007) Adaptive significance of flexistily in *Alpinia blepharocalyx* (Zingiberaceae): a hand-pollination experiment. *Ann. Bot.* 99: 661–666.
- Sun, S., D.Y. Zhang, A.R. Ives and Q.J. Li (2011) Why do stigmas move in a flexistylous plant? *J. Evol. Biol.* 24: 497–504.
- Takano, A., J. Gisil, M. Yusoff and T. Tachi (2005) Floral and pollinator behaviour of flexistylous Bornran ginger, *Alpinia nieuwenhuizii* (Zingiberaceae). *Plant Syst. Evol.* 252: 167–173.
- Thomson, J.D. (1989) Deployment of ovules and pollen among flowers within inflorescences. *Evol. Trends Plants* 3: 65–68.
- Wang, Y.Q., D.X. Zhang and Z.Y. Chen (2005a) Pollination biology of *Alpinia hainanensis* (Zingiberaceae). *Acta Phytotaxon. Sin.* 43: 37–49.
- Wang, Y.Q., D.X. Zhang and Z.Y. Chen (2005b) A preliminary study of the pollination biology of *Alpinia oxyphylla* (Zingiberaceae). *Acta Phytotaxon Sin.* 29: 599–609.
- Wu, Z.Y. and P.H. Raven (eds.) (2000) *Flora of China*, Vol. 24 (Flagellariaceae through Marantaceae). Science Press, Beijing, and

- Missouri Botanical Garden Press, St. Louis, p. 351.
- Zhang, D.Y. and X.H. Jiang (2001) Mating system evolution, resource allocation, and genetic diversity in plants. *Acta Phytoecol. Sin.* 25: 130–143.
- Zhang, D.Y. (2004) The evolution of plant life history and reproductive ecology. Science Press, Beijing, p. 101.
- Zhang, L. and Q.J. Li (2002) Flexistylis and its evolutionary ecological significance. *Acta Phytoecol. Sin.* 26: 385–390.
- Zhang, L., Q.J. Li, X.B. Deng, P.Y. Ren and J.Y. Gao (2003) Reproductive biology of *Alpinia blepharocalyx* (Zingiberaceae): another example of flexistylis. *Plant Syst. Evol.* 241: 67–76.