

Pollen Histochemistry and Pollen : Ovule Ratios in Zingiberaceae

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• **Background and Aims** Pollen grains of 37 species from 11 genera in the family Zingiberaceae were examined to assess qualitatively starch or lipid contents; the pollen grain and ovule numbers per flower and pollen : ovule ratios were also counted and calculated. Pollen : ovule ratios were studied to reveal patterns of variation in the Zingiberaceae.

• **Methods** Freshly open flowers with dehiscent anthers were collected at random from plants growing in natural habitats or in botanical gardens. Presence of lipids or starch in pollen grains was tested by Sudan solution and IKI solution, respectively, and examined under a microscope. To estimate the pollen and ovule numbers per flower, one anther from each bud was carefully dissected and all pollen grains were counted; ovaries were carefully dissected out of each flower and counted. Whenever possible, at least 10–15 buds were used in the determination.

• **Key Results** Thirty-three of all the 37 species examined had starchy pollen. Starch was not found in only four species and lipid was not found in only one species; among the four tribes in subfamily Zingiberoideae, all species of Zingibereae and Globbeae had pollen with no starch, Alpineae and Hedychieae had pollen with and without starch, whereas, all species of subfamily Costoideae had starchy pollen with abundant lipids. The mean pollen : ovule ratios in the members of the Zingiberaceae investigated range from 3.25 ± 1.56 to 616.52 ± 117.83 .

• **Conclusions** The pollen nutrition types seemed not related to mating systems. The pollen : ovule ratios in members of the Zingiberaceae with the same breeding system are noticeably lower than those recorded by previous authors. The lower pollen : ovule ratios in this family are presumed to be related to the highly efficient pollination systems, mediated by pollen which can be quite glutinous and the relatively large stigma area. In most of the *Alpinia* species the anaflexistylous flowers have much larger numbers of pollen grains and higher pollen : ovule ratios than the cataflexistylous flowers. There are significant differences in mean pollen grain numbers and pollen : ovule ratios between different life forms but ovule numbers are approximately the same.

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Key words: Zingiberaceae, pollen, starch, lipid, pollen-ovule ratio, breeding system.

INTRODUCTION

Dafni (1992) commented that pollen histochemistry is possibly related to pollination mode, pollinator foraging behaviour and phylogeny. All angiosperm pollen grains contain stored food reserves in the form of starch and/or lipids and can be classified as two classes, 'starchy' and 'starchless' (Baker and Baker, 1979). Many studies have shown that all angiosperm pollen contains some lipids, while starches are not always present; possible ecological and taxonomic (including phylogenetic) correlations with their presence or absence have also been discussed (Baker and Baker, 1979). The tendency for whole families characterized by having either starchy or starchless pollen, and hence the potential phylogenetic usefulness of this character, was recognized at an early date (Grayum, 1985). Baker and Baker (1979) refuted the suggestion that 'primitive' angiosperms would be characterized by starchy pollen and revealed that there is no evidence that 'primitive' families are any more likely to be made up of taxa with starchy pollen than others. However, Grayum (1985) suggested that starchless pollen is the primitive type in Araceae, and the same is true of monocots as a whole.

Generally, starchy pollen has been considered a feature of wind-pollinated (anemophilous) flowering plants, whereas insect-pollinated (entomophilous) species show a greater or

lesser replacement of starch by sugar or lipids (Baker and Baker, 1979). Baker and Baker (1979) also concluded that selection in favour of starchless (oil-rich) pollen grains is where these are part or all of the reward received by bees and flies visiting the flowers and, conversely, where insect nutrition is not involved selection is in favour of starchy pollen. They also proposed that the mean diameter of the starchy grains is significantly larger than that of the starchless grains. Darwin (1877) put forward that pollen size was related to the length of the style in connection with pollen dimorphism. Baker and Baker (1979) elucidated that both starchy and starchless groups of species showed that mean diameters are significantly larger than those of their shorter-styled counterparts and they suggested that this was an indication that copious reserves are necessary to produce long pollen tubes required in these long-styled species.

After Cruden (1977) suggested that the pollen : ovule (P/O) ratio indicates plant breeding systems, many subsequent studies tried to relate P/O ratios with species' breeding systems, and P/O ratios are widely used in breeding system studies. Nevertheless, it is still unclear what the exact factors are that determine P/O ratios (Wyatt, 1984). It has been suggested that the P/O ratios reflect the likelihood of sufficient pollen grains reaching each stigma to result in maximum seed set, and thus, the more efficient the transfer of pollen, the lower the P/O ratio should be (Cruden, 1977).

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Charnov (1982), however, argued that pollen-ovule ratios can be explained more directly as a result of local mate competition, with allocation ratios in hermaphroditic plants being driven by male/female gain curves for fitness. Cruden and Miller-Ward (1981) added that pollen grain size is a factor influencing pollen-ovule ratios and stated that species with relatively large stigma areas compared with the pollen-bearing area of the pollinator often have lower P/O ratios. Furthermore, Preston (1986) suggested that the interpretation of the P/O ratio must take into account sources other than the breeding system and proposed there can be no single general standard for evaluating P/O ratios, and any study that includes P/O ratios must discuss the criteria used to evaluate them. Vasek and Weng (1988) commented that, in any event, the standards for evaluating breeding systems need to be set at the level of family (or perhaps tribe) and each genus or other major taxon requires its own standard, and its breeding system cannot necessarily be judged on the standard for a mixture of other major taxa. Recently, pollen grain numbers and ovule numbers were found to correlate with style number, life form and breeding system, and that there are significant differences between some taxonomic groups in pollen grain numbers and ovule numbers but not in P/O ratios (Jürgens and Gottsberger, 2002). In fact, many different factors influence the pollen-ovule ratio, and species with different evolutionary histories may have very different P/O ratios despite having similar breeding systems (Preston, 1986).

The shift from predominant cross-pollination to predominant self-pollination is a recurrent theme in the evolutionary history of the angiosperms (Stebbins, 1970). The evolution of inbreeding in flowering plants has commonly been accompanied by a reduction in the number of pollen grains produced per flower (Vasek and Weng, 1988).

Zingiberaceae is a large family of animal-pollinated tropical monocotyledons, and members of this family display a broad range of pollination and breeding systems (Sakai *et al.*, 1999; Zhang *et al.* 2003). A new behavioural outbreeding mechanism, 'flexistily', was found recently in some species from the genera *Amomum* and *Alpinia* (Zingiberaceae) (Cui *et al.*, 1996; Li *et al.*, 2001). In this paper, pollen grains of 37 species from 11 genera in Zingiberaceae, were examined to assess starch or lipid contents, the pollen grain and ovule numbers per flower, and the P/O ratios to reveal patterns of variation in the family, aiming to shed more light on the relationship between pollination mechanism, phylogenetic constraints, breeding systems and the pollen nutrition types and the P/O ratios.

MATERIALS AND METHODS

Pollen histochemistry

Between April and August 2003, freshly open flowers with dehiscing anthers were collected at random from plants. The pollen samples were immersed in a drop of IKI solution or a drop of Sudan IV solution, and examined under a microscope. A dark bluish-black colour (stained with IKI) indicates the presence of starch, and a red colour (stained with Sudan IV) indicates the presence of lipids (Dafni, 1992).

The colour of stained starch or lipid was recorded and more than one sample was examined for each species. Baker and Baker (1979) have emphasized the necessity of using only mature pollen to test for starch content, inasmuch as even starchless pollen may accumulate starch at premature stages of development. For this reason, only fresh and mature pollen from dehiscing anther was surveyed during this study. In all, 32 species from 10 genera of four tribes in subfamily Zingiberoideae, and five species from one genus of subfamily Costoideae were tested. Information on aperture type, exine sculpturing and the diameters of pollen grains were cited from Liang (1988a, b).

Pollen : ovule ratios

During March 2002 to August 2003, flower buds 1 or 2 d prior to anthesis, were collected for a total of 22 Zingiberaceae taxa from natural or cultivated populations in Guangdong and Yunnan provinces in China and were placed in FAA solution, and refrigerated until needed. Only a single population was sampled for most of the taxa and only one bud was collected per individual (but more than one population was sampled for *Amomum villusom* and *Caulokaempferia coenobialis*). One anther from each bud was carefully dissected and all pollen grains were transferred to calibrated tubes and made up to 0.5 or 1 mL with staining solution. The suspension was stirred with a vortex mixer for 60 s, then 10–15 separate samples of 1 µL each were transferred onto slides and the pollen grain numbers were counted with the aid of a microscope. Ovaries were carefully dissected out of each flower and placed in a drop of water on a microscope slide. The entire placenta with attached ovules was removed via a longitudinal incision in the ovary wall. The ovules were carefully loosened from the placenta and spread in the drop of water to be counted at ×40 magnification under a dissecting microscope. For each flower, the P/O ratio was calculated as the number of pollen grains in one anther divided by the number of ovules. Whenever possible, at least 10–15 buds were used in the determination.

RESULTS

Pollen histochemistry

The results of pollen histochemistry are presented in Table 1. Thirty-three of all the 37 species examined had starchy pollen. Starch was not found in only four species (*Amomum villosom*, *Globba racemosa*, *Hedygium coronarium* and *H. flavum*) and lipid was not found in only one species (*Stahlianthus involucratus*). Except for these five species, pollen of all the other species examined had both starch and lipid, and the starchy pollen of some species always stored rich lipids at the same time, e.g. the phylogenetically primitive taxa, *Alpinia guinanensis*, *A. platytilus*, *A. zerumbet*, and the phylogenetically derived taxa *Pyrgophyllum yunnanense*, *Curcuma kwangsiensis* and all species of *Costus* (Wu, 1994).

Among the four tribes in subfamily Zingiberoideae, all species of Zingibereae (only two species examined) and

TABLE 1. Pollen histochemistry of some plants in Zingiberaceae

Taxon	Source	Starchy	Lipid	Pollen diameters* (µm)	Ornamentation	Breeding system
Subfamily Zingiberoideae						
Tribe I. Alpinaceae						
<i>Alpinia blepharocalyx</i> var. <i>glabrior</i> (Handel-Mazzetti) T.L. Wu	WYQ3, S. China Botanical Garden	+++	+	?	Spinose	Obligate xenogamy
<i>A. chinensis</i> (Retz.) Rose.	WYQ6, S. China Botanical Garden	+++	+	66.5	Spinose*	Obligate xenogamy
<i>A. densibracteata</i> T.L. Wu & Senjen	WYQ7, Nan Kun Shan	+++	+	?	Spinose	Obligate xenogamy
<i>A. galanga</i> (Linnaeus) Willdenow	WYQ5, S. China Botanical Garden	+++	+	75.2	Spinose*	Obligate xenogamy
<i>A. guinanensis</i> D. Fang & X.X. Chen	WYQ4, S. China Botanical Garden	+++	+++	76.1	Spinose	Obligate xenogamy
<i>A. hainanensis</i> K. Schumann	WYQ2, S. China Botanical Garden	+++	+	73.2	Spinose	Obligate xenogamy
<i>A. intermedia</i> Gagnepain	WYQ8, S. China Botanical Garden	++	+	80.1	Spinose	Obligate xenogamy
<i>A. oxyphylla</i> Miquel	WYQ1, S. China Botanical Garden	+++	+	80.8	Spinose	Obligate xenogamy
<i>A. platytilus</i> K. Schumann	WYQ9, S. China Botanical Garden	+++	+++	?	Spinose	Obligate xenogamy
<i>A. polyantha</i> D. Fang	WYQ11, S. China Botanical Garden	+++	+	78.8	Spinose	Obligate xenogamy
<i>A. tonkinensis</i> Gagnepain	WYQ10, S. China Botanical Garden	+++	+	72	Spinose	Obligate xenogamy
<i>A. zerumbet</i> (Persoon) B.L. Burt & R.M. Smith	WYQ12, S. China Botanical Garden	+++	+++	68.3	Spinose	Obligate xenogamy
<i>Amomum maximum</i> Roxb.	WYQ14, S. China Botanical Garden	+++	+	73.5	Spinose	Obligate xenogamy
<i>A. villousum</i> Lour.	WYQ13, S. China Botanical Garden	–	+	61	Spinose	Facultative xenogamy
Tribe II. Zingibereae						
<i>Zingiber striolatum</i> Diels	WYQ31, Kunming Botanical Garden	+	+	111.65	Striate*	Facultative xenogamy
<i>Z. zerumbet</i> (L.) Smith	WYQ21, S. China Botanical Garden	+	+++	92.35	Verrucate	Facultative xenogamy
Tribe III. Hedychieae						
<i>Caulokaempferia coenobialis</i> K. Larsen	WYQ29, Nankunshan, Guangdong	+++	+	82.2	Psilate*	Autogamy
<i>Curcuma kwangsiensis</i> S.G. Lee & C.F. Liang	WYQ16, S. China Botanical Garden	+++	+++	79.15	Psilate	Facultative xenogamy
<i>C. longa</i> Linnaeus	WYQ19, Kunming, Yunnan	+++	+	69	Psilate*	Facultative xenogamy
<i>C. phaeoaulis</i> Valetton	WYQ17, S. China Botanical Garden	++	+	?	Psilate	Facultative xenogamy
<i>C. wenyujin</i> Y.H. Chen & C. Ling	WYQ18, S. China Botanical Garden	+	+	?	Psilate	Facultative xenogamy
<i>C. zanthorrhiza</i> Roxburgh	WYQ15, S. China Botanical Garden	++	+	70.3	Psilate	Facultative xenogamy
<i>Hedychium coccineum</i> Smith	WYQ30, Kunming Botanical Garden	+	+	71.5	Psilate*	Facultative xenogamy
<i>H. coronarium</i> J. König	WYQ20, S. China Botanical Garden	–	+	72.4	Psilate*	Facultative xenogamy
<i>H. flavum</i> Roxburgh	WYQ22, S. China Botanical Garden	–	+	75	Psilate*	Facultative xenogamy
<i>H. spicatum</i> Smith	WYQ32, Kunming Botanical Garden	+++	+	70.4	Psilate*	Facultative xenogamy
<i>H. spicatum</i> var. <i>acuminatum</i> (Roscoe) Wallich	WYQ37, Ji Zu Shan, Yunnan	+++	+	75.5	Psilate*	Facultative xenogamy
<i>Pyrgophyllum yunnanense</i> T.L. Wu & Z.Y. Chen	WYQ35, Ji Zu Shan, Yunnan	+++	+++	67.3	Spinose	Autogamy
<i>Roscoea blanda</i> K. Schum	WYQ36, Ji Zu Shan, Yunnan	+++	+	?	?	Obligate xenogamy
<i>R. humeana</i> I.B. Balfour & W.W. Smith	WYQ34, Ji Zu Shan, Yunnan	+++	+	83.6	Spinose*	Obligate xenogamy
<i>Stahlianthus involucratus</i> (King ex Bak.) Graib	WYQ23, S. China Botanical Garden	+++	–	67.5	Psilate*	Facultative xenogamy

TABLE 1. Continued

Taxon	Source	Starchy	Lipid	Pollen diameters* (µm)	Ornamentation	Breeding system
Tribe IV. Globbeae						
<i>Globba racemosa</i> Smith	WYQ33, Kunming Botanical Garden	–	+++	43.1	Spinose*	Facultative xenogamy
Subfamily Costoideae						
K. Schum.						
<i>Costus barbatus</i> Suess.	WYQ28, S. China Botanical Garden	+++	+++	?	?	Facultative xenogamy
<i>C. curvibracteatus</i> Mass	WYQ27, S. China Botanical Garden	+++	+++	?	?	Facultative xenogamy
<i>C. dubius</i> (Afzel.) K. Schum.	WYQ26, S. China Botanical Garden	+++	+++	115.5	Verrucate*	Facultative xenogamy
<i>C. igneus</i> N.E. Br.	WYQ25, S. China Botanical Garden	+++	+++	?	?	Facultative xenogamy
<i>C. speciosus</i> (J. Koen.) Smith	WYQ24, S. China Botanical Garden	+++	+	99.0	Verrucate*	Facultative xenogamy

*Data from Liang (1988a, b).

Starch and lipid reaction: –, negative; +, positive; ++, strong; +++, very strong.

Globbeae (only once species examined) showed entirely starchless pollen, Alpineae and Hedychieae show both starchless pollen and starch pollen. Whereas, all species of subfamily Costoideae showed starchy pollen with abundant lipids. Except for *Amomum* and *Hedychium*, all species within the same genera studied showed identical pollen nutrition reservoir types.

Pollen: ovule ratios

The pollen grain numbers, ovule numbers, and P/O ratios for the species investigated are given in Table 2. The mean P/O ratios in *Zingiberaceae* investigated range from 3.25 ± 1.56 in *Caulokaempferia coenobialis* to 616.52 ± 117.83 in the anaflexistylous flower of *Alpinia stachyodes*. The maximal mean pollen grain number is 51270 ± 1197.51 in the anaflexistylous flower of *Alpinia hainanensis*; and the minimal pollen grain number per flower, 113.75 ± 41.63 , is found in *Caulokaempferia coenobialis*. Ovule numbers ranged from 7.1 ± 1.3 in cataflexistylous flower of *Alpinia stachyodes* to 182.22 ± 28.36 in the cataflexistylous flower of *Alpinia hainanensis*. The data show that pollen grain numbers, ovule numbers and P/O ratios from different species in *Zingiberaceae* even in a same genus are greatly varied (Table 2). But the mean pollen grain numbers, ovule numbers and P/O ratios of genera from the same tribe are not significantly different (Table 3). The degree of intraspecific differences in the P/O ratios from different populations varies greatly in different species: the pollen grain numbers, ovule numbers and P/O ratios of *Caulokaempferia coenobialis* from different populations are very different, but the pollen grain numbers and P/O ratios of *Amomum villosum* from different populations are approximately equivalent, although their ovule numbers are different (Table 4).

The species investigated were classified as autogamous, facultatively xenogamous and obligately xenogamous

based on the observations given here and Cruden's outcrossing index (Cruden, 1977), which is the sum of assigned values for three characteristics of the flower and floral behaviour: diameter of the flower; temporal separation of anther dehiscence and stigma receptivity; and spatial relationship. According to our data, the pollen grain numbers, ovule numbers, and P/O ratios in species with the same breeding system are very different (Table 2). The mean P/O ratios of species with obligate xenogamy, facultative xenogamy and autogamy are 345.39 ± 125.07 , 108.07 ± 62.93 and 58.70 ± 91.78 , respectively (Table 5). *Caulokaempferia coenobialis* is classified as actively autogamous based on its mucilaginous pollen moving onto the stigma and carrying out selfing (Y.Q. Wang, D.-X. Zhang and Z.-Y. Chen, unpubl. res.) and *Pyrgophyllum yunnanense* as passively autogamous based on the fact that its anthers and stigmas have a very close position which leads to selfing (Y.-Q. Wang, D.X. Zhang and Z.-Y. Chen, unpubl. res.). Their P/O ratios are 5.73 ± 3.50 and 164.63 ± 36.15 , respectively.

DISCUSSION

Starch in pollen

Franchi *et al.* (1996) proposed that a positive starch reaction was categorized by three colour reactions, brown, blue and black, together with the presence or absence of birefringence. In our studies, starch reaction with IKI shows two colours (blue and black), and an unwanted phenomenon occurred with most or all pollen grains of *Globbeae racemosa*, *Zingiber striolatum*, and a few pollen grains of *Hedychium coronarium*, *H. flavum* and *H. spicatum* stained red. Previous studies revealed that starch reserves stored during pollen development give rise to carbohydrates at maturity and combinations of different types of carbohydrates in mature pollen may depend on the extent of starch hydrolysis (Speranza *et al.*, 1997). After total or partial starch hydrolysis, insoluble periodic acid-Schiff reaction

TABLE 2. The pollen grain numbers, ovule numbers, and P/O ratios for each of the species investigated (mean \pm s.d.)

Taxa	Population	Pollen grain nos/flower	Ovule nos/flower	P/O ratios/flower	Breeding system
Subfamily Zingiberoideae					
Tribe I. Alpineae					
<i>Alpinia blepharocalyx</i> var. <i>glabrior</i> (anaflexistyly)	S. China Botanical Garden	29090 \pm 5526.02	60 \pm 8.53	488.69 \pm 87.45	Obligate xenogamy
<i>A. blepharocalyx</i> var. <i>glabrior</i> (cataflexistyly)	S. China Botanical Garden	20040 \pm 3513.46	77.3 \pm 5.06	259.89 \pm 45.72	Obligate xenogamy
<i>A. guinanensis</i> (anaflexistyly)	S. China Botanical Garden	17740 \pm 5364.55	43.0 \pm 35.08	412.27 \pm 132.79	Obligate xenogamy
<i>A. guinanensis</i> (cataflexistyly)	S. China Botanical Garden	7100 \pm 1948.33	33.0 \pm 83.57	214.55 \pm 67.63	Obligate xenogamy
<i>A. hainanensis</i> (anaflexistyly)	S. China Botanical Garden	51270 \pm 1197.51	179 \pm 36.80	301.63 \pm 108.21	Obligate xenogamy
<i>A. hainanensis</i> (cataflexistyly)	S. China Botanical Garden	32490 \pm 6328.42	182.22 \pm 28.36	184.00 \pm 29.57	Obligate xenogamy
<i>A. oxyphylla</i> (anaflexistyly)	S. China Botanical Garden	8890 \pm 2402.69	25.8 \pm 1.99	345.67 \pm 90.64	Obligate xenogamy
<i>A. oxyphylla</i> (cataflexistyly)	S. China Botanical Garden	6380 \pm 779.49	26.3 \pm 3.87	246.79 \pm 41.87	Obligate xenogamy
<i>A. polyantha</i> (anaflexistyly)	S. China Botanical Garden	8085 \pm 1361.63	15.4 \pm 1.91	542.10 \pm 154.59	Obligate xenogamy
<i>A. polyantha</i> (cataflexistyly)	S. China Botanical Garden	4985 \pm 1515.76	17 \pm 2.28	297.79 \pm 96.44	Obligate xenogamy
<i>A. stachyodes</i> (anaflexistyly)	Nan Kun Shan, Guangdong	4600 \pm 1126.7	7.5 \pm 1.5	616.52 \pm 117.83	Obligate xenogamy
<i>A. stachyodes</i> (cataflexistyly)	Nan Kun Shan, Guangdong	3265 \pm 828.27	7.1 \pm 1.3	462.22 \pm 86.65	Obligate xenogamy
<i>A. zerumbet</i> (anaflexistyly)	S. China Botanical Garden	24710 \pm 9013.37	90.8 \pm 8.23	269.46 \pm 86.79	Obligate xenogamy
<i>A. zerumbet</i> (cataflexistyly)	S. China Botanical Garden	30950 \pm 8495.44	93.0 \pm 56.75	331.75 \pm 87.94	Obligate xenogamy
<i>Amomum maximum</i>	S. China Botanical Garden	14236.67 \pm 2390.70	44.6 \pm 3.75	320.82 \pm 54.56	Obligate xenogamy
<i>A. villosum</i>	S. China Botanical Garden	15360 \pm 1487.41	70.6 \pm 7.09	220.23 \pm 34.07	Facultative xenogamy
	Pan Long, Yang Chun, Guangdong	13890 \pm 2433.29	55.5 \pm 7.05	252.81 \pm 44.96	
	Chun Wan, Yang Chun, Guangdong	15150 \pm 3457.82	61.3 \pm 5.71	246.49 \pm 47.53	
Tribe II. Zingibereae					
<i>Zingiber striolotum</i>	Kunming Botanical Garden	5191.667 \pm 780.89	66.6 \pm 7.97	79.01 \pm 14.71	Facultative xenogamy
Tribe III. Hedychieae					
<i>Caulokaempferia coenobialis</i>	Ding Hu Shan, Guangdong	437.18 \pm 144.65	52.8 \pm 6.145	8.21 \pm 2.36	Active autogamy
	Nan Kun Shan, Guangdong	113.75 \pm 41.63	36.8 \pm 5.62	3.25 \pm 1.56	Active autogamy
<i>Curcuma kwangsiensis</i>	S. China Botanical Garden	8300 \pm 655.74	50.6 \pm 6.97	167.49 \pm 28.07	Facultative xenogamy
<i>C. phaeocalis</i>	S. China Botanical Garden	1150 \pm 220.45	64.1 \pm 5.84	13.51 \pm 2.96	Facultative xenogamy
<i>C. wenyujin</i>	S. China Botanical Garden	3680 \pm 554.40	49.2 \pm 6.52	78.33 \pm 15.56	Facultative xenogamy
<i>C. zanthorrhiza</i>	S. China Botanical Garden	2985 \pm 642.48	39.3 \pm 642.48	81.02 \pm 25.79	Facultative xenogamy
<i>Hedychium coccineum</i>	Kunming Botanical Garden	2142.86 \pm 417.45	27.5 \pm 4.90	82.67 \pm 24.67	Facultative xenogamy
<i>H. coronarium</i>	S. China Botanical Garden	19480 \pm 2705.85	141.1 \pm 5.49	138.49 \pm 21.35	Facultative xenogamy
<i>H. spicatum</i>	Kunming Botanical Garden	2379.63 \pm 945.79	61.0 \pm 18.68	41.85 \pm 21.91	Facultative xenogamy
<i>Pyrgophyllum yunnanense</i>	Ji Zu Shan, Yunnan	3319.44 \pm 2303.52	20.67 \pm 3.45	164.63 \pm 36.15	Passive autogamy
<i>Roscoeia blanda</i>	Ji Zu Shan, Yunnan	15110 \pm 4651.98	69.33 \pm 11.35	232.07 \pm 86.71	Obligate xenogamy
Tribe IV. Globbeae					
<i>Globba racemosa</i>	Kunming Botanical Garden	5195.83 \pm 1370.68	39.7 \pm 6.68	127.27 \pm 31.41	Facultative xenogamy
Subfamily Costoideae					
<i>Costus speciosus</i>	S. China Botanical Garden	9275 \pm 1838.31	67.25 \pm 10.12	139.27 \pm 26.30	Facultative xenogamy

TABLE 3. Comparison of P/O ratios from taxonomic groups (mean \pm s.d.)

Taxa	Pollen grain nos/flower	Ovule nos/flower	P/O ratios	No. of species
Tribe Alpineae				
<i>Alpinia</i>	17828.21 \pm 14297.90	61.36 \pm 58.25	355.24 \pm 130.04	7
<i>Amomum</i>	14518.34 \pm 398.34	53.53 \pm 12.63	280.33 \pm 57.26	2
<i>T</i>	0.6117	0.2584	1.1743	
<i>P</i>	0.270	0.400	0.120	
Tribe Hedychieae				
<i>Curcuma</i>	4028.75 \pm 2633.53	50.8 \pm 8.83	85.09 \pm 54.72	4
<i>Hedychium</i>	8000.83 \pm 9941.96	76.57 \pm 58.36	87.67 \pm 48.52	3
<i>T</i>	0.6745	0.5359	0.0659	
<i>P</i>	0.250	0.295	0.475	

T, Student's *T*-test value; *P*, probability.

TABLE 4. Comparison of P/O ratios from different populations in the same species (mean \pm s.d.)

Species	Population	Pollen grain no./flower	Ovule no./flower	P/O ratios
<i>Amomum villosum</i>	South China Bot. Garden	15360 \pm 1487.41	70.6 \pm 7.09	220.23 \pm 34.07
	Pan Long, Yang Chun Co., Guangdong	13890 \pm 2433.29	55.5 \pm 7.05	252.81 \pm 44.96
	Chun Wan, Yang Chun Co., Guangdong	15150 \pm 3457.82	61.3 \pm 5.71	246.49 \pm 47.53
	d.f.	2	2	2
	<i>F</i>	3.0380	11.8237	1.4814
	<i>P</i>	>0.05	<0.05	>0.05
	Mean	14800 \pm 795.05	62.47 \pm 7.62	239.84 \pm 17.28
<i>Caulokaempferia coenobialis</i>	Ding Hu Shan, Zhaoqing, Guangdong	437.18 \pm 144.65	52.8 \pm 6.15	8.21 \pm 2.36
	Nan Kun Shan, Longmen, Guangdong	113.75 \pm 41.63	36.8 \pm 5.62	3.25 \pm 1.56
	<i>N</i>	10	10	10
	<i>T</i>	6.7949	6.0733	5.5444
	<i>P</i>	<0.005	<0.005	<0.005
	Mean	275.47 \pm 228.70	44.8 \pm 11.31	5.729 \pm 3.50

F, *F*-test showing significance.

TABLE 5. Comparison of breeding-system P/O ratios (mean \pm sd)

	Autogamy			Obligate	Facultative
	Active autogamy	Passive autogamy	Mean	xenogamy	xenogamy
P/O ratios	5.73 \pm 3.50	164.63 \pm 36.15	58.70 \pm 91.78	345.39 \pm 125.07	108.07 \pm 62.93
No. of species	1	1	2	9	11
CV	61.08	21.96	156.35	36.21	58.23

CV, coefficient of variation.

(PAS)-positive oligo/polysaccharides were found in the cytoplasm associated with much soluble sugar, and the pollen grains were dehydrated at dispersal (Speranza *et al.*, 1997). The red colour in the pollen may suggest that they contain some protein or pollen starch hydrolysis.

Baker and Baker's (1979) studies on developing pollen grains showed that *Fuchsia* pollen, passes through a starchless phase, followed by one of starch accumulation, followed by its reduction to virtual absence by the time the anthers dehisce. They also mentioned that, generally, in the Rosaceae there is little difficulty in classifying pollen as starchy or starchless. In cases where starch had a very strong presence in immature grains but not disappear entirely from mature grains it was decided that the grains should be classified as 'starchless' (Baker and Baker, 1979). In

Zingiberaceae, three species of *Hedychium* (*H. coccineum*, *H. flavum* and *H. coronarium*) exhibited phenomena similar to those they described, although the pollen of the other two species (*H. spicatum* and *H. spicatum* var. *acuminatum*) was typically starchy.

Previous studies indicated that members of a particular family are usually uniform regarding the possession of starchy or starchless pollen (Baker and Baker, 1979). Zona (2001) revealed that the order Zingiberales is the most uniform order as regards starchy pollen within the Commelinoid monocots, and all of the taxa in the Zingiberaceae he studied (19 species from ten genera) had starchy pollen, stained either brown or black, with two species of *Alpinia* having birefringent starchy pollen. The present results, however, are only in partial agreement with theirs. Of all the

species in this study, four (*Amomum villosum*, *Globba racemosa*, *Hedychium coronarium* and *H. flavum*) have entirely starchless pollen, while all other species have pollen at least some reserves of starch. Also, no lipid was found in the pollen of one species (*Stahlianthus involucratus*), a result which contradicts Baker and Baker (1979), who considered that all pollen grains contain some lipid.

In rare instances, there may be two kinds of pollen produced by the same flower (Baker and Baker, 1979). Mangelsdorf (1932) suggested that within a single anther there may be segregation of alleles for starch production when the sporophyte itself is heterozygous for a mutation; also there may be a proportion of 'bad' pollen admixed with the 'good' pollen in the anther as the result of some upset in the meiotic process, and the 'bad' pollen may give no starch reaction while the 'good' pollen gives a positive reaction. Among the species examined, *Zingiber zerumbet* shows two types of pollen (large pollen and small pollen), with the much fewer smaller ones showing a starch reaction, although both types apparently contain lipids. In *Curcuma zanthorrhiza*, *C. wenyujin* and *C. phaeocalis*, roughly half of the pollen grains are starchy and the other half starchless.

Among 37 species studied, two (*Caulokaempferia coenobialis* and *Pyrgophyllum yunnanense*) were regularly self-pollinated, 15 were exclusively cross-pollinated and 20 were facultatively xenogamous. The two autogamous, all the 15 obligate xenogamous and 14 of 20 facultatively xenogamous species have starchy pollen, while all the lipid pollen comes from the facultatively xenogamous species. The results shown here indicate that there is no correlation between mating system and pollen nutrition reserve type, although previously Baker and Baker (1979) commented that the pollen of the selfers are more frequently starchy than those of outcrossers.

Almost all species studied (only *Caulokaempferia coenobialis* and *Pyrgophyllum yunnanense* being self-pollinated) were entomophilous, with most of the pollinators being bees and, in a few instances, butterflies or moths. Baker and Baker (1979) elucidated that, when pollen grains are part or all of the reward received by bees and flies visiting flowers, selection favoured starchless (oil-rich) pollen and, when insect nutrition is not involved, it favoured starchy pollen. Insects visiting flowers of Zingiberaceae in this study mostly forage on nectar and only accidentally on pollen grains (bees), with the exception of *Hedychium* species, *Amomum villosum* and *Globba racemosa* to which the pollinators came for both nectar and pollen grains. As almost all species of Zingiberaceae in the present study produced a large amount of nectar, according to Baker

and Baker's hypothesis, species in this family should mostly produce starchy pollen grains. Our results are in full accordance with this hypothesis.

Grayum (1985) reported that in Araceae, 91 % of all spinose pollen is starchy, 83 % of all psilate genera are exclusively starchy and the figures for verrucate and striate pollen types are comparable, but genera in the most primitive, foveolate-reticulate category are equally likely (10/10) to be starchy or starchless. In the studies carried out by the authors, 89.5 % of all spinose pollen is starchy (17 of 19 species), 50 % of all psilate pollen is starchy (six of 12 species, with the exception that three produce starchless pollen and the other three produce a mixture of starch and starchless). All the verrucate and striate pollen types (100 %) are starchy. This result does not agree with what Grayum (1985) suggested as the correlation between exine sculpture and pollen nutrition type.

Baker and Baker (1979) also suggested that in many cases, pollen grain size followed family classification. In Zingiberaceae, the pollen grain sizes are highly correlated with generic classification (Table 1). Generally, the mean pollen grain diameter of the species with starchy pollen is significantly greater than that of the species with starchless pollen (Grayum, 1985). The present study, however, shows that there is no significant difference in size between starchy and starchless pollens in Zingiberaceae, although there are more variations in starchless pollens (see Table 6).

Pollen: ovule ratios

According to Cruden (1977), the P/O ratios range from 2.7 to 6.7 in cleistogamous flowers, from 18.1 to 39.0 in obligate autogamous flowers, from 31.9 to 396.9 in facultative autogamous flowers, from 244.7 to 2558.6 in facultative xenogamous flowers, and from 2108 to 19523 in xenogamous flowers. The present data show that P/O ratios of xenogamous species in the family are above 184.00 (mean 345.39 ± 125.07), while those of most facultatively xenogamous species (with the exception of *Amomum villosum*) are less than 168 (mean 108.07 ± 62.93). The P/O ratios of autogamous species range from 5.73 ± 3.50 to 164.63 ± 36.15 . These data are obviously lower than the numbers in the respective breeding systems summarized by Cruden (1977). Previous studies have shown that species with special pollen-transporting mechanisms (pollinia, viscin threads, polyads) have much lower P/O ratios than species lacking these mechanisms (Cruden, 1977; Cruden and Jensen, 1979; Mehrhoff, 1983; Kopture, 1984; Vasek

TABLE 6. Comparison of P/O ratios and pollen size (diameters) in species of Zingiberaceae with starchy and starchless pollen

	Pollen grain no./flower	Ovule no./flower	P/O ratios	Pollen size (μm)
Starchy pollen	12423.26 ± 12450.79 ($n = 17$)	56.59 ± 43.36 ($n = 17$)	248.95 ± 165.31 ($n = 17$)	77.55 ± 11.51 ($n = 21$)
Starchless pollen	9362.07 ± 7396.12 ($n = 5$)	67.47 ± 44.20 ($n = 5$)	133.46 ± 65.06 ($n = 5$)	75.29 ± 21.90 ($n = 7$)
<i>T</i> (<i>t</i> -test)	0.6835	0.0217	2.3313	0.2235
<i>P</i>	0.250	0.490	0.01	0.410

TABLE 7. Comparison of P/O ratios from different life forms

	Pollen grain no./flower	Ovule no./flower	P/O ratios	No. of species
Evergreen perennials	15614-01	60-22	302-82	12
Deciduous perennials	5372-53	55-68	91-96	10
<i>T</i>	2-5219	0-2663	4-5523	
<i>P</i>	<0-005	0-395	<0-005	

and Weng, 1988; Wyatt *et al.*, 2000). The low P/O ratios may be due to very efficient pollinating mechanisms ensuring that sufficient numbers of pollen grains are deposited on the stigmas (Cruden, 2000; Jürgens and Gottsberger, 2002). The lower P/O ratios in the family may be another indication of its much specialized, sophisticated pollination mechanisms. Cruden and Miller-Ward (1981) postulated that species with relatively larger stigma areas compared with the pollen-bearing area of the pollinator often have lower P/O ratios; the low P/O ratios in Zingiberaceae could also be explained by the large stigma areas.

Dimorphic flowers (cataflexistylous vs. anaflexistylous) have been described recently in *Amomum* and *Alpinia* (Cui *et al.*, 1996; Li *et al.*, 2001). The present observations on the P/O ratios of the two phenotypes in *Alpinia* showed that in most taxa in the genus (except *A. zerumbe*), the anaflexistylous flowers have larger pollen numbers and higher P/O ratios than the cataflexistylous flowers; the ovule numbers in the anaflexistylous flowers, however, are approximately equivalent to, or less than those in the cataflexistylous flowers (except in *A. guianensis*) (see Table 2), a result in contradict with Li (2002) and Zhang (2003) who also measured the P/O ratios of the two phenotypes and further speculated that it may indicate that flexistylous be an intermediate from hermaphrodite to dioecy.

While studying the P/O ratios in Caryophyllidae, Jürgens and Gottsberger (2002) found significant differences between some taxonomic groups in pollen grain numbers and ovule numbers but not in P/O ratios. The same phenomenon is found in Zingiberaceae (see Table 3). It was also noticed that there is a correlation between the breeding system and life-form types in the family, although with significant differences in mean pollen grain numbers and P/O ratios, approximately constant ovule numbers were recorded in species with different life forms (see Table 7). The result is consistent with that of Caryophyllidae as reported by Jürgens and Gottsberger (2002). All of the two autogamous and most facultative xenogamous species are deciduous perennials. Most of the obligated xenogamous species (the only exception being *Roscoea blanda*) are evergreen perennials. The pollen number and the P/O ratios were obviously higher in evergreen perennials than in deciduous perennials (see Table 7).

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LITERATURE CITED

- Baker HG, Baker I. 1979. Starch in angiosperm pollen grains and its evolutionary significance. *American Journal of Botany* 66: 591–600.
- Charnov EL. 1982. *The theory of sex allocation*. Princeton: Princeton University Press.
- Cruden RW. 1977. Pollen-ovule ratios: a conservative indicator of breeding systems in flowering plants. *Evolution* 31: 32–46.
- Cruden RW. 2000. Pollen grains: why so many? *Plant Systematics and Evolution* 222: 143–165.
- Cruden RW, Jensen KG. 1979. Viscin threads, pollination efficiency and low pollen-ovule ratios. *American Journal of Botany* 66: 875–879.
- Cruden RW, Miller-Ward S. 1981. Pollen-ovule ratio, pollen size, and the ratio of stigmatic area to the pollen-bearing area of the pollinator: a hypothesis. *Evolution* 35: 964–974.
- Cui X-L, Wei R-C, Huang R-F. 1996. A study on the breeding system of *Amomum tsaoko*. In: Wu T-L, Wu Q-G, Chen Z-Y, eds. *Proceedings of the second symposium on the family Zingiberaceae*. Guangzhou: Zhongshan University Press.
- Dafni A. 1992. *Pollination ecology, a practical approach*. New York: Oxford University Press.
- Darwin C. 1877. *The different forms of flowers on plants of the same species*. London: John Murray.
- Franchi GG, Bellani L, Nepi M, Pacini E. 1996. Types of carbohydrate reserves in pollen: localization, systematic distribution and ecophysiological significance. *Flora* 191: 143–159.
- Grayum MH. 1985. Evolutionary and ecological significance of starch storage in pollen of the Aracea. *American Journal of Botany* 72: 1565–1577.
- Jürgens A, Gottsberger TWG. 2002. Pollen grain numbers, ovule numbers and pollen-ovule ratios in Caryophyllidae: correlation with breeding system, pollination, life form, style number, and sexual system. *Sexual Plant Reproduction* 14: 279–289.
- Kopture S. 1984. Outcrossing and pollinator limitation of fruit set: breeding systems of neotropical *Inga* trees. *Evolution* 38: 1130–1143.
- Li Q-J, Xu Z-F, Xia Y-M, Zhang L, Deng X-B, Gao J-Y. 2001. Study on the flexistylous pollination mechanism in *Alpinia* plants (Zingiberaceae). *Acta Botanica Sinica* 43: 364–369 [in Chinese].
- Li Q-J. 2002. *Study on the flexistylous outcrossing mechanism in Alpinia plants (Zingiberaceae)*. PhD Thesis, Kunming Institute of Botany, Chinese Academy of Sciences [in Chinese].
- Liang Y-H. 1988a. Pollen morphology of *Alpinia* (Zingiberaceae) from China. *Acta Botanica Austro Sinica* 4: 103–107 [in Chinese].
- Liang Y-H. 1988b. Pollen morphology of the family Zingiberaceae in China—pollen types and their significance in the taxonomy. *Acta Phytotaxonomica Sinica* 26: 265–281 [in Chinese].
- Mangelsdorf PC. 1932. Mechanical separation of gametes in maize. *Journal of Heredity* 23: 288–295.
- Mehrhoth LA. 1983. Pollination in the genus *Isotria* (Orchidaceae). *American Journal of Botany* 70: 1444–1453.
- Preston RE. 1986. Pollen-ovule ratios in the Cruciferae. *American Journal of Botany* 73: 1732–1740.
- Sakai S, Kato M, Inoue T. 1999. Three pollination guilds and variation in floral characteristics of Bornean gingers (Zingiberaceae and Costaceae). *American Journal of Botany* 86: 646–658.
- Speranza A, Calzoni GL, Pacini E. 1997. Occurrence of mono- or disaccharides and polysaccharide reserves in mature pollen grains. *Sexual Plant Reproduction* 10: 110–115.
- Stebbins GL. 1970. Adaptive radiation in angiosperms. I. Pollination mechanisms. *Annual Review of Ecology and Systematics* 1: 307–326.
- Vasek FC, Weng V. 1988. Breeding systems of *Clarkia* sect. *Phaeostoma* (Onagraceae). I. Pollen-ovule ratios. *Systematic Botany* 13: 336–350.
- Wu T-L. 1994. Phytogeography of the Zingiberaceae. *Journal of Tropical and Subtropical Botany* 2: 1–14 [in Chinese].

- Wyatt R. 1984.** Evolution of self-pollination in granite outcrop species of *Arenaria* (*Caryophyllaceae*). III. Reproductive effort and pollen-ovule ratios. *Systematic Botany* **9**: 432–440.
- Wyatt R, Steven BB, Sara RL. 2000.** Pollen-ovule ratios in milkweeds (*Asclepiadaceae*): an exception that proves the rule. *Systematic Botany* **25**: 171–180.
- Zhang L, Li Q-J, Deng X-B, Ren P-Y, Gao J-Y. 2003.** Reproductive biology of *Alpinia blepharocalyx* (*Zingiberaceae*): another example of flexistyly. *Plant Systematics and Evolution* **241**: 67–76.
- Zona S. 2001.** Starchy pollen in commelinoid monocots. *Annals of Botany* **87**: 109–116.