

Age at pollination modifies relative male and female reproductive success in a monoecious fig tree

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Abstract Plants that depend on a single species of insect pollinator must often contend with infrequent and unpredictable visitation. Prolongation of floral receptivity comes at the cost of reduced male and/or female reproductive success among older flowers. Fig trees (*Ficus* spp.) have a highly specific pollination symbiosis and individual inflorescences (syconia) that remain receptive for days or weeks. Reproductive success in monoecious fig trees involves production of both seeds and fig wasp offspring. We assessed whether the reproductive output of individual syconia changes with the length of time they waited for pollination, and whether the relative female and male reproductive success also changes. A pollination experiment was conducted in an SE Asian monoecious fig tree *Ficus curtipes*, in which receptive syconia were covered with mesh bags to exclude wasps and pollinated by single pollinators of this fig tree at their different receptive ages. When the syconia matured their size and contents were recorded. Seed quality was also assessed. The results showed that pollinators entered syconia that had been waiting for up to 36 days. The frequencies of abortions among syconia pollinated at different ages were low throughout. The number of un-utilised flowers increased progressively in older syconia. Seed production was highest in syconia entered on the first day of receptivity,

whereas pollinator production peaked in syconia pollinated on day 12, then declined in older syconia. Consequently, overall reproductive efficiency declined with syconium age and floral sex allocation became more male-biased in older syconia. Older syconia also produced lighter seeds. These results suggest that un-pollinated syconia of *F. curtipes* can remain receptive for several weeks. This makes pollination of each syconium more likely, but at the cost of reduced productivity and with more ovules allocated to male function. However, the prolongation of floral receptivity has significance for the co-adaptation between syconia and fig wasps and for the evolution of the fig tree-fig wasp symbiosis.

Keywords *Ficus* · Fig wasp · Floral sex allocation · Flower age · Pollination · Reproduction

1 Introduction

Floral receptivity (the period when a flower can be pollinated successfully) is highly variable, ranging in duration from a few hours to several months (Primack 1985; van Doorn 1997; Steinacher and Wagner 2010). The longevity of receptivity is clearly adaptive, with a strong influence on plant reproductive success (Evanhoe and Galloway 2002; Rathcke 2003; Itagaki and Sakai 2006; Steinacher and Wagner 2010), and reflects a balance between the costs of flower maintenance and new flower production (Primack 1985; Schoen and Ashman 1995). Flowers at the beginning of receptivity will differ from older flowers in their physiological condition, especially in species where receptivity is lengthy. Marshall et al. (2010) catalogue numerous changes that may occur during the life spans of individual flowers. The relative benefits of pollen receipt and donation may show corresponding changes, and patterns of investment may change accordingly (Ashman and Schoen 1994).

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Long lived flowers with extended periods of receptivity are expected when the costs of flower maintenance are low and rates of pollen receipt and donation are also low (Schoen and Ashman 1995). High initial costs of production of flowers and their supporting structures may also be significant. Together, these factors explain the extended periods of floral receptivity exhibited by fig trees (*Ficus*). The more than 800 species of fig trees are characterised by their unique inflorescence, by having highly specific pollinators and also in most species by an all year flowering pattern that results in only a small proportion of the trees in a population releasing or requiring pollen-carrying fig wasps at any one time. All-year flowering has made fig trees a keystone resource in tropical forests, because they provide syconia for vertebrates to eat during seasons when other plants do not (McKey 1989; Shanahan et al. 2001). However, they often remain at low densities (Mawdsley et al. 1998) and synchronise flowering within individual trees, and their pollinator specificity results in an unpredictable and routinely limiting supply of pollinators (Bronstein 1988).

Syconia are enclosed globular inflorescences containing hundreds or thousands of tiny flowers. Each *Ficus* species often depends on a single species of host specific fig wasp (Agaonidae) for pollination and in return provides the wasps with a nursery for their larval development (Weiblen 2002; Herre et al. 2008). The short-lived adult female fig wasps (Kjellberg et al. 1988; Dunn et al. 2008) enter the syconia through a narrow ostiole and once inside they pollinate the uniovular flowers while also galling and laying eggs in some of them (Janzen 1979). The females typically fail to re-emerge and so pollinate just a single syconium. Floral receptivity ends shortly after entry by the pollinators, though in some species with larger syconia it may continue for several days if they are only entered by a single pollinator (Khadari et al. 1995). Each flower can produce a single seed or a single pollinator offspring, which mature a few weeks later. This means a wasp gall produced with one potential seed lost. Adults of the next generation of fig wasps emerge from their galls, mate, become loaded with pollen and immediately fly off in search of receptive syconia on another tree. Protogyny and within-tree fruiting synchrony prevent self-pollination, and force the pollinators to disperse in search of receptive syconia on other trees, which may be far away (Ahmed et al. 2009). The length of time that syconia can remain receptive varies between a few days to several weeks (Khadari et al. 1995; Zhang et al. 2011). This variability suggests that syconium longevity may be an adaptive trait, perhaps associated with differences in the reliability of pollinator visitation between different fig tree species (Suleman et al. 2011).

Fig trees and their pollinators are a convenient system for studying the costs of floral longevity because pollination depends on entry of a single wasp, which can be controlled experimentally, and because both male and female reproductive

functions are easily monitored (Marshall et al. 2010). Each female flower can produce either a single seed, nothing, or a single pollen-carrying fig wasp female, or a single fig wasp male that is not of direct value to pollen dispersal of fig trees (but is essential to allow female pollinators to escape the syconia). The relative success of syconia that have been waiting different lengths of time for pollinators has been described for two species of dioecious fig trees in two controlled pollination experiments (Suleman et al. 2011; Zhang et al. 2011), in which the receptive syconia were pollinated at their different ages. Fig trees with a dioecious breeding system have functionally distinct male and female trees with syconia that produce only pollinators or seeds respectively. Productivity among syconia that were pollinated later was found to decline in the two dioecious species, with seed numbers declining more rapidly than pollinator numbers (Suleman et al. 2011; Zhang et al. 2011).

Here we describe a similar controlled pollination experiment with a monoecious fig tree where pollinators and seeds develop in the same syconia and potentially compete for resources. Specifically, we investigated the effects of age at pollination on the numbers and quality of seeds produced by the syconia, and compared seed productivity with the numbers of pollinators produced by the same syconia to determine whether male and female reproductive functions responded differently.

2 Materials and methods

2.1 Study organisms

Ficus curtipes is a monoecious tree reaching about 5–10 m in height. It is widely distributed in seasonal tropical forests of Southeast Asia and is also cultivated in cities and villages as an ornamental tree. The syconia form in the leaf axils, reaching about 12 mm in diameter when receptive to pollination (Zhang et al. 2009). They are produced throughout the year and typically develop as well-synchronized crops, with individual trees producing syconia at different times of the year. Like other monoecious fig trees, the syconia of *F. curtipes* contain both male and female flowers and produce both seeds (female reproductive function) and fig wasps (male reproductive function).

F. curtipes is pollinated by foundress females of an undescribed species of *Eupristina* (Hymenoptera, Agaonidae) that is only associated with this *Ficus* species. Because pollination is passive in *F. curtipes* (with large quantities of pollen produced; see Kjellberg et al. 2001), two further non-agaonid fig wasps that enter the syconia to oviposit can act as supplementary pollinators, but they only develop in syconia that also contain *Eupristina* (Zhang et al. 2008, 2009). *Eupristina* sp. adult females are short lived, rarely surviving more than one day under natural conditions and once they enter a syconium

they rarely re-emerge (Gu D., pers. obs.). Under natural conditions *Eupristina* sp. foundress numbers per syconium number one or two (Gu et al. 2012).

2.2 Pollination experiments

Experimental introductions of pollinators were performed between 20 Sept. and 26 Oct. 2010 on one synchronously-fruiting tree at the Xishuangbanna Tropical Botanical Garden, Yunnan Province, China (21°55'N, 101°25'E). Twigs bearing pre-receptive syconia were covered with mesh bags to prevent oviposition by pollinators and other fig wasps. Syconium receptivity was judged using the method described in Khadari et al. (1995). The pollinators for pollination experiments had been obtained 12 h before each introduction from a single tree bearing mature syconia. On the morning of the first day when the syconia became receptive (day 0), we deposited single pollinator female on the surface of a syconium and waited for the wasp to enter it. The syconium entered by the wasp was numbered with a tag and covered with a mesh bag. This was repeated on syconia at different receptive ages (day, 0, 4, 8, 12, etc.) until the pollinators were no longer willing to enter. According to the number of foundresses observed within syconia of this species under natural conditions (Gu et al. 2012), a single wasp was introduced per syconium at any receptive age. At least thirty syconia at each receptive age group were randomly chosen for the pollination experiment. A total of 422 syconia were pollinated in this way, of which 389 completed their development and the remainder aborted.

The pollinated syconia were collected once they ripened. Their equatorial and polar diameters were measured with a digital calliper and their volume was estimated using the formula for an oblate spheroid ($V = \frac{1}{6}\pi A^2 B \approx 0.523A^2 B$, where A is the equatorial diameter, and B is the polar diameter). The uniovulate female flowers were scored as either producing a seed or a wasp (a normal gall), as hollow empty galls ('bladders') where pollinator larvae had failed to survive (Ghana and Compton 2012) or as having been unutilised (withered flowers). Any misshapen seeds were recorded separately. The numbers of healthy and aborting/unhealthy male flowers (there is one anther per flower) were also recorded.

Multiple sub-samples of ten apparently healthy air dried seeds were collected at random and weighed to the nearest 0.001 g in groups of ten. Two groups of 100 randomly selected seeds from each age group were also germinated on moist filter paper in Petri dishes at 25 ± 1 °C with a 12/12 h light/dark period. The number of seeds that had germinated was recorded.

Sex allocation of monoecious syconia actually indicates the allocation of ovules between female (seeds) and male (pollinator progeny or galls) function. Therefore, it was

evaluated by the number of seeds and galls and the index of ovule allocation between male and female function (IOAMFF, the proportion of total seeds to total utilised female flowers) and the effective index of ovule allocation between male and female function (EIOAMFF, the proportion of healthy seeds to female flowers that successfully produce healthy seeds or pollinators). Technically, sex allocation of monoecious syconia should involve the number of male flowers (anthers), but the number of male flowers was determined at the beginning of the syconia' development, thereby being excluded when involving syconium sex allocation in this study.

2.3 Statistical analysis

Data were analysed by one-way analysis of variance (ANOVA), and Duncan's multiple range test was used to determine significant differences between age groups ($P < 0.05$). When heterogeneity of variances and non-normality, even after transformation, was present, we used nonparametric Kruskal–Wallis tests followed by Tamhane post hoc tests ($P < 0.05$) to detect differences among syconia pollinated at different ages. Mann–Whitney tests were used to identify differences between ages when Kruskal–Wallis tests indicated significant differences ($P < 0.05$) and Tamhane tests failed to detect the differences, with P -values adjusted using the Holm method (Norman and Streiner 2008). Pollinators were increasingly unwilling to enter syconia that had been receptive for longer than 28 days and the contents of syconia aged 28 days ($n=46$), 32 days ($n=5$) and 36 days ($n=4$) were combined throughout. All statistical analyses were conducted using the program SPSS 13.0 for Windows software (SPSS 2004), and all figures were plotted using the Sigmaplot 10.0 software (Systat Software 2006).

3 Results

3.1 Overall reproductive efficiency

Most un-pollinated syconia continued to be receptive for at least 28 days. The frequencies of abortions among syconia pollinated at different ages were low throughout, ranging between about 5 % and 10 %, and did not differ significantly between age groups ($\chi^2=1.82$, d.f. = 7, $P=0.969$). Reflecting changes in their contents, syconia that had been pollinated at a later date had a smaller volume when mature (Table 1), despite consistently containing about 180 female flowers and 140 male flowers per syconium (Table 1). The number of un-utilised flowers that contained neither seeds nor galls increased progressively in older syconia, from about 40 to 80 (i.e. from nearly 25 % to 50 %) flowers per syconium, suggesting that the pollinators laid fewer eggs, as

Table 1 Variation in the size and contents of *F. curtipyes* syconia in relation to their age when they were pollinated. One-way ANOVA or Kruskal-Wallis tests were applied to each row. Age groups with different superscripts were significantly different

	Age at pollination (days since becoming receptive) (mean \pm SD)										F/H	P
	0	4	8	12	16	20	24	$\geq 28^{\dagger}$				
Number of syconia	33	50	50	51	50	46	54	55				
Syconium size (cm ³)	1.72 \pm 0.33 ^a	1.51 \pm 0.26 ^{ab}	1.43 \pm 0.19 ^{bc}	1.41 \pm 0.21 ^{bc}	1.31 \pm 0.17 ^{cd}	1.27 \pm 0.18 ^d	1.33 \pm 0.20 ^{cd}	1.32 \pm 0.20 ^{cd}	77.18 [#]	< 0.0001		
Total female flowers	185.55 \pm 13.02 ^a	185.86 \pm 17.30 ^a	184.44 \pm 15.77 ^{ab}	183.76 \pm 13.99 ^{ab}	181.44 \pm 15.33 ^{abc}	176.54 \pm 18.19 ^c	181.46 \pm 14.84 ^{abc}	177.73 \pm 15.93 ^{bc}	2.32 [*]	0.0252		
Un-utilised flowers (%)	24.58 \pm 10.71 ^a	32.69 \pm 15.99 ^{abc}	33.32 \pm 11.19 ^{bc}	31.33 \pm 9.85 ^{ab}	39.51 \pm 14.88 ^{cd}	40.26 \pm 13.77 ^{cd}	46.04 \pm 14.37 ^d	48.73 \pm 14.72 ^d	98.27 [#]	< 0.0001		
Total seeds	52.85 \pm 19.99 ^a	37.66 \pm 14.99 ^b	36.74 \pm 18.53 ^b	27.27 \pm 26.35 ^{bc}	19.50 \pm 16.05 ^c	22.48 \pm 17.09 ^c	24.15 \pm 15.80 ^c	18.82 \pm 13.94 ^c	100.91 [#]	< 0.0001		
Healthy seeds (%)	98.00 \pm 2.61	97.41 \pm 3.61	96.37 \pm 6.55	97.23 \pm 5.36	96.16 \pm 9.31	97.87 \pm 4.29	95.29 \pm 8.57	94.25 \pm 9.96	6.77 [#]	0.4535		
Total galls	87.73 \pm 24.16 ^{abc}	88.62 \pm 34.37 ^{abc}	86.66 \pm 20.68 ^{abc}	99.29 \pm 20.66 ^a	90.92 \pm 29.54 ^{ab}	83.15 \pm 23.02 ^{bc}	74.28 \pm 25.27 ^{bc}	72.84 \pm 24.18 ^c	45.21 [#]	< 0.0001		
Empty galls	37.76 \pm 34.90 ^a	25.92 \pm 28.74 ^{ab}	21.98 \pm 25.39 ^{ab}	19.20 \pm 26.76 ^b	23.62 \pm 28.04 ^{ab}	22.33 \pm 24.98 ^{ab}	18.30 \pm 20.07 ^b	24.22 \pm 23.41 ^{ab}	18.08 [#]	0.0116		
Normal galls (%)	54.02 \pm 39.21 ^a	65.58 \pm 35.57 ^{ab}	73.10 \pm 30.13 ^{ab}	79.73 \pm 27.22 ^b	69.79 \pm 35.36 ^{ab}	67.87 \pm 36.78 ^{ab}	71.35 \pm 31.17 ^{ab}	65.78 \pm 28.78 ^{ab}	24.49 [#]	0.0009		
Total male flowers	148.18 \pm 9.22 ^a	142.02 \pm 13.11 ^{bc}	145.82 \pm 11.32 ^{abc}	145.80 \pm 11.64 ^{abc}	142.76 \pm 13.34 ^{abc}	146.65 \pm 10.72 ^{ab}	146.00 \pm 12.29 ^{abc}	140.51 \pm 15.24 ^c	2.13 [*]	0.0401		
Healthy male flowers	128.45 \pm 45.05 ^{ab}	122.84 \pm 46.42 ^{ab}	138.58 \pm 27.11 ^a	139.75 \pm 30.81 ^a	128.00 \pm 42.02 ^{ab}	128.74 \pm 42.76 ^{ab}	134.52 \pm 35.80 ^{ab}	123.98 \pm 36.76 ^b	18.00 [#]	0.0120		
Healthy male flowers (%)	86.56 \pm 5.22 ^{ab}	86.65 \pm 4.53 ^{ab}	95.01 \pm 2.37 ^{ab}	95.70 \pm 2.74 ^b	89.61 \pm 3.91 ^{ab}	87.49 \pm 4.01 ^a	92.10 \pm 3.13 ^{ab}	87.85 \pm 3.21 ^a	24.47 [#]	0.0009		
IOAMFF	0.38 \pm 0.14 ^a	0.34 \pm 0.23 ^{ab}	0.30 \pm 0.12 ^{ab}	0.20 \pm 0.17 ^c	0.18 \pm 0.13 ^c	0.20 \pm 0.13 ^c	0.25 \pm 0.17 ^{bc}	0.20 \pm 0.13 ^c	69.98 [#]	< 0.0001		

IOAMFF, index of ovule allocation between male and female function (proportion of total seeds to total utilised female flowers)

*F: ANOVAs

#H: Kruskal-Wallis tests

[†]Data from age 28 days (sample size, $n=46$ syconia), age 32 day (sample size, $n=5$ syconia) and age 36 days (sample size, $n=4$ syconia) were pooled during the analyses

well as pollinating fewer flowers, in the oldest syconia (ANOVA, $F_{7, 381}=15.80$, $P<0.0001$, Fig. 1; Table 1). Taken together, these suggested that overall reproductive efficiency declined with syconium age.

3.2 Reproductive efficiency of female function

The proportion of misshapen seeds in the syconia was consistently low in all age groups and germination rates of normal, round seeds remained consistently above 90 % (Tables 1 and 2). Seed weights nonetheless declined with syconium age (Table 2), with potential consequences for their post-germination survivorship. Given the decline in the weight of seeds from old syconia (Table 2), the age-related changes in female reproductive success may be greater than indicated.

3.3 Reproductive efficiency of male function

Reproductive efficiency of male function is determined by the proportion of normal galls and that of healthy anthers. Failed, empty galls were more common in the youngest, day 0 syconia, but consistently comprised only a small proportion of the flowers in syconia of all age groups, with little difference between each age group (Table 1), suggesting that increased mortalities among fig wasp larvae were not responsible for the decline in numbers of wasps produced by the oldest syconia. The proportion of anthers that failed to complete their development ranged between about 5–15 %, and was not related to the age of the syconia (Table 1).

The sexes of *Eupristina* sp. were recorded in a sub-sample of the syconia (Table 3). Only females transfer pollen between syconia and are of direct value to the plants. Females were much more numerous than males (proportion males=0.24, $n=22,014$ wasps in 312 syconia) and their numbers varied more

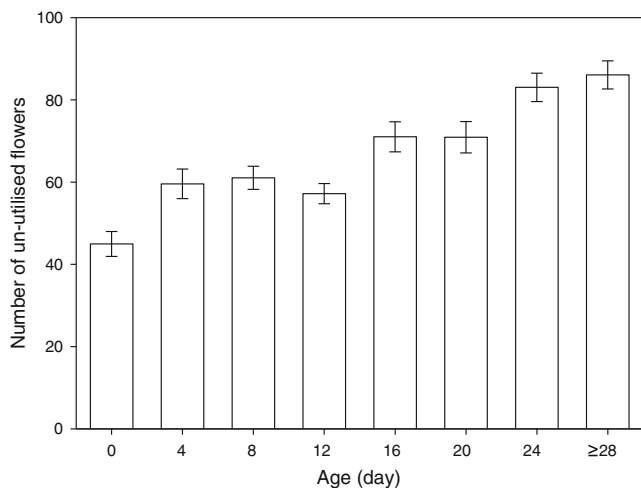


Fig. 1 Effect of age at pollination on overall reproductive efficiency of *F. curtipes* syconia. Data are shown with means \pm SE (n (syconia)=33, 50, 50, 51, 50, 46, 54, 55)

Table 2 Variation in seed quality in relation to the age when *F. curtipes* syconia were pollinated. Groups of 100 seeds were included in each germination trial. One-way ANOVA or Kruskal-Wallis tests were applied to each row. Age groups with different superscripts were significantly different

	Age at pollination (days since becoming receptive) (mean \pm SD)								F/H	P
	0	4	8	12	16	20	24	$\geq 28^{\ddagger}$		
Seed mass										
Number of replicates	37	32	39	44	39	39	42	36		
Mass of ten seeds (mg)	11.32 \pm 1.38 ^a	11.25 \pm 1.41 ^{ab}	10.82 \pm 1.39 ^{ab}	10.23 \pm 1.43 ^{bc}	9.31 \pm 1.22 ^{cd}	8.82 \pm 1.07 ^d	8.95 \pm 0.94 ^d	7.86 \pm 1.36 ^e	36.10*	<0.0001
Germination										
Germinated seeds Trial 1	96	95	97	95	92	94	95	93		
Germinated seeds Trial 2	96	97	94	95	97	93	94	93		
Combined germination rate (%)	96.00 \pm 0.00	96.00 \pm 1.41	95.50 \pm 2.12	95.00 \pm 0.00	94.50 \pm 3.54	93.50 \pm 0.71	94.50 \pm 0.71	93.00 \pm 0.00	7.50 [#]	0.3787

* F: ANOVAs

H: Kruskal-Wallis tests

[‡]Data from the mixed samples of age 28 days (sample size, $n=46$), age 32 day (sample size, $n=5$) and age 36 days (sample size, $n=4$)

Table 3 Variation in pollinator offspring sex ratios in relation to the age when their natal syconia were pollinated. One-way ANOVA or Kruskal–Wallis tests were applied to each row. Age groups with different superscripts were significantly different

	Age at pollination (days since becoming receptive) (mean \pm SD)										F/H	P
	0	4	8	12	16	20	24	$\geq 28^{\dagger}$				
Number of syconia	14	37	40	48	41	35	47	50				
Number of female pollinators	44.79 \pm 29.76 ^{bc}	65.92 \pm 30.33 ^a	56.43 \pm 23.69 ^{ab}	62.81 \pm 30.91 ^a	63.15 \pm 29.34 ^a	55.77 \pm 25.72 ^{ab}	46.53 \pm 24.68 ^{bc}	39.12 \pm 24.73 ^c			5.29*	<0.0001
Number of male pollinators	17.57 \pm 16.14	17.97 \pm 16.74	16.10 \pm 11.18	19.54 \pm 15.76	13.24 \pm 7.06	13.80 \pm 8.34	16.40 \pm 13.89	14.04 \pm 10.39			1.31*	0.2459
Sex ratio (proportion males)	0.32 \pm 0.27 ^{ab}	0.22 \pm 0.20 ^{ab}	0.24 \pm 0.18 ^{ab}	0.26 \pm 0.26 ^{ab}	0.17 \pm 0.07 ^a	0.20 \pm 0.11 ^{ab}	0.26 \pm 0.20 ^{ab}	0.28 \pm 0.22 ^b			21.53 [#]	0.0310

* F: ANOVAS

[#] H: Kruskal–Wallis tests

[†] Data from the mixed samples of age 28 days (sample size, $n=46$), age 32 day (sample size, $n=5$) and age 36 days (sample size, $n=4$)

widely, from a maximum of about 66 in intermediate age syconia, declining to 40 in the oldest syconia (Table 3). Male fig wasp numbers did not differ with syconium age.

3.4 Floral sex allocation

Syconia pollinated on the first day of receptivity averaged over 50 seeds per syconium, but this declined rapidly with age and syconia pollinated at day 12 days or older contained only about half as many seeds, or less (Kruskal–Wallis test, $H=100.91$, d.f. = 7, $n=389$, $P<0.0001$, Fig. 2). Syconia pollinated on the first day of receptivity also contained about 50 galled ovules with pollinator wasp adults, but their numbers progressively increased in slightly older syconia before declining back to an average of about 50 wasps in syconia that had been waiting 28 days or longer (Kruskal–Wallis test, $H=27.53$, d.f. = 7, $n=389$, $P<0.0001$, Fig. 2). All average IOAMFFs were less than 0.5 and decreased significantly with the increase of the syconium receptive age (Table 1), demonstrating that floral sex allocation in *F. curtipes* was male-biased and became more male-biased with the aging of the receptive syconia. However, the average EIOAMFFs at the age 0 day and 4 days were about 0.6 and near to 0.5, respectively (Fig. 3), although these also decreased significantly within age range studied (Kruskal–Wallis test, $H=51.60$, d.f. = 7, $n=389$, $P<0.0001$, Fig. 3). This showed that the effective allocation of ovules between male and female function was almost in balance when syconia pollinated were at an early receptive age.

4 Discussion

The syconia of *F. curtipes* remained receptive for an extended period, with pollinators willing to enter some 36 day old

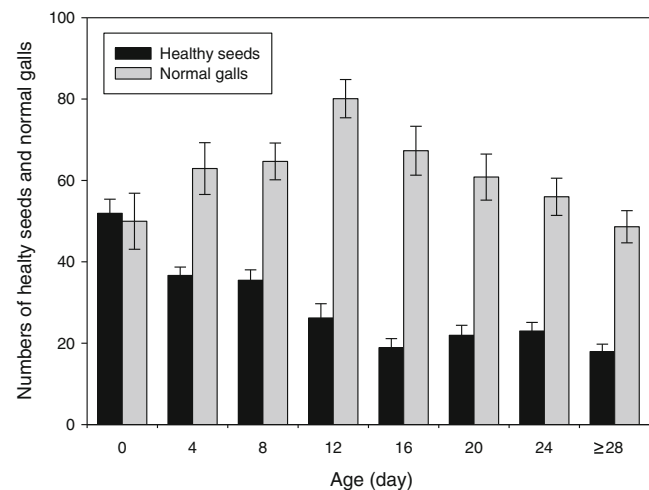


Fig. 2 Effect of age at pollination on seeds and galls produced of *F. curtipes* syconia. Data are shown with means \pm SE (n (syconia)=33, 50, 50, 51, 50, 46, 54, 55)

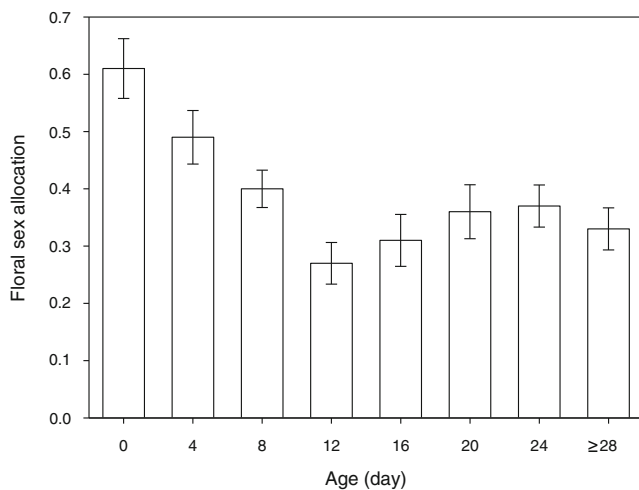


Fig. 3 Realised floral sex allocation to female reproductive function [EIOAMFF, effective index of ovule allocation between male and female function, i.e. healthy seeds/(healthy seeds + normal galls)] in *F. curtipes* syconia entered by pollinators at different ages. Data are shown with means \pm SE (n (syconia)=33, 50, 50, 51, 50, 46, 54, 55)

and most 28 day old syconia, which then successfully produced seeds and pollinator progeny. This is the longest duration of receptivity recorded among *Ficus* species (Khadari et al. 1995; Suleman et al. 2011; Zhang et al. 2011). Although this could be interpreted as a response to a particularly unpredictable supply of pollinators, almost all *F. curtipes* syconia at Xishuangbanna are pollinated within 3 to 5 days of becoming receptive (Gu D., pers. obs.). *F. curtipes* is also the first monoecious species examined for the length of receptivity, and there may be a link between floral longevity and breeding system. Furthermore, it is also the first passively-pollinated species. *F. curtipes* syconia contain about equal numbers of male and female flowers, a much higher proportion than in actively-pollinated syconia, because pollen transfer is much less efficient (Kjellberg et al. 2001). The extra initial investment required to produce these male flowers should favour extended longevity. Floral longevity in *Ficus* also varies with temperature (Zhang et al. 2011). Our experiments were not carried out during the cold season and floral longevity in *F. curtipes* is likely to be even longer then.

F. curtipes is one of the few *Ficus* species that have both a passive pollination system and the presence of non-agaonid fig wasps that enter its syconia to oviposit, a combination which results in non-agaonids also acting as pollinators (Jousselin et al. 2001; Zhang et al. 2008). These non-agaonids cannot reproduce in syconia that lack *Eupristina* sp., but routinely supplement the pollen carried into syconia by the usual pollinator (Zhang et al. 2008). However, they often mistakenly enter some syconia that lack *Eupristina* sp., and therefore act as independent pollinators of the syconia. Entry into older *Eupristina*-free syconia is particularly common (Gu D., unpubl. res.), a behaviour that will favour extended floral longevity in *F. curtipes*.

Eupristina sp. females were unwilling to attempt to enter most syconia that were older than 32 days, showing that production of attractant volatiles may have declined or ceased. The reproductive success of each syconium declined long before this, with an increasing proportion of flowers failing to produce seeds or pollinators. Empty gall numbers changed little with syconium age (Table 1), but the youngest syconia contained the most empty galls, which may be related to the time-related change of plant defences (Dunn et al. 2011), showing that the decline in efficiency among older syconia was not due to higher rates of mortality among the pollinators. Rather, it resulted from a smaller proportion of the flowers in older syconia being utilised for either female or male reproductive functions. Either the pollinators that entered the older syconia were less active, pollinating fewer flowers or, and more likely given that pollination is passive in this species, some female flowers were senescing and no longer able to respond to the pollen deposited on their stigmas.

The age-related changes in relative success of seed and pollinator production altered the balance of male and female reproductive success. The youngest receptive syconia contained the highest proportion of seeds. Declines in seed production in older syconia were not matched by falling pollinator numbers. This resulted in the male component contributing more to the overall reproductive success of older syconia. Consequently, the floral sex allocation became more male-biased in older syconia. However, although the floral sex allocation became more male-biased in older syconia, there was always a certain proportion of flowers able to produce seeds. This may be another evidence for the unbeatable seed hypothesis, suggesting that some female flowers in a monoecious syconium may be reserved for seeds (West and Herre 1994).

In other species the numbers of pollinator progeny displayed a linear decline over time, with syconia on the first day of their receptive period containing the most seeds and most pollinators (Zhang et al. 2011). This was not the case in *F. curtipes*, where the most pollinator progeny were present in syconia that had been receptive for several days. It may be no coincidence that *F. curtipes* syconia that have been receptive for several days are also more attractive to its pollinator than recently receptive syconia (Gu D., unpubl. res.), in contrast to *F. montana* and *F. semicordata*, which are most attractive on their first days of receptivity (Suleman et al. 2011; Zhang et al. 2011). This means there may be a positive relationship between the suitability of receptive syconia for pollinator reproduction and their attractiveness to pollinators.

The extended receptive period in *F. curtipes* increases the proportion of its syconia that are pollinated, and also increases the number of trees that can donate pollen to a particular crop, but is achieved at the cost of reduced productivity in syconia that have waited longer for pollinators. Changes in relative success of seed and pollinator production over time also mean

that optimal ratios of male and female reproductive function in each inflorescence will change with age at pollination, which may be related to the time-related change of plant defences (Dunn et al. 2011). The decline in productivity with age at pollination in *F. curtipes* was much more evident in female than male reproductive function. Seeds and pollinator progeny are produced on separate female and male plants of dioecious fig trees, where sexual differences in productivity have been shown to be more rapid among females of one species, and males of another (Suleman et al. 2011; Zhang et al. 2011). This variation between species suggests that aging differences between the sexes are not necessarily governed by physiological constraints, and are potentially adaptive.

Among plants in general, female flowers tend to be maintained for longer than male flowers (Marshall et al. 2010), a pattern reflecting sexual differences in investment. Female reproduction is thought to generally require a greater investment in resources than male reproduction (Obeso 2002), but this difference is less evident in nursery pollination systems, where resources are required to support developing larvae of the insects that will eventually disperse the pollen. Among fig trees, investment in male function will also be more expensive for passively-pollinated species such as *F. curtipes*, because their low efficiency of pollen transfer requires the production of many more male flowers than in actively pollinated species. Age-related changes in seed production similar to those in *F. curtipes* have been recorded in *Raphanus sativus*, where seed production in flowers that were newly receptive was greater than in flowers pollinated a day later (Marshall et al. 2007), but whether rates of decline in female reproductive function are typically faster than male function is unclear.

In conclusion, our results indicate that un-pollinated syconia of *F. curtipes* can remain receptive for several weeks. This makes pollination of each syconium more likely, but at the cost of reduced productivity and with more ovules allocated to male function. Nevertheless, in the case of *F. curtipes*, the extended receptive period may have important consequences not only for adaptation to pollinator shortages but also for the evolution of the interaction between the fig tree and its three internally ovipositing wasps. However, because the work was only carried out on one tree, repetition of the work in future, involving more individuals, is required to confirm our interpretation on the results.

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