

Parasites exert conflicting selection pressures to affect reproductive asynchrony of their host plant in an obligate pollination mutualism

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Summary

1. Plant reproductive phenology is generally viewed as an individual's strategy to maximize gamete exchange and propagule dispersal and is often considered largely dependent on patterns of floral initiation. Reproductive phenology, however, can be affected by proximate responses to pollinators, parasites and herbivores which could influence floral longevity or fruit development time.

2. We examined the influence of insect interactants on within-plant reproductive phenology in the fig–fig wasp nursery pollination mutualism in *Ficus racemosa* (Moraceae). Most figs support a wasp community comprised of a mutualistic pollinator, with several host-plant-specific non-pollinating herbivorous gallers and parasitoids. These wasps reproduce within enclosed inflorescences called syconia, which develop into fruit after pollination. While different wasp species oviposit into syconia at varying times during its ontogeny, all wasp progeny are constrained to exit syconia simultaneously just prior to fruit ripening. Developing larvae of early-ovipositing wasps may hasten syconium ontogeny through formation of earlier and larger nutrient sinks, whereas larvae of late-arriving parasites may lengthen syconium ontogeny to complete their development successfully. Seeds are also important nutrient sinks. The number of seeds and the type and number of developing wasps may therefore be expected to influence syconium development times, thereby affecting the reproductive synchrony of syconia on a plant.

3. Observations on naturally pollinated and parasitized syconia indicated that their seed and wasp content affected syconium development time. Experimental manipulations of syconia to produce only seeds or various combinations of wasps confirmed this finding. Early-ovipositing galler progeny reduced syconium development times, while gallers ovipositing concurrently with pollinators had no effect on syconium development. Late-ovipositing parasitoid progeny, the presence of only seeds within the syconium, or delayed pollination increased syconium development time. The differential development of syconia, which was influenced by mutualistic or parasitic progeny, accordingly contributed to within-tree reproductive asynchrony.

4. *Synthesis.* Individual reproductive units in fig trees called syconia, which also function as brood sites for pollinating and parasitic fig wasps, have plastic development durations dependent on pollination timing and species of wasps developing within them. Syconium development times are a likely compromise between conflicting demands from developing seeds and different wasp species.

Key-words: asynchrony, brood-site pollination mutualism, development time, *Ficus racemosa*, nursery pollination, nutrient sink, parasites, pollinators, reproductive ecology, reproductive phenology

Introduction

In plants, reproductive phenology is an important factor in determining reproductive success (Rathcke & Lacey 1985; Elzinga *et al.* 2007; Rafferty & Ives 2012). Reproductive

synchrony at a plant or population level could enhance pollinator or fruit disperser attraction (Augsburger 1981; Albert *et al.* 2008), satiate seed predators (Janzen 1971; Żywiec *et al.* 2012) or facilitate efficient use of predictably short-lived resources (Ims 1990; Silveira, Martins & Araújo 2013). On the other hand, reproductive asynchrony at plant and population levels could encourage outcrossing while avoiding

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geitonogamy (pollen movement within plants) (Cruden & Hermann-Parker 1977; Rathcke & Lacey 1985), spread the risk of uncertain pollination (Rathcke & Lacey 1985; Newstrom *et al.* 1994), or avoid pollinator and seed disperser satiation (Howe & Estabrook 1977; Gomez 1993). These are some evolutionary explanations for patterns in reproductive phenology. However, ecological conditions and mechanisms involving proximate responses to animal interactants can also affect these patterns. For example, floral longevity may be extended by lack of pollinators and thereby pollen limitation (Rathcke 2003; Castro, Silveira & Navarro 2008). Plant–pollinator interactions could modulate asynchrony in a fruit crop through variations in nutrient sink strength by variable seed number per fruit (Gorchov 1985; Marcelis & Hofman-Eijer 1997). Fruit development time can also be influenced by developing insect parasites (Lodos 1971; Xiao & Fadamiro 2010). Plants may also respond to herbivory with positive (Kessler, Diezel & Baldwin 2010) or negative impacts on flowering and fruiting phenology (Heil 2002).

The interplay of plant–pollinator–herbivore/parasite interactions in influencing reproductive synchrony is best investigated in obligate nursery or brood-site pollination mutualisms, in which pollinators reproduce within the plants they pollinate. In such systems, pollinators are often herbivores, and the mutualism between plants and pollinators is commonly exploited by parasites whose offspring also develop within the brood sites (Pellmyr & Leebens-Mack 2000; Cook & Rasplus 2003). We examined these conflicts in the fig–fig wasp system in which pollinator and parasite offspring develop within globular enclosed inflorescences called syconia. In such systems, the reproductive phenology of the plant partner, at individual and population levels, is crucial in maintaining the mutualism by affecting breeding site availability for pollinators (Janzen 1979; Bronstein *et al.* 1990). Most monoecious fig species exhibit within-tree reproductive synchrony and population-level asynchrony coupled with year-round reproduction to encourage outcrossing, which also helps maintain pollinator populations (Janzen 1979; Bronstein 1989). However, within-tree reproductive asynchrony in *Ficus* does occur (Janzen 1979; Bronstein 1989; Gates & Nason 2012) such that the pollen donation male phase of syconia overlaps with the pollen receptive female phase of flowers in other syconia within trees. This can also enhance the probability of sexual phase overlap between trees due to elongated phenophases. These overlaps were assumed to arise via variable initiation times of syconium primordia. Therefore, instances of within-tree reproductive asynchrony were hitherto regarded as plant-mediated mechanisms (i) to maintain pollinator populations during harsh conditions (Ramírez 1970; Janzen 1979) or within small local populations (Bronstein 1989; Bronstein *et al.* 1990; Gates & Nason 2012) or (ii) to have arisen as a by-product of the temporal availability of resources for reproduction (Frank 1989; Bronstein & Patel 1992).

The impact of parasites on within-plant asynchrony of the syconia has never been examined. Since the brood-site syconium functions first as an inflorescence and then as a fruit

(post-pollination), events occurring within a syconium before, during and after pollination could potentially have considerable impact on the development of individual syconia and accordingly on reproductive asynchrony. Syconia are independent physiological entities such that inadequate pollination services to a syconium compared to oviposition by pollinators may trigger host sanctions resulting in abortion of that particular syconium by the plant (Jandér, Herre & Simms 2012). The syconia of most *Ficus* spp generally host a single pollinator species, with several fig species-specific parasitic, non-pollinating fig wasps consisting of herbivorous galls and parasitoids of the galls and pollinators (Cook & Rasplus 2003; Herre, Jandér & Machado 2008; Cook & Segar 2010) that oviposit at different stages of syconium development (Kerdelhué, Rossi & Rasplus 2000; Cook & Segar 2010; Ranganathan, Ghara & Borges 2010). Irrespective of when the many species of fig wasps oviposit into syconia, their offspring are constrained to complete their development by the time the pollinator offspring eclose because (i) usually only male pollinators can cut exit holes in syconium walls to release pollen-carrying females (Janzen 1979; Bronstein 1991) and (ii) syconia rapidly enter seed dispersal stage after the exit of pollinating wasps; consequently, undeveloped offspring would be devoured by frugivores or insect predators entering syconia through these exit holes (Bronstein 1988; Y. Ranganathan, M. Ghara & A. Krishnan, pers. obs.).

We examined the impact on syconium asynchrony of wasp parasites that begin their development within the brood site at three different stages: (i) pre-pollination stage syconia into which large herbivorous galler wasps oviposit, (ii) pollination stage syconia which receive oviposition from galls of a size similar to pollinators and (iii) post-pollination syconia into which parasitoids of the galls and pollinators oviposit. The early-ovipositing, large galls could be expected to decrease syconium development time due to the earlier (and perhaps larger) nutrient sink formation by their developing offspring. The late-ovipositing parasitoids could be expected to delay syconium development to allow their progeny to reach maturity before the onset of the wasp dispersal or exit phase. Therefore, conflicting pressures to influence the growth rate and development time of a shared receptacle may be fuelled by inhabitants with variable requirements of nutrient sink strengths and opposing development times. Coupled with the observation that syconia within a single reproductive cycle in a tree vary greatly, qualitatively and quantitatively, in composition of nursery inhabitants (Cook & Power 1996; Ghara *et al.* 2014), the potential of variable syconium development time as a mechanism contributing to within-tree asynchrony in *Ficus* spp can be readily postulated. Such a mechanism has not been explored before.

In this paper, we have used *Ficus racemosa* and its fig wasp community to examine the influence of syconium initiation and development time on within-tree reproductive asynchrony. Fine-scale temporal observations on unmanipulated syconia were used to address the following questions: (i) Does variation in syconium initiation time account for all instances of within-tree reproductive asynchrony? (ii) What is

the natural variation in syconium development time due to variation in pollination time or seed and wasp composition of syconia? Wasp introduction and manipulative experiments were employed to address the following questions: (iii) How do developing pollinators and seeds affect syconium development time? (iv) What effects do developing offspring of specific non-pollinating fig wasps, ovipositing at different stages of syconium ontogeny, have on syconium development time?

Materials and methods

SPECIES BIOLOGY AND STUDY SITE

The monoecious *Ficus racemosa* (subgenus *Sycomorus*) is distributed throughout the Indo-Australasian region. Trees reproduce aseasonally and annually produce 2–6 crops of cauliflorous figs borne in bunches (racemes) on tree trunks and larger branches. Syconial development is divided into five phases (Galil & Eisikowitch 1968): A or pre-floral phase (flowers undeveloped), B or female floral phase (female flowers receptive to pollination), C or interfloral phase (wasp progeny and seeds develop within syconia), D or male floral phase (wasps eclose and mate, pollinator females collect pollen from mature anthers and leave natal syconia through exit holes chewed by male pollinators) and E or post-floral phase (syconia ripen and attract seed dispersers). Individual syconia have varying development times for each phase (A-phase = 16–30 days, B-phase = 2–8 days, C-phase = 25–40 days, D-phase = 1 day, E-phase = 1–3 days) with total development times ranging from 40 to 80 days. Each syconium can contain 2000–7000 flowers with 0–1200 pollinators, 0–300 non-pollinators and 0–3000 seeds developing inside them (Wang *et al.* 2008; A. Krishnan, unpubl. data).

The Indian population of *Ficus racemosa* used in this study is pollinated by the mutualistic agaonid wasp *Ceratosolen fusciceps* Mayr and is also host to six other species of fig host-specific non-pollinating fig wasps in the subfamilies Sycophaginae and Sycoryctinae (gallers – *Apocryptophagus stratheni* Joseph, *Apocryptophagus testacea*

Mayr, *Apocryptophagus fusca* Girault and the parasitoids – *Apocryptophagus agraensis* Joseph, *Apocrypta westwoodi* Grandi and *Apocrypta* sp. 2) (Ghara & Borges 2010) that develop within syconia. The various fig wasps can be categorized into groups based on their oviposition windows and biology (Fig. 1). The gallers *A. stratheni* and *A. testacea* are larger-bodied than pollinators and oviposit in A-phase syconia with oviposition windows lasting 4 and 6 days, respectively, followed by the galler *A. fusca* (similar body size as the pollinator) and the pollinator *C. fusciceps* in B-phase (oviposition windows 8 and 4 days, respectively) (Ranganathan, Ghara & Borges 2010; Ghara 2012). The parasitoid *Apocrypta* sp. 2 (known to parasitize *A. stratheni*, *A. testacea* and *A. fusca*, and suspected to parasitize pollinators also; Wang & Zheng 2008; P. Yadav, unpubl. data) has an oviposition window of 20 days spanning late A- to mid-C-phase, whereas *Apocrypta westwoodi* (known to parasitize the large gallers *A. stratheni* and *A. testacea*; Wang & Zheng 2008; P. Yadav, unpubl. data) and *A. agraensis* (suspected parasitoid/inquiline of pollinators) oviposits in C-phase over 7 and 8 days, respectively (Ranganathan, Ghara & Borges 2010; Ghara 2012). Syconia containing all seven species of developing wasps are generally rare, but those containing 3–4 wasp species are quite common (Ghara *et al.* 2014). All non-pollinating wasps oviposit from the outside of the syconium; only pollinating wasps enter the syconium for pollination and oviposition. All fig wasps of *F. racemosa* are dependent upon pollinator males to chew exit holes through the syconium walls.

EFFECT OF NATURAL VARIATION IN POLLINATION TIME AND SEED AND WASP COMPOSITION OF SYCONIA ON SYCONIUM DEVELOPMENT TIME

The flowering/fruiting phenologies of 16 trees within the campus of the Indian Institute of Science (12°58' N, 77°35' E), Bangalore, India, were observed for 20 months (November 2008 to August 2010). Fig bunches (20 per tree) were marked; patterns of initiation and development of syconia within each bunch were noted every 2–3 days. For every reproductive episode, within-tree asynchrony was calculated using a modified form of Augspurger's index of synchrony

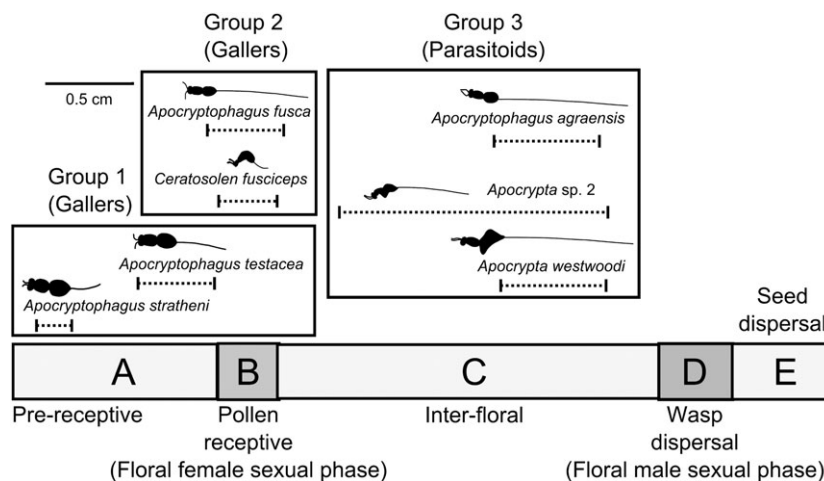


Fig. 1. Schematic diagram showing oviposition windows (dashed lines) of the various fig wasp fauna of *Ficus racemosa*. Each of the labelled grey boxes represents the relative duration of that phase (A–E) on an individual representative tree. The single undashed line indicates a scale bar (0.5 cm) applicable to wasp sizes. Group 1 encompasses early-arriving herbivorous gallers, larger-bodied than pollinators that oviposit in the early phase (A-phase) of syconium development. Group 2 includes pollinators and a herbivorous galler of similar size, both of which oviposit in the pollen receptive phase (B-phase). Group 3 includes the late-arriving parasitoids of groups 1 and 2, which oviposit in the later stages of syconium development (early to mid-C-phase).

(Augspurger 1983) where 0 indicates complete synchrony and 1 indicates complete asynchrony (see Appendix S1 in Supporting Information). Data on syconium volume and number of wasps and seeds within 116 D-phase syconia from 7 trees (observed and collected between November 2008 and January 2009) were obtained from a previous study (Ghara *et al.* 2014). These data were supplemented with development time (DT) and length of B-phase for individual syconia (as an indicator of pollination time) from observations (every 2–3 days) following their progress through different phenological phases from initiation till their collection in D-phase. The D-phase syconia which were collected before male pollinators cut exit holes, were a subset of those monitored for within-tree asynchrony and were collected from single crops borne on seven trees between November 2008 and January 2009. Each of the 10–20 syconia collected from each tree was from different monitored bunches and was selected haphazardly. We obtained the ostiole–insertion of stalk distance and two measures of syconium diameter (D1, D2, orthogonal to each other) using a vernier caliper. Since syconia were often ellipsoidal and not spherical, we calculated their volumes (in cm^3) assuming them to be ellipsoids. Individual syconia were placed in separate capped 50 mL containers to allow wasps to exit naturally, after which they were split into two halves to further facilitate wasp emergence. The syconia were then dissected into quarters and examined to collect and count all seeds as well as exited and unexited wasps (such as males of non-pollinators, some pollinator males and unmated female wasps trapped within galls) to obtain an accurate estimate of syconium contents (i.e. the total seed and wasp composition of the syconium). All wasps were stored in 70% ethanol and later identified. These data were used to explore relationships between syconium contents and syconium DT in syconia exposed to natural pollination and parasitic events using path analysis (see Statistical Analysis).

An *a priori* model was constructed using known biological facts about the system (Fig. 2). Path analysis is especially useful as it allows the use of non-independent explanatory variables which abound in this system. For example, the number of pollinators that develop in a syconium functions as an explanatory variable for the number of seeds in a syconium (Fig. 2), but, as the number of pollinators itself is dependent upon various other factors such as syconium volume, it becomes a non-independent explanatory variable. Syconium volume is expected to affect all syconium contents (seeds and wasps) positively as larger syconia have more ovules (see Appendix

S2) and thus provide more development sites for seeds and wasps; syconium volume is also expected to have a negative effect on syconium development time since larger fruit with more contents tends to grow faster (Gorchov 1985; Marcelis & Hofman-Eijer 1997; Heuvelink & Körner 2001). Progeny of the early- and late-arriving gallers are expected to have negative relationships with each other as well as with pollinator progeny and seeds as they compete for development space and resources. Predatory parasitoid progeny can have negative or positive relationships with pollinator and galler progeny which are their prey. A positive or negative relationship between pollinator progeny and seeds would depend on the ratio between the number of available ovules and the number of pollinator wasps entering a syconium as this determines the severity of conflict between the mutualists (Anstett, Bronstein & Hossaert-McKey 1996; Wang *et al.* 2008). Since length of syconium receptivity also contributes to total syconium development time, the B-phase length is expected to affect syconium development time positively. A prolonged B-phase could also have a positive effect on the progeny of the gallers ovipositing concurrently with pollinators. Early-ovipositing gallers are expected to decrease syconium development time to either avoid larval/pupal predation by parasitoids or as a result of greater nutrient sink strength. Late-ovipositing parasitoids are expected to delay syconium development to allow their progeny enough time to mature fully. Both above hypotheses regarding early-ovipositing gallers and late-ovipositing parasitoids are naturally constrained by the availability of fully developed pollinator males to effect the production of exit holes in the syconium for the release of wasps.

EXPERIMENTS TO MANIPULATE POLLINATION TIME AND SEED AND WASP CONTENTS OF SYCONIA

Wasp introduction experiments were also conducted to further demonstrate the effect of different factors on syconium development time. All wasps used for introductions in the experiments were obtained from D-phase syconia collected 1–2 days before or on the day of the introduction from surrounding trees. In all experiments (unless otherwise specified), pollination was carried out by allowing 1–2 pollinators to enter each syconium 2–4 days after onset of B-phase. For pollination, pollinators were placed on B-phase syconia with camel-hair brushes and monitored till they entered the ostiole. Non-pollinators were collected in 50 mL vials and released into the bags (1 for every 2 syconia in a

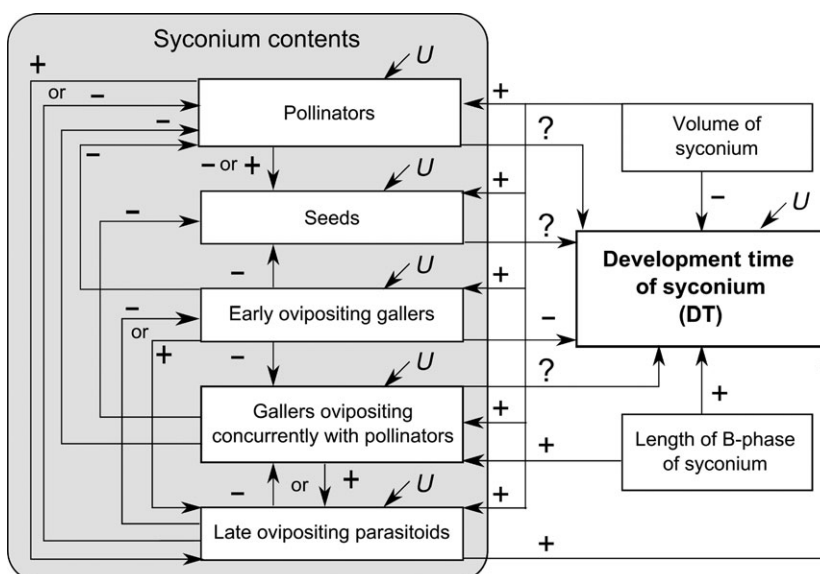


Fig. 2. Representative path diagram describing the *a priori* model. It outlines the effects of syconium volume, length of B-phase of syconium and various syconium contents (seeds and wasps) on syconium development time. The symbols '+' and '-' beside arrows indicate those relationships that are expected to be positive or negative, respectively, whereas '?' indicates an unknown relationship (it could be positive, negative or may not exist). The magnitude of unexplained variance for a factor is indicated by 'U'.

bagged bunch). Non-pollinator introductions were carried out over 2 days during their peak oviposition windows. Dates of syconium initiation, onset of B-phase and pollination were noted, from which two measures of development in days for every syconium in each experiment were recorded: (i) total development time (TDT) – time taken to develop to E-phase from time of initiation (this includes the duration of the A- through D-phases); (ii) post-pollination development time (PPDT) – time taken to develop till E-phase after pollination (equivalent to duration of C- and D-phases). Each set of experiments was repeated at least thrice on different trees (see Appendix S3). Experiments were conducted opportunistically throughout the study period (December 2009 to August 2012) based on availability of suitable syconia (details of the time periods available in Appendix S3). Bunches having 30–60 pre-A-phase syconia were selected, covered with cloth bags (i.e. bagged) to exclude unwarranted wasp oviposition and subjected to several treatments to examine several effects as discussed below.

Effect of mutualistic pollinators on syconium development time

1 *Effect of pollination time* – lengths of B-phase of syconia were varied by pollinating one set of syconia within 1–2 days after initiation of B-phase, while the second set was pollinated after 5–7 days of B-phase initiation. The experiment was replicated on 3 trees, with a total of 78 early-pollinated syconia (18, 23 and 37 syconia in Trees 1, 2 and 3, respectively) and 68 late-pollinated syconia (29, 17 and 22 syconia in Trees 1, 2 and 3, respectively).

2 *Effect of foundress number* – in one set, each syconium received one introduced pollinator (foundress), while in a second set, each syconium received six introduced pollinators. It was expected that syconia receiving one foundress would have fewer pollinator progeny than those receiving six foundresses (Wang *et al.* 2008). The experiment was replicated on 4 trees, with a total of 148 syconia receiving one foundress (31, 44, 34 and 39 syconia in Trees 1, 2, 3 and 4, respectively) and 121 syconia receiving six foundresses (24, 34, 30 and 33 syconia in Trees 1, 2, 3 and 4, respectively).

3 *Effect of presence of pollinator progeny* – in one set, each syconium received one to two introduced pollinators with intact ovipositors; these syconia would develop with pollinator progeny and seeds. In another set, each syconium received 1–2 pollinators with excised ovipositors; these would develop with only seeds since such pollinators cannot oviposit. The experiment was replicated on three trees, with a total of 74 syconia receiving pollinators with intact ovipositors (32, 20 and 22 syconia in Trees 1, 2 and 3, respectively) and 63 syconia receiving pollinators with excised ovipositors (26, 19 and 18 syconia in Trees 1, 2 and 3, respectively).

Effect of non-pollinators on syconium development time

1 *Effect of oviposition by early-arriving gallers* – one set of syconia was exposed to ovipositing *A. testacea* females in A-phase followed by pollination in B-phase, one set was exposed only to ovipositing *A. testacea* females in A-phase without being pollinated, and the control set received only introduced pollinators in the B-phase. The experiment was replicated on five trees, with a total of 143 syconia exposed to *A. testacea* oviposition and pollinated (53, 23, 39 and 28 syconia in Trees 1, 2, 4 and 5, respectively), 70 syconia exposed only to *A. testacea* oviposition (19, 15, 20 and 16 syconia in Trees 2, 3, 4 and 5, respectively) and 100 syconia in the control set which were unexposed to *A. testacea* oviposition (36, 18, 25 and 21 syconia in Trees 1, 3, 4 and 5, respectively).

2 *Effect of oviposition by gallers laying eggs concurrently with the pollinator* – one set of syconia was exposed to ovipositing *A. fusca* females and pollinators in the B-phase, while the control set received only introduced pollinators. The experiment was replicated on three trees, with a total of 78 syconia exposed to *A. fusca* oviposition and pollinated (35, 27 and 16 syconia in Trees 1, 2 and 3, respectively) and 75 syconia in the control set which were unexposed to *A. fusca* oviposition (30, 27 and 18 syconia in Trees 1, 2 and 3, respectively). A set of 19 syconia on Tree 3 that received only *A. fusca* oviposition without being pollinated were also obtained, but these data were not utilized as the sample size was too low for analysis.

3 *Effect of oviposition by late-ovipositing parasitoids* – all bagged syconia were pollinated in B-phase, following which one set was exposed to ovipositing *A. agransensis* females (a known parasitoid of the pollinator) (Ghara 2012; Ranganathan 2012) during mid-C-phase of syconium development. The experiment was replicated on four trees, with a total of 98 syconia in the control set which were unexposed to *A. agransensis* oviposition (20, 18, 23 and 37 syconia in Trees 1, 2, 3 and 4, respectively) and 82 syconia exposed to *A. agransensis* oviposition (10, 29, 14 and 29 syconia in Trees 1, 2, 3 and 4, respectively).

STATISTICAL ANALYSIS

The effects of syconium volume (measured in D-phase), length of B-phase and syconium contents (seeds and wasps) on the syconium DT of syconia exposed to natural pollination and parasitic events were investigated via path analysis using the software LISREL 9.1 (Jöreskog & Sörbom 2012). Path analysis is a multiple regression technique allowing estimations of the magnitude and sign of directional relationships in complex explanatory models incorporating multiple dependent and independent variables (Wright 1934; Mitchell 1992). Models were evaluated based on goodness-of-fit measures that included chi-square values, *P*-values, root mean square error of approximation (RMSEA) and parsimony (degrees of freedom). All variables in the data set were square-root-transformed to improve normality, although multivariate normality was not achieved. Consequently, robust maximum likelihood (RML) estimation was used to fit structural equation models to the transformed data. Further details of the methodology for obtaining the best-fit and most parsimonious path model describing the data are provided in Appendix S4. In all results provided in this paper, the pollinators, gallers or parasitoids mentioned refer to the numbers of fully developed and enclosed offspring of these groups. Data obtained from each experimental bagging were analysed separately using linear mixed models (LMMs), with tree identity as a random factor. These analyses were conducted with the software R version 2.15.2 (R Core Team, Vienna, Austria) using the package *nlme*.

Results

EFFECT OF NATURAL VARIATION IN POLLINATION TIME AND SEED AND WASP COMPOSITION ON SYCONIUM DEVELOPMENT TIME

There were 90 reproductive episodes (i.e. syconial crops) over 20 months on 16 trees, from which three patterns of within-tree flowering/fruitlet phenology emerged (Fig. 3). Crop numbers per tree were variable (3–8 over the 20-month monitoring period). The most common pattern (56 episodes)

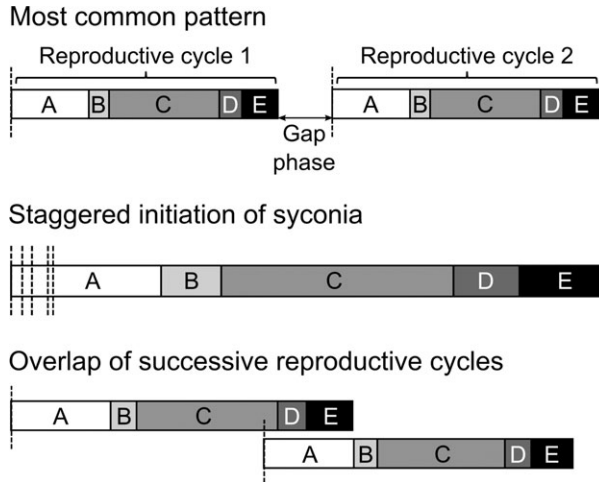


Fig. 3. Schematic diagram describing within-tree syconium initiation patterns observed in *Ficus racemosa*. Each labelled box represents the relative duration of that phase (A–E) on an individual tree. Dotted lines indicate times of syconium initiation.

involved initiation of syconia over 2–3 days and a gap phase (during which no reproductive structures were observed on the tree) between successive reproductive cycles. The second most common pattern (21 episodes) was staggered initiation of syconia where syconium initiation events continued up to 3 weeks from the first initiation event, thereby lengthening the duration of each phase (A–E) on the tree. The third pattern (13 episodes) was overlap between successive reproductive cycles where new syconia were initiated before the previous reproductive cycle was completed (any time between mid-C- and E-phases). Of these, sexual phase overlap (overlap between male D- and female B-phases) between two successive reproductive cycles occurred only five times. Values of the modified Augspurger’s index ranged from 0.22 to 0.43 (mean \pm SD = 0.34 \pm 0.04) for the most common pattern, which had almost synchronous initiation of syconia over 2–3 days. Within-tree asynchrony values ranged from 0.31 to

0.53 (0.41 \pm 0.07) for staggered initiations and 0.34 to 0.85 (0.47 \pm 0.07) for overlaps between successive reproductive cycles. As mentioned in the Materials and methods, low values of this modified index (whose values range from 0 to 1) indicate high synchrony, whereas high values indicate high asynchrony (see Appendix S1). Although the 34 reproductive episodes with variations in syconium initiation times (staggered initiations plus overlaps with successive cycles) generally showed higher values of Augspurger’s index, reproductive episodes with synchronous initiation of syconia also exhibited within-tree asynchrony.

In all results, pollinators, gallers or parasitoids mentioned refer to the numbers of fully developed and enclosed offspring of these groups. The results obtained from path analysis provided us with two acceptable models based on goodness-of-fit measures (Appendix S2). However, the conclusions from the best-fit model (χ^2 value = 8.63, P = 0.28, RMSEA = 0.5, d.f. = 7) and the most parsimonious model (χ^2 value = 10.46, P = 0.23, RMSEA = 0.5, d.f. = 8) were identical as the two differed only in the exclusion of one factor (the effect of gallers ovipositing concurrently with pollinators on syconium DT, Appendix S2), the path coefficient of which was positive as predicted in Fig. 2, but was found to be non-significant (Fig. 4).

As predicted by the *a priori* model (Fig. 2), syconium volume and early-ovipositing gallers had significant negative effects on syconium DT, whereas length of B-phase of syconium and late-ovipositing parasitoids had significant positive effects (Fig. 4). The positive effect of syconium volume on all syconium contents (seeds and wasps), the positive effect of B-phase length on gallers ovipositing concurrently with pollinators, the negative effect of early-ovipositing gallers on pollinators and the negative effect of gallers ovipositing concurrently with pollinators on seeds were also as predicted by the *a priori* model (Figs 2 and 4). Unexpected results included the positive relationship between pollinators and gallers ovipositing concurrently with pollinators as well as the

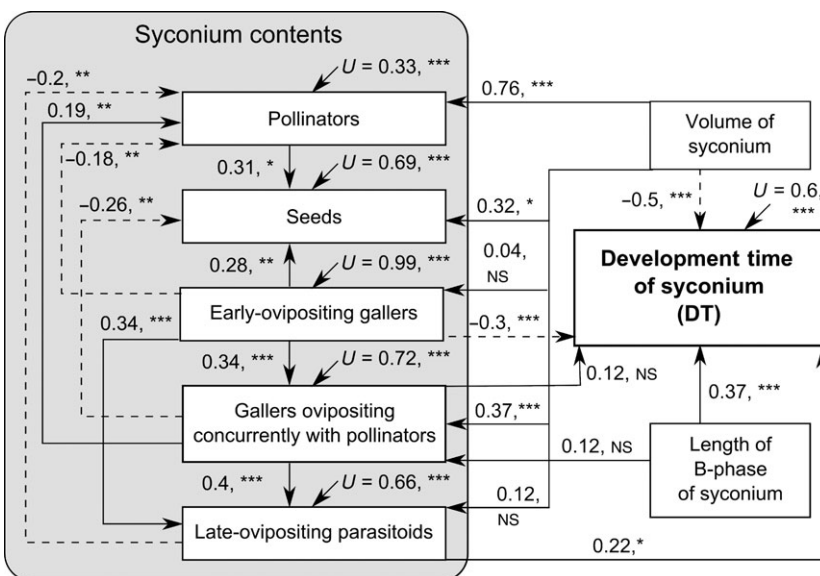


Fig. 4. Path diagram describing the best-fit model. Solid arrows indicate positive relationships, and dotted arrows indicate negative relationships. Numbers next to these arrows indicate standardized path coefficients. U = unanalysed variance from unknown causes. *** P < 0.001, ** P < 0.01 and > 0.001, * P < 0.05 and > 0.01, NS P > 0.5.

positive relationships of these galls and seeds with early-ovipositing galls (Fig. 4). Among factors whose relationships were not predicted *a priori*, the following results were obtained: the number of developing pollinators had a positive effect on seeds; the two galls had a positive effect on their parasitoids; and parasitoids had a negative effect on pollinators. The bagging experiments demonstrated that the effect of mutualists and parasites on syconium development time could range from a low of 1–2 days to a high of 6–12 days (see Appendix S3).

EXPERIMENTS TO MANIPULATE POLLINATION TIME AND WASP AND SEED COMPOSITION OF SYCONIA

Effect of mutualistic pollinators on syconium development time

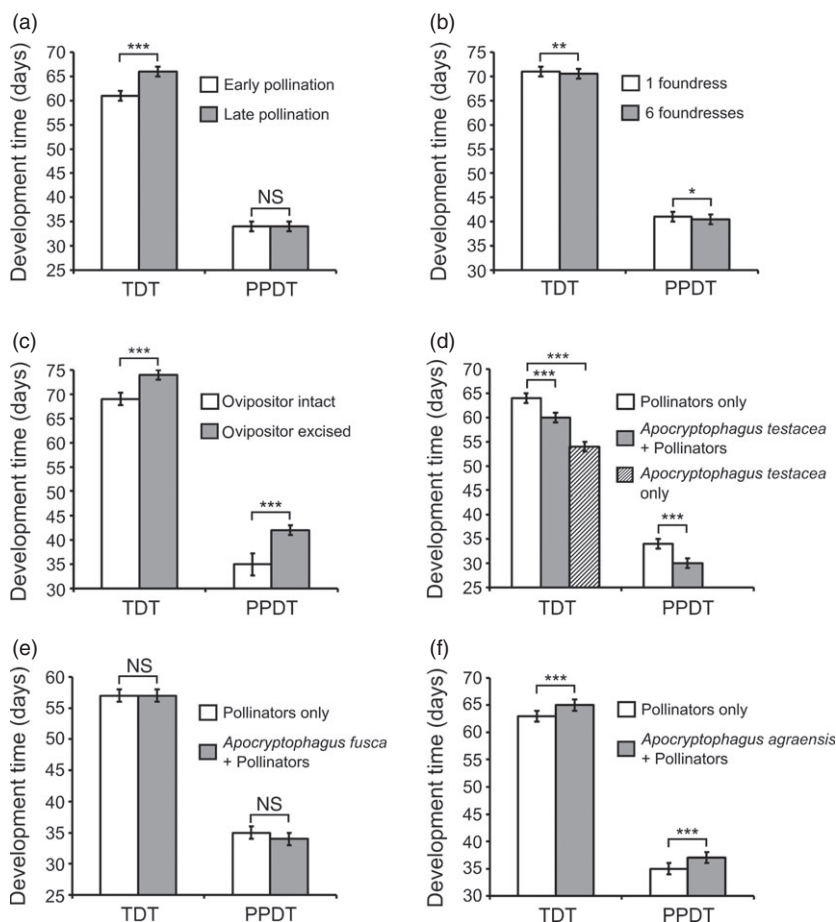
1 Effect of pollination time – Pollinating syconia late in B-phase significantly increased TDT ($\beta = 5.8 \pm 0.21$ SE, d.f. = 142, $t = 27.4$, $P < 0.001$, Fig. 5a, Table S5a of Appendix S5), though the effect on PPDT was non-significant ($\beta = 0.43 \pm 0.22$ SE, d.f. = 142, $t = 1.9$, $P = 0.054$; Fig. 5a, Table S5a of Appendix S5). Furthermore, the difference in TDT between early- and late-pollinated syconia was concordant with the differences in their pollination times (5–7 days, as explained in Materials and methods). Therefore, pollinator

arrival into syconia can influence development time of the syconium by affecting the length of the syconium receptive phase.

2 Effect of foundress number – Syconia receiving six pollinating wasps had significantly lower TDT ($\beta = -0.5 \pm 0.16$ SE, d.f. = 264, $t = -3.15$, $P = 0.002$, Table S5b of Appendix S5) and PPDT ($\beta = -2.9 \pm 0.14$ SE, $t = -2.04$, $P = 0.04$, Table S5b of Appendix S5) than syconia receiving one pollinating wasp, but the effect size was very low (Fig. 5b). This indicates that the number of foundresses, and perhaps number of pollinator progeny, does not have a strong influence on syconium development time.

3 Effect of presence of pollinator progeny – Syconia receiving pollinators with excised ovipositors contained only seeds and had significantly longer PPDTs ($\beta = 6.8 \pm 0.53$ SE, d.f. = 133, $t = 12.82$, $P < 0.001$, Table S5c of Appendix S5) than those containing wasps and seeds (syconia receiving pollinators with intact ovipositors) (Fig. 5c). Examining the TDTs in this experiment was not relevant, as any difference in TDTs must be attributed to differences in PPDTs since all conditions between the two sets of syconia were identical and pollination treatments were carried out either on the same day or within 1–2 days of each other. As syconia containing only seeds had longer development times (20–30% longer than syconia with pollinator progeny), the presence of pollinator progeny shortened syconium development time considerably.

Fig. 5. Results of bagging and wasp introduction studies demonstrating the effect of mutualists and parasites on syconium development time (DT). Variations in syconium development time produced by mutualists include (a) early pollination ($N = 78$) versus late pollination ($N = 68$); (b) variation in pollinator progeny number by introducing one foundress ($N = 148$) versus six foundresses ($N = 121$) per syconium; and (c) pollinator progeny presence ($N = 74$) or absence ($N = 63$). White and grey boxes for (a), (b) and (c) indicate syconium treatments. Effects of parasites were investigated by comparing syconium development times of syconia containing (d) only pollinators ($N = 100$), pollinators + *Apocryptophagus testacea* ($N = 143$) and *Apocryptophagus testacea* only ($N = 70$); (e) pollinators only ($N = 75$) and pollinators + *Apocryptophagus fusca* ($N = 78$); and (f) pollinators only ($N = 98$) and pollinators + *Apocryptophagus agragensis* ($N = 82$). White, grey and hatched boxes here represent syconium contents (seeds and wasps). Vertical bars indicate mean syconium development times, and error bars indicate SE. *** $P < 0.001$, ** $P < 0.01$ and * $P < 0.05$ and > 0.01 , NS $P > 0.5$.



Effect of non-pollinating fig wasps on syconium development time

1 *Effect of oviposition by early-ovipositing galls* – Syconia containing only *A. testacea* had significantly lower TDTs ($\beta = -7.2 \pm 0.27$ SE, d.f. = 306, $t = -27.35$, $P < 0.001$, Table S5d of Appendix S5) than those with only pollinators (Fig. 5d). No PPDTs for syconia containing only *A. testacea* were recorded as these were not pollinated (contained no seeds or pollinators); yet their survival to D-phase demonstrated the ability of *A. testacea* to drive unpollinated syconia to develop without aborting. Syconia containing developing pollinators and *A. testacea* also had significantly lower TDTs ($\beta = -2.6 \pm 0.21$ SE, d.f. = 306, $t = -12.17$, $P < 0.001$, Table S5d of Appendix S5) and PPDTs ($\beta = -4.1 \pm 0.26$ SE, d.f. = 306, $t = -15.48$, $P < 0.001$, Table S5d of Appendix S5) than syconia containing only pollinators (Fig. 5d), with the differences in TDTs mainly caused by differences in PPDTs (as both sets of syconia had similar DTs until B-phase and were pollinated either on the same day or within 1–2 days of each other). Therefore, progeny of the early-ovipositing galler *A. testacea* are capable of decreasing syconium development time, although presence of pollinator progeny reduced the magnitude of the effect.

2 *Effect of galls ovipositing concurrently with the pollinator* – Syconia containing pollinators and *A. fusca* did not have significantly different TDTs ($\beta = -0.01 \pm 0.18$ SE, d.f. = 149, $t = -0.07$, $P = 0.95$, Table S5e of Appendix S5) or PPDTs ($\beta = 0.008 \pm 0.17$ SE, d.f. = 149, $t = 0.46$, $P = 0.65$, Table S5e of Appendix S5) than syconia with only pollinators (Fig. 5e). *Apocryptophagus fusca*, like *A. testacea*, was also able to inhibit syconium abortion and drive the development of unpollinated syconia to D-phase, but as very few syconia containing only *A. fusca* were obtained ($N = 19$, see Appendix S3), their development times were not analysed. Syconia containing pollinators and progeny of galls ovipositing concurrently with pollinators (*A. fusca*) had syconium development times similar to syconia containing only pollinators.

3 *Effect of late-ovipositing parasitoids* – Syconia containing pollinators and *A. agragensis* had significantly longer TDTs ($\beta = 2.7 \pm 0.21$ SE, d.f. = 175, $t = 12.67$, $P < 0.001$, Table S5f of Appendix S5) and PPDTs ($\beta = 2.8 \pm 0.19$ SE, d.f. = 175, $t = 14.58$, $P < 0.001$, Table S5f of Appendix S5) than syconia with only pollinators (Fig. 5f), with the difference in TDTs attributable to differences in PPDTs since both sets of syconia had similar DTs until B-phase and were pollinated either on the same day or within 1–2 days of each other. Late-ovipositing parasitoids were therefore able to extend the development time of the syconium.

Discussion

Insect pollinators and parasites can affect the development of plant reproductive structures by affecting floral longevities (Rathcke 2003; Castro, Silveira & Navarro 2008; Fründ, Dormann & Tschamtker 2011) and fruit development times

(Lodos 1971; Gorchov 1985; Marcelis & Hofman-Eijer 1997; Xiao & Fadamiro 2010). Our results reaffirm the importance of insect interactants on the development schedule of plant reproductive structures especially in a tightly co-evolved nursery pollination mutualism. All previous hypotheses attempting to explain within-tree asynchrony in *Ficus* spp (Ramírez 1970; Janzen 1979; Bronstein 1989; Frank 1989; Bronstein & Patel 1992; Gates & Nason 2012) assumed that variation in syconium initiation patterns was the only proximate causal mechanism. Our observations on *F. racemosa* showed that reproductive episodes with synchronous initiation of syconia also exhibited within-tree asynchrony, indicating that syconium development times were important contributors to this phenomenon. Path analyses (Fig. 4) and wasp introduction experiments (Fig. 5) demonstrated that (i) delayed pollination due to delayed pollinator arrival lengthened syconium development time, (ii) galls ovipositing concurrently with the pollinator had no effect on syconium development time, (iii) galls ovipositing before the pollinators could shorten syconium development time, and finally (iv) parasitoids ovipositing after pollination could lengthen development time. Thus, parasites of the mutualism are capable of causing either a lengthened or shortened syconium development based on the contents of the developing syconia whose net effect could be within-tree reproductive asynchrony.

EFFECT OF POLLINATORS AND SEEDS ON SYCONIUM DEVELOPMENT TIME

Syconium pollination time can affect total syconium development time through (i) the length of its receptivity period as an inflorescence and (ii) its post-pollination development time (PPDT) as a fruit (Gorchov 1985; Marcelis & Hofman-Eijer 1997). Since the total development times (TDTs) of early- and late-pollinated syconia were found to vary, but their PPDTs were not significantly different (Fig. 5a), syconium development time in this case was affected by pre-pollination processes only. Since syconia are known to remain receptive for extended periods of time waiting for adequate pollen receipt (Khadari *et al.* 1995; Anstett, Kjellberg & Bronstein 1996; Wang *et al.* 2009; Gu *et al.* 2012; Liu *et al.* 2013), the length of the receptivity period had a predictably important effect on syconium development. Although path analysis indicated no effect of pollinator progeny and seed numbers on syconium development time (Fig. 4), syconia receiving six foundresses (expected to have more wasps and seeds than those with one foundress; Wang *et al.* 2008) could be stronger nutrient sinks and therefore should have lower development times. Fruit growth rates and hence maturation times are dependent on seed numbers and nutrient sink strength (Gorchov 1985; Heuvelink & Körner 2001). Bagging studies indicated that 6-foundress syconia developed significantly faster than 1-foundress syconia, but the effect size was very small (on the order of 0.5 days, Fig. 5b, Appendix S3). One reason for this small effect size could be that interference competition between foundresses (Wang *et al.* 2009) in the 6-foundress syconia in our study site could have led to similar final

pollinator progeny and seed numbers as in syconia with single foundresses. The presence of developing pollinators in syconia lowered their development times after pollination as compared to syconia containing only seeds (Fig. 5c). Monoecious *F. racemosa* syconia manipulated to be functionally female (containing only seeds) took longer to develop than syconia that were functionally hermaphrodite. This closely resembles syconium development time patterns observed in syconia in monoecious figs that contain only seeds (Galil & Eisikowitch 1971) or in dioecious fig species wherein female syconia that contain only seeds take longer to develop than male syconia that contain only pollinators (Patel 1996). Despite the fact that seeds and pollinator progeny begin development together (as pollinator oviposition and pollination happen almost simultaneously), pollinator progeny and their galls are larger than seeds (Ghara 2012), probably making syconia with pollinator progeny stronger nutrient sinks which therefore develop faster than syconia containing only seeds. Ripening of the syconium is probably triggered by changes in the syconium resulting from wasp development since syconia that do not contain wasps take longer to ripen (Galil & Eisikowitch 1971; Kjellberg *et al.* 2005). Furthermore, cross-introductions of pollinators into syconia of host and non-host *Ficus* species indicated that the host syconium may also modulate its own development time (Kjellberg *et al.* 2005). Therefore, the development time of syconia is probably controlled by a combination of factors involving nutrient sink strengths affected by wasp progeny, presence of seeds and endogenous syconial characteristics.

CONFLICTING EFFECTS OF EARLY- AND LATE-OVIPOSITING PARASITES ON SYCONIUM DEVELOPMENT

Early-ovipositing gallers such as *A. testacea* develop in very large galls (Ranganathan, Ghara & Borges 2010; Ghara *et al.* 2014). This could lead to the formation of stronger and earlier nutrient sinks explaining why syconia containing *A. testacea* progeny developed faster than those with only pollinator progeny (Fig. 5d). A possible by-product of this accelerated syconium development is predator avoidance by these early gallers. Since pre-adult fig wasps constrained to develop within individual galls are essentially immobile, predation pressure by parasitoids on developing wasps could drive the evolution of faster development times in these early-ovipositing gallers to reduce time spent in these vulnerable juvenile stages. This result would be facilitated by early-oviposition and consequent early nutrient sink formation. Evolution of enhanced growth rates to reduce mortality due to predation in susceptible juvenile stages is a well-known strategy in several animal taxa (Benrey & Denno 1997; Kingsolver *et al.* 2012). Although shortened larval development time would decrease predator pressure on *A. testacea*, these gallers are dependent upon pollinator males to chew exit holes in the syconia; therefore, the magnitude of the effect of accelerated development would be limited by the development time of pollinator males. The reduction in syconium development time due to

A. testacea presence would consequently be under opposing selective forces. Development times of syconia containing progeny of *A. fusca* (the non-pollinator that oviposits concurrently with pollinators) and those of control syconia with only pollinator progeny were not significantly different (Fig. 5e), probably due to similar initiation times and similar strengths of nutrient sink formation; this is because both species oviposit concurrently (Ranganathan, Ghara & Borges 2010) and have similar-sized galls (Ghara *et al.* 2014). The late-ovipositing parasitoid, *A. agragensis*, increased TDT by increasing PPDT (Fig. 5f), ensuring that their progeny had sufficient time to complete development before wasp dispersal D-phase. Consequently, their undeveloped progeny are not consumed by seed-dispersing frugivores or by predators entering syconia through syconium exit holes (Bronstein 1988; Y. Ranganathan, M. Ghara & A. Krishnan, pers. obs.). Since *A. agragensis* larvae feed on developing pollinators (Ranganathan 2012), essentially replacing them with their own larvae, perturbations in syconium nutrient sink strength are an unlikely explanation for this phenomenon. Although parasitoid larvae can retard the development of their insect hosts (Dahlman *et al.* 2003; Pennacchio & Strand 2006), it is not certain how these factors could affect development times of whole syconia. The mechanism behind delayed syconium development caused by *A. agragensis* is still unresolved and needs further investigation.

CONCORDANCE BETWEEN PATH ANALYSIS PREDICTIONS AND WASP INTRODUCTION EXPERIMENTS

The effects of B-phase length, pollinators and non-pollinating parasites on syconium development time as predicted by path analysis were confirmed in the wasp introduction experiments. Although experiments to demonstrate the significantly negative effect of syconium volume on its development time are extremely difficult, the path analysis results are valid since larger syconia are likely to be stronger nutrient sinks with faster growth rates since they generally contain more ovules (see Appendix S2) and tend to have more inhabitants whether of the same or different species (Fig. 4). Path analysis also provided important information about the myriad relationships between various syconium inhabitants apart from their effect on syconium development time. Pollinators, which could have either a positive or negative effect on seeds (Fig. 2), were found to affect seed numbers positively (Fig. 4). This was probably due to high ovule availability coupled with low numbers of foundresses entering each syconium (Anstett, Bronstein & Hossaert-McKey 1996; Wang *et al.* 2008). All the non-pollinating gallers were expected to have negative relationships with each other and with pollinators and seeds (Fig. 2) since they compete for syconium development space and resources. Galls of early-ovipositing gallers often fill B-phase syconium cavities (Ghara *et al.* 2014), which, by hindering pollinator movement and oviposition, could explain the unexpected positive relationship between these gallers and numbers of seeds (Fig. 4). Gallers ovipositing concurrently

with pollinators were found to have a surprisingly positive relationship with pollinators (Fig. 4). However, these galls could be choosing to oviposit preferentially in pollinated syconia (which have lower chances of aborting) (Ghara *et al.* 2014). Late-arriving parasitoids, which prey upon galls, had positive relationships with the two classes of non-pollinating galls and affected pollinators negatively. Since predator–prey relationships can involve positive effects of prey on predator populations or negative effects of predators on prey, these results were unsurprising.

WHAT DOES VARIATION IN SYCONIUM DEVELOPMENT TIME MEAN FOR THE FIG TREE AND FIG WASPS?

Our results suggest that the development time of a syconium likely results from a tug-of-war between its wasp inhabitants. Host-plant-specific wasp inhabitants are capable of causing syconium development time variations ranging from as low as 1–2 days to as high as 6–12 days (see Appendix S3), which coupled with innate levels of within-tree asynchrony (caused by variations in time of syconium bud initiation), could extend the duration of each phenophase on a tree. This not only affects the pattern and timing of wasp dispersal and thus the male function of the tree, but also the temporal availabilities of oviposition sites.

Effect on the fig tree: One of the main effects of intra-tree variation in syconium development time due to host-plant-specific syconium inhabitants would be extended duration of the male floral phase (D-phase) on a single tree. This could increase the probability of male–female sexual phase overlaps within the same tree, assuring it some reproductive success in conditions of pollinator scarcity. However, as the number of failed reproductive episodes due to non-arrival of pollinators was only seven out of 90 (A. Krishnan, unpubl. data), pollinator limitation does not seem to be a major problem in this system. Male–female sexual phase overlaps within the same tree could also lead to inbreeding. However, this does not appear to be a major effect of the phenomenon, as within-tree sexual phase overlap was rare, occurring in only five reproductive episodes out of the 90 observed. Nonetheless, extended D-phase durations caused by variable syconium development times could have a potentially positive effect on the reproduction of the tree by increasing the probability of male–female sexual phase overlap between trees (Bronstein *et al.* 1990). Two major negative effects of within-tree asynchrony caused by extended durations of the different phases are possible. First, extended D-phase durations would lead to higher within-tree asynchrony in pollinator and seed dispersal, which could diminish the dual advantages of fruit disperser attraction and seed/pollinator wasp predator satiation (Bronstein 1988). Secondly, extended durations of the non-sexual A- and C-phases could expose trees to galler and parasitoid non-pollinators for longer periods of time, resulting in higher parasitism rates on the mutualism.

Effect on pollinators and non-pollinators: Since adult fig wasp life spans in this system can range from 1 day (pollinators) to 25 days (*A. westwoodi*) (Ghara & Borges 2010), even

small variations in wasp dispersal time from natal syconia could play a major role in the reproductive success of individual wasps by affecting the probabilities of their finding appropriate oviposition sites. Pre-adult development of the different wasps is also dramatically affected by reductions or increases in only a few hours of nursery life at critical development times (P. Yadav, unpubl. data). Therefore, even small effect sizes of the order of just a single day in syconial development time can make an important difference to the survival of the different wasps. Extended durations of the various sexual and non-sexual phases of syconium development (A through C) could also translate to temporally more constant availabilities of oviposition sites for both pollinators and non-pollinators. Although parasitoids have been shown to have a negative effect on pollinator reproduction within syconia in our path analysis results, it is possible that they have an overall beneficial effect on pollinator population survival by enhancing the probability of between-tree sexual phase overlap (Bronstein *et al.* 1990). Therefore, non-pollinators, which have generally been viewed as having negative effects on the fig–fig wasp mutualism (Janzen 1979; Bronstein 1991; Cook & Rasplus 2003; Herre, Jandér & Machado 2008; Segar & Cook 2012; but see Dunn *et al.* 2008; Al-Beidh *et al.* 2012), could be contributing to stabilizing and maintaining the system through their effects on within- and between-tree reproductive phenology. Since many developing wasps are herbivores, it is also possible that the phenotypic plasticity seen in syconium development is mediated by changes in ethylene (Galil 1968) as a result of herbivory (Von Dahl & Baldwin 2007). The elucidation of such mechanisms awaits further investigations.

Syconium initiation patterns are therefore not the sole proximate mechanisms responsible for within-tree reproductive asynchrony. Individual syconia have plastic development durations dependent on pollination timing and species of wasp progeny developing within them. Syconium development times are a likely compromise between conflicting demands from developing seeds and wasp species.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Description of modifications to Augspurger's synchrony index (Augspurger 1983) to obtain a within-tree index of asynchrony for *Ficus*.

Appendix S2. Relationship between syconium volume in D-phase and number of ovules.

Figure S2. Positive correlation between syconium volume (a measure for syconium size) and number of ovules.

Appendix S3. Detailed results of tree-wise analyses of bagging experiments using unpaired two-way *t*-tests or Kruskal–Wallis ANOVAs to confirm results obtained through LMMs.

Table S3. Total development time (TDT) and post-pollination development time (PPDT) of syconia (in days) from bagging and wasp introduction experiments.

Appendix S4. Methods of obtaining and details of resulting best-fit and most parsimonious path models from the *a priori* model.

Table S4. Descriptions of path models and their goodness-of-fit measures.

Appendix S5. Detailed LMM outputs for all bagging studies with tree ID taken as random factor.

Table S5a. Effect of pollination time on TDT (total development time) and PPDT (post-pollination development time) of syconia.

Table S5b. Effect of number of pollinator progeny (by manipulating number of foundresses entering syconia) on TDT (total development time) and PPDT (post-pollination development time) of syconia.

Table S5c. Effect of pollinator progeny presence on TDT (total development time) and PPDT (post-pollination development time) of syconia.

Table S5d. Effect of progeny of early-ovipositing gallers (*Apocryptophagus testacea*) on TDT (total development time) and PPDT (post-pollination development time) of syconia.

Table S5e. Effect of progeny of gallers ovipositing concurrently with pollinators (*Apocryptophagus fusca*) on TDT (total development time) and PPDT (post-pollination development time) of syconia.

Table S5f. Effect of progeny of a late-ovipositing parasitoid (*Apocryptophagus agransensis*) progeny on TDT (total development time) and PPDT (post-pollination development time) of syconia.