INVITED PAPER

For the Special Section: The Ecology, Genetics, and Coevolution of Intimate Mutualisms

Host sanctions in Panamanian *Ficus* are likely based on selective resource allocation[1]

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PREMISE OF THE STUDY: Fig trees and their pollinators, fig wasps, present a powerful model system for studying mutualism stability: both partners depend on each other for reproduction, cooperation levels can be manipulated, and the resulting field-based fitness quantified. Previous work has shown that fig trees can severely reduce the fitness of wasps that do not pollinate by aborting unpollinated figs or reducing the number and size of wasp offspring. Here we evaluated four hypotheses regarding the mechanism of sanctions in four Panamanian fig species.

METHODS: We examined wasp and fig samples from field experiments with manipulated levels of pollination.

KEY RESULTS: In unpollinated figs, the fig wall and the wasp offspring had a lower dry mass. Unpollinated figs had as many initiated wasp galls as pollinated figs but fewer galls that successfully produced live wasp offspring. Across three experimentally increasing levels of pollination, we found nonlinear increases in fig wall mass, the proportion of wasp galls that develop, and wasp mass.

CONCLUSIONS: Our data did not support the hypotheses that lack of pollination prevents gall formation or that fertilized endosperm is required for wasp development. While our data are potentially consistent with the hypothesis that trees produce a wasp-specific toxin in response to lack of pollination, we found the hypothesis that sanctions are a consequence of trees allocating more resources to better-pollinated figs more parsimonious with the aggregate data. Our findings are completely analogous to the selective resource allocation to more beneficial tissues documented in other mutualistic systems.

KEY WORDS coevolution; cooperation; *Ficus*; fig wasp; Moraceae; mutualism; partner choice; pollination; resource allocation; sanctions; species interaction

Mutualisms are ecologically important and widespread—examples include the mycorrhizal fungi that help forest trees take up nutrients, pollinators that help flowering plants reproduce, and gut microbes that help many animals, including humans, take up nutrients (Herre et al., 1999; Bäckhed et al., 2005; Douglas, 2010; Ollerton et al., 2011). Despite their ecological importance, the evolutionary maintenance of mutualisms is a puzzle (Sachs et al., 2004; West et al., 2007; Leigh 2010). How are partners in the mutualism prevented from taking the benefits of the interaction without paying the cost? Such cheaters would gain a relative fitness advantage and would threaten to break down the mutualistic interaction. Mechanisms suggested to align the interests of the two partners include

vertical transmission of symbionts (here used to mean the smaller partner in the mutualism) and repeated interactions with automatic fitness benefits (Herre et al., 1999; Sachs et al., 2004; West et al., 2007; Leigh, 2010). However, in systems where multiple symbionts interact with each host, symbiont transmission is horizontal, or partners do not interact long term, other mechanisms appear to be needed to prevent mutualism breakdown (Herre et al., 1999; Sachs et al., 2004; West et al., 2007; Leigh, 2010). In such systems, host sanctions are often reported, here defined as mechanisms that reduce the fitness of uncooperative symbionts.

Host sanctions have been documented in a number of ecologically important mutualisms, such as in the plant–mycorrhiza and legume–rhizobium mutualisms, and several plant–pollinator systems including the yucca–yucca moth, leafflower–leafflower moth and the fig tree–fig wasp mutualisms (Pellmyr and Huth, 1994; Kiers et al., 2003, 2011; Simms et al., 2006; Bever et al., 2009; Goto et al., 2010; Jandér and Herre, 2010; Wang et al., 2014). In some mutualistic systems, we know that host sanctions are a consequence of reduced resource allocation to less cooperative partners. For example, mycorrhizal fungi allocate less phosphorous to plants that

give them less carbon and vice versa (Kiers et al., 2011; Werner and Kiers 2015). Likewise, legumes seem to allocate less resources to root nodules containing rhizobia that fix less nitrogen (Kiers et al., 2003; Simms et al., 2006; Sachs et al., 2010). In contrast, the mechanism underlying host sanctions in plant–pollinator systems is less well studied.

In this paper, we address the mechanism of sanctions in the ecologically important mutualism between fig trees and their pollinating wasps (Shanahan et al., 2001). Fig wasps are the sole pollinators of fig trees, and fig wasp offspring can only develop in galled fig flowers. Two thirds of fig species are associated with actively pollinating wasp species that expend time and energy collecting (using their front legs), transporting (in special pollen pockets), and depositing (using their front legs) pollen (Frank, 1984; Kjellberg et al., 2001; Cruaud et al., 2012). However, not all wasp individuals of actively pollinating wasp species transport pollen (Jandér and Herre, 2010), and some fig wasp species that originate from actively pollinating wasp lineages have stopped pollinating altogether (Compton et al., 1991; Peng et al., 2008). By now, it is well established that in most actively pollinated fig species, fig wasps that do not pollinate have reduced fitness compared to wasps that pollinate: (1) some unpollinated figs abort, which kills all developing wasp offspring within (Jandér and Herre, 2010; Jansen-González et al., 2012), (2) generally fewer wasp offspring emerge from unpollinated figs that mature on the tree (Herre et al., 2008; Jandér and Herre, 2010; Jandér et al., 2012; Wang et al., 2014, 2015), and (3) those offspring that do emerge are often smaller and therefore less likely to themselves become foundresses (Jandér et al., 2016), and likely suffer additional fitness consequences (Herre, 1989; Moore and Greef, 2003; Dunn et al., 2015; Jandér, 2015). Together, these three components of *Ficus* host sanctions can reduce the fitness of pollen-free wasps to 63–0.001% of that of pollinating wasps (Jandér and Herre, 2010; Jandér et al., 2016). However, the mechanism behind these sanctions is unknown. Hypothesized mechanisms for the reduced fitness of fig wasps that do not pollinate include: (H_1) Pollination increases the chance of successful gall formation (Jousselin et al., 2003). (H_2) Wasp larvae developing in galled fig flowers need fertilized endosperm as a food source during development (Jansen-González et al., 2012). (H_3) Trees secrete a wasp-specific toxin that kills some of the developing wasp offspring in response to lack of pollination (Ibanez et al., 2009; Wang et al., 2014). (H_4) Resources are selectively allocated by the tree to more profitable (better-pollinated) fruits, thereby increasing resources to the developing wasp larvae (Herre and West, 1997; Jandér et al., 2012).

Here we used data from manipulative field experiments on four actively pollinated Panamanian fig species to address these four hypotheses. For figs that either are (P+) or are not (P-) pollinated, we examined information from already published studies (fig abortions, number and size of wasp offspring) (Jandér and Herre, 2010; Jandér et al., 2012, 2016), and added newly collected data from samples originating from those previously published experiments. Our newly collected data include (1) the number of wasps that die in their galls part way through development (bladders), which together with the previously published wasp numbers allowed us to calculate the proportion of wasp galls that develop to maturity, and (2) the fig wall mass (mass of the fig fruit minus the mass of seeds and wasps). All figures in this paper are based on newly collected data (in some cases combined with previously published offspring numbers). Predictions for the four hypotheses are listed in Table 1.

For comparison, we also examined data from two passively pollinated species. Passively pollinating fig wasps do not actively expend time or energy collecting or depositing pollen. The passively pollinated fig species investigated so far appear to lack host sanctions based on abortion and offspring reduction (Jandér and Herre, 2010). We therefore would expect passively pollinated species to not show any differences between pollinated and unpollinated figs in the newly collected data sets (bladders, and proportion of wasps that develop).

Additionally, we examined data from an experiment on *F. nymphaeifolia* with two foundresses in each fig combined into three different degrees of pollination—P-P-, P-P+ or P+P+ (Jandér et al., 2012)—to determine whether trees are able to gradually increase their resource allocation as the proportion of mutualists in a fig, and therefore pollination, increases. We combined newly collected data on bladder number, wasp mass, mass of fig wall, and mass of seeds, with previously published data on wasp offspring numbers (Jandér et al., 2012). We also used this data to hint at whether the effect on fig and wasp offspring characteristics (such as wasp offspring number and mass, fig wall mass, etc.) is a linear or saturated function of the proportion of mutualists in the fig (Archetti and Scheuring, 2013; Steidinger and Bever, 2016).

MATERIALS AND METHODS

Study species—We studied samples collected from natural populations of trees and wasps near the Panama Canal, Republic of Panama. The actively pollinated fig species *F. citrifolia* is here pollinated by *Pegoscapus tonduzi*, *F. nymphaeifolia* by *P. piceipes*, *F. obtusifolia* by the two cryptic species *P. hoffmeyeri A* and *B*, and *F. popenoei* by the two cryptic species *P. gemellus A* and *B* (Wiebes, 1995; Molbo et al., 2003). The passively pollinated fig species *F. insipida* is here pollinated by *Tetrapus costaricana* and *F. maxima* by *T. americanus*. For simplicity, we will here use the fig species name as a proxy for its associated wasp species.

P-/P+ experiments—The samples for the single and multiple-foundress experiments originated from the field experiments described by Jandér and Herre (2010) and Jandér et al. (2012), respectively; experimental setup is described in detail there. Briefly, we matched a flowering tree with a wasp-producing tree. To create P- (pollen-free) female wasps, we removed the pollen from half of the wasp-producing figs with fine forceps before the females had emerged from their galls. The remaining figs were untouched, allowing P+ (pollen-carrying) wasps to collect pollen. The P- treatment does not reduce the number of eggs a female carries or can lay (Jandér and Herre, 2010). Prereceptive figs on the experimental (flowering) trees were covered with fine mesh bags to prevent uncontrolled pollination. When the experimental figs were receptive, we introduced either a single P- or P+ wasp, or two wasps: P-P-, P-P+, or P+P+. The single foundress experiments were done on all six fig species; the two-foundress experiment was done on *F. nymphaeifolia*, chosen because it has strong sanctions combined with the practical possibility of experimentally creating multifoundress figs. In the two-foundress experiment, each independent vial of foundresses was used to set up maximum two experimental figs (Jandér et al., 2012). Some weeks later, right before wasp offspring emergence, the figs were collected, brought to the laboratory, wasps allowed to emerge in petri dishes, and figs dissected. Number of

TABLE 1. Predictions from the four alternate hypotheses regarding the mechanism of host sanctions in figs maturing on the tree, and some published findings for the Panamanian fig species. If gall formation fails in unpollinated flowers (H_1), there should be fewer total galls in unpollinated (P-) figs than in pollinated (P+) figs. If wasp larvae die because fertilized endosperm (produced by pollination of the flower in which they develop) is the required food for developing wasps (H_2), bladder numbers will be higher and wasp sizes smaller, but seed size ought to be equal in P- and P+ figs. Importantly, in both H_1 and H_2, the effect on wasp number and size would be on the level of individual flowers, whereas in H_4 and possibly H_3 the effect would be on the fig level. If wasp larvae die because of a toxin (H_3), bladder number would be higher in P- than in P+ figs, but seed mass should be the same because plant structures ought not to be affected by a wasp-specific toxin. Depending on the mechanism of the toxin, emerging P- wasp offspring may or may not be smaller than P+ wasp offspring. If sanctions are a consequence of selective resource allocation (H_4), developing wasp larvae in P- figs fail to get sufficient nutrition during development and either die part way through development and/or emerge smaller than wasps that develop in P+ figs. The reduced resource allocation to P- figs would also result in a less-developed fruit overall, leading to a lighter fig wall, and if any stray seeds are present (occasionally a few seeds might be present in "unpollinated" figs due to passive transfer of pollen grains (Jandér and Herre, 2010), they would be lighter than normal seeds unless seeds can pull in sufficient resources. Fig wall mass should also be less in P- than in P+ figs under H_1, H_2, and H_3 because P+ figs support seeds.

Hypothesis	Wasp offspring no.	Bladder no. (*B*)	Total galls (bladders + wasps) (*G*)	Prop. wasp galls developed (*D*)	Fig wall mass (*F*)	Wasp offspring size	Seed size	Level of precision
H_1: Gall formation	P- < P+	P- = P+	P- < P+	P- = P+	P- < P+ side effect of seed support	P- = P+	P- = P+	Flower
H_2: No endosperm for food	P- < P+	P- > P+	P- = P+	P- < P+	P- < P+ side effect of seed support	P- < P+	P- = P+	Flower
H_3: Wasp-specific toxin	P- < P+	P- > P+	P- = P+	P- < P+	P- < P+ side effect of seed support	P- < P+ or P- = P+, depending on mechanism	P- = P+	Fig or flower?
H_4: Less resources to fig	P- < P+	P- > P+	P- = P+	P- < P+	P- < P+ direct effect of reduced resources	P- < P+	P- < P+; if seeds can override resource allocation: P- = P+	Fig
Published data	P- < P+ Jandér and Herre, 2010; Jandér et al., 2012					P- < P+ Jandér et al., 2016		Fig Jandér et al., 2012, 2016

adult wasp offspring, bladders (empty wasp galls where wasp larvae died during development; sometimes containing remnants of a wasp larva), and seeds were counted. Some samples from the original experiments by Jandér and Herre (2010) and Jandér et al. (2012) were lost due to a faulty freezer. Where available, the fig fruit, 10 female offspring and 10 seeds from each fig were dried at 60°C for 48 h and weighed to the nearest $0.001 \times g$ (fruit) or 0.001 mg using a microbalance (wasps and seeds).

Calculations and statistical tests—For each fig, we calculated the total initiated number of galls (*G*) as (bladders + wasps), and the proportion of galls that develop into adult wasps (*D*) as (wasps / *G*). Calculating the proportion of galls that develop is particularly useful in nonexperimental situations when foundress wasps are likely to be of different age and/or have used different amounts of energy on their way to the tree, and therefore differ in their lifespan and total number of eggs deposited. It is also helpful when experimental figs differ in foundress numbers (Wang et al., 2014). We here present this data to enable comparisons with current and future data sets. Because we previously established that the fitness effect for wasps acts on the fig level in *F. nymphaeifolia*, we here analyzed the outcome for the entire fig (both foundresses within the fig) in the two-foundress experiment rather than for individual wasp lineages inside the fig (Jandér et al., 2012). Where available, we calculated the fig wall mass (*F*) as (total dry fruit mass) – (dry mass of seeds + dry mass of wasps). Each fig was used as an independent sample in the *t* tests and Mann–Whitney *U* tests. Data were log-transformed when needed to meet assumptions of normality and heteroscedasticity; *t* tests used equal or unequal variances depending on the data. Data that were not normally distributed, and the proportion

of galls that matured to adult wasps, were analyzed with Mann–Whitney *U* tests. Mass of wasps were examined in detail and linked to fitness by Jandér et al. (2016). Mass of (rare) seeds in P- figs are only available for two figs in *F. nymphaeifolia*, not the other species.

For each tree, we then calculated: F_R = relative dry mass of the fig wall in P- figs compared with P+ figs = F_{P-} / F_{P+}; G_R = relative number of total initiated wasp galls in P- figs compared with P+ figs = G_{P-} / G_{P+}; B_R = relative number of bladders in P- figs compared with P+ figs = B_{P-} / B_{P+}; D_R = relative proportion of wasp galls that produced adult wasp offspring in P- figs compared with P+ figs = D_{P-} / D_{P+}.

Because we know from previous experiments that experimentally produced P- foundresses carry and are able to lay as many eggs as P+ foundresses (Jandér and Herre, 2010), we expect G_R to be close to 1 (unless H_1 is true). When comparing across species, each tree was used as an independent sample, and only species with >1 tree were included in the ANOVAs/Welch tests. SPSS version 22 (IBM, Armonk, New York, USA) was used for the statistical analyses; all tests were two-tailed.

RESULTS

Effects of lack of pollination in single foundress introductions, across species

Fig wall mass—The fig wall, *F*, was clearly and significantly heavier in P+ figs than in P- figs in three of four examined actively pollinated species—*F. nymphaeifolia*, *F. obtusifolia*, *F. popenoei* (Table 2, Fig. 1A), as would be expected if P+ figs were allocated more resources. A similar pattern was seen in *F. citrifolia*, but the difference

TABLE 2. The dry mass of the fig wall (mass of fig fruit minus seeds and wasps) across the two pollination treatments on each experimental tree for the four studied actively pollinated fig species. Equal or unequal variances were used in *t* tests depending on the data; boldface indicates significant *P* values.

Species	Tree	N	Mean mass of fig wall (g) (sem)	t (df)	P
F. citrifolia	BCI17	3 P-	0.1812 (0.0039)	−1.72 (11)	0.113
		10 P+	0.2042 (0.0070)		
F. nymphaeifolia	BN67	23 P-	0.7192 (0.0190)	−7.49 (22.00)	**<1E-6**
		15 P+	1.0209 (0.0355)		
	BS1	11 P-	0.7687 (0.0234)	−7.13 (24)	**<1E-6**
		15 P+	1.0825 (0.0334)		
F. obtusifolia	BN64	4 P-	1.1184 (0.1247)	−3.39 (12)	**0.0053**
		10 P+	1.561 (0.0665)		
	MIL	32 P-	0.9907 (0.0331)	−2.37 (49)	**0.022**
		19 P+	1.1431 (0.0623)		
F. popenoei	BV11	32 P-	0.5461 (0.0142)	−2.52 (42)	**0.016**
		12 P+	0.5985 (0.0196)		

was not significant (Table 2). Data on fig wall mass were not available for the two passively pollinated species.

Number of initiated galls—For six of our nine experimental trees, there was no significant difference in the number of initiated galls (*G*) between the P- and P+ treatments (Table 3). For the remaining three trees, there were slightly fewer galls in P- figs than in P+ figs (Table 3). The relative number of total galls in P- compared with P+ figs, G_R, did not significantly differ between passive and active species ($t_7 = 1.57$, $P = 0.16$), nor (as expected) across the actively pollinated species (ANOVA, $F_{2,5} = 0.013$, $P = 0.99$; Fig. 1B).

Number of bladders (failed development of initiated galls)—In all actively pollinated species examined (*F. citrifolia*, *F. nymphaeifolia*, *F. obtusifoli*a, *F. popenoei*), there were more bladders in P- figs than in P+ figs, as would be expected if wasp larvae died partway through development (Fig. 1C, Table 3). In contrast, in the two passively pollinated species, *F. maxima* and *F. insipida*, bladders were very few and did not significantly differ between P- and P+ figs (Fig. 1C, Table 3). When each tree was used as an independent sample, the relative number of bladders in P- figs compared with P+ figs, B_R, was significantly higher in actively pollinated figs species than in passive (unequal variances *t* test, $t_{6.28} = -3.28$, $P = 0.016$; Fig. 1C). There was no significant difference in B_R across the actively pollinated species, although this was likely due to the small sample size for each species (Welch robust ANOVA, test statistic = 8.81, df = 1.35, $P = 0.17$; Fig. 1C).

Proportion of galls that successfully produced adult wasp offspring—The proportion of wasp galls that produced adult wasp offspring, *D*, was dramatically lower in P- figs than in P+ figs for all actively pollinated species (Fig. 1D, Table 3). In the passively pollinated fig species, the proportion of galls that produced adult offspring was consistently high and did not differ across the P- and P+ treatments (Fig. 1D, Table 3). The relative proportion of wasp galls that produced adult offspring, D_R, was significantly lower for actively than for passively pollinating species (unequal variances *t* test, $t_{3.38} = 6.02$, $P = 0.015$). As expected, D_R varied across the active species consistent with previous measurements of sanction strength (Jandér and Herre, 2010), although not significantly due to the small sample size for each species (Welch robust ANOVA, test statistic = 5.71, df =2, 1.78, $P = 0.17$; Fig. 1d).

Effects of stepwise changes in pollination level—Similar to the results in the experiments with just two pollination levels (P- or P+),

the number of wasp offspring was lowest in P-P- figs and highest in P+P+ figs (Fig. 2A) in the multifoundress experiment on *F. nymphaeifolia*. In figs with an intermediate pollination level (P-P+) the number of wasp offspring was intermediate (Tree BCI: ANOVA $F_{2,11} = 5.53$, $P = 0.027$; tree BS#1: Welch robust test statistic 33.69, df = 2,32.62, $P = 1.1E-8$; Fig. 2A). Consistent with the single-foundress experiments, the number of bladders was highest in P-P- figs, lowest in P+P+ figs, and intermediate for the P-P+ figs with intermediate pollination level (Tree BCI: ANOVA $F_{2,11} = 156.50$, $P = 1.0E-7$; tree BS1: ANOVA $F_{2,60} = 121.84$, $P = 1.7E-21$; Fig. 2B). As a consequence, the proportion of wasp offspring that developed to maturity was lowest in P-P- figs, highest in P+P+ figs, and intermediate in P-P+ figs (ANOVAs, tree BCI: $F_{2,11} = 139.98$, $P = 1.7 E-7$; tree BS1: $F_{2,60} = 84.58$, $P = 6.4 E-18$; Fig. 2C). As expected, the number of total initiated galls (sum of bladders and wasps) did not differ across the treatments (ANOVAs, tree BCI: $F_{2,11} = 0.63$, $P = 0.55$; tree BS1: $F_{2,60} = 0.064$, $P = 0.94$; Fig. 2D).

The mean mass of a wasp offspring was lower in P-P- figs than in the other two groups, but only significantly so in the tree with the larger sample size (ANOVAs, tree BCI: $F_{2,7} = 0.91$, $P = 0.46$; tree BS1: $F_{2,47} = 13.88$, $P = 2.0E-5$; Fig. 2E). The mass of the fig wall was lower in P-P- figs than in P-P+ and P+P+ figs for both trees (Welch robust ANOVAs, tree BCI: Welch statistic = 9.94, df = 2, 4.31, $P = 0.024$; tree BS1: Welch statistic = 49.47, df = 2, 24.94, $P = 2.1E-9$; Fig. 2F). The mean mass of one seed did not significantly differ between P-P+ figs and P+P+ figs (*t* tests, tree BCI: $t_7 = 0.91$, $P = 0.39$; tree BS1: $t_{30} = 1.99$, $P = 0.06$; Fig. 2G). Two of the P-P- figs had some seeds (1 and 3 seeds, respectively), but because of the low sample size (1 fig per tree), P-P- seeds could not be meaningfully included in the statistical analyses. The one seed on tree BCI was very light, the three seeds on BS1 had on average a similar mass as those in the P-P+ and P+P+ treatments (Fig. 2G).

DISCUSSION

The data presented here in conjunction with already published studies suggest that the most likely mechanism underlying host sanctions in figs is relatively higher resource allocation to better-pollinated figs. The evidence for these species allows us to discard both the hypothesis that pollination is required for successful gall formation (H[1]) and the hypothesis that fertilized endosperm is required for developing larvae (H[2]). While we do not have data to exclude the hypothesis that wasp offspring die because the tree emits wasp-specific toxins to unpollinated figs (H[3]), the hypothesis that sanctions are a consequence of relatively higher resource allocation to better-pollinated figs (H[4]) is simpler and more parsimonious. Indeed, higher resource allocation to better-pollinated figs can explain all three components of host sanctions that have been previously reported: abortions of unpollinated figs and reduced number and size of offspring in unpollinated figs that mature. Further, these findings are completely analogous
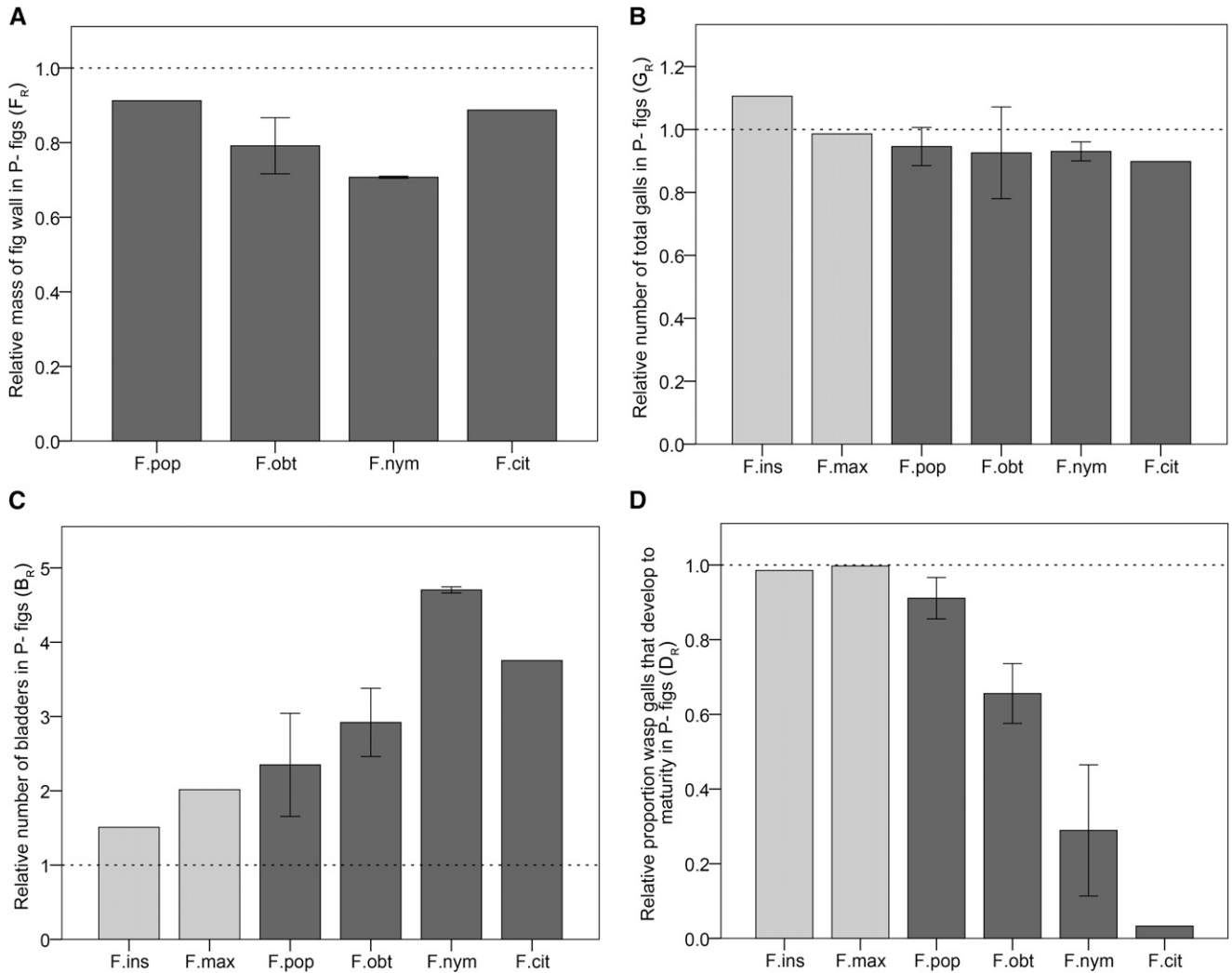
FIGURE 1 (A) The mass of the fig wall and (D) the proportion of wasp galls that developed to maturity were lower in single-foundress, unpollinated (P-) figs than in pollinated (P+) figs across four actively pollinated fig species (dark gray bars), but not across the two passively pollinated species (light gray bars). (B) The number of initiated wasp galls was equal or slightly less in P- figs than in P+ figs and did not differ between active and passively pollinated species. (C) The number of bladders was higher in P- than in P+ figs across all actively pollinated species, but not the passively pollinated species. The horizontal dashed line at 1.0 indicates the level of no difference between the P+ vs P- treatments. Each experimental tree is treated as one sample in the ANOVAs (ins: 1, max: 1, pop: 2, obt: 2, nym: 2, cit: 1), error bars represent 1 standard error of the mean. Source data are in Tables 2 and 3. *Abbreviations:* F. ins = *Ficus insipida*, F. max = *F. maxima*, F. pop = *F. popenoei*, F. obt = *F. obtusifolia*, F. nym = *F. nymphaeifolia*, F. cit = *F. citrifolia*.

to the selective resource allocation to more beneficial tissues that has been documented in other mutualistic systems (Kiers et al., 2003, 2011; Simms et al., 2006; Bever et al., 2009; Bever, 2015; Werner and Kiers, 2015).

Selective resource allocation in Ficus—The most parsimonious explanation that is consistent with the data is H_4: that more resources are selectively allocated by the plant to more profitable modules, in this case better-pollinated figs that produce more seeds (Herre and West, 1997; Jandér et al., 2012). It is obvious that pollinated figs are allocated more resources from the tree: pollinated figs are (1) less likely to abort and (2) always heavier than conspecific unpollinated figs because they contain seeds. Pollinated figs also have a heavier fig wall than unpollinated figs. Therefore, without doubt, pollinated figs are allocated more resources than unpollinated figs. We suggest that developing wasps can benefit from the increased resources pulled in by developing seeds; the increased resources available allow a larger proportion of wasp offspring to develop to maturity (Jandér and Herre, 2010), and those offspring that do develop have a larger body size (Jandér et al., 2016). Although we have focused on examining the reasons for the reduced number of offspring in unpollinated figs that mature on the tree, the resource allocation hypothesis can also explain the selective abortions of unpollinated figs previously observed (Jandér and Herre, 2010), as aborting a fig represents the most extreme form of reducing resource allocation. Therefore, a single mechanism, selective resource allocation to more profitable figs, could explain all three components of host sanctions that we hitherto have observed: abortions of unpollinated figs, and reduced number and size of offspring in unpollinated figs that mature (Fig. 3).

TABLE 3. Number of bladders, total galls (bladders + wasps), and the proportion of wasp galls that matured to adult wasps (wasps / galls) across the two pollination treatments on each experimental tree for the six studied species. Data were analyzed with *t* tests or Mann–Whitney *U* tests depending on the data; boldface indicates significant *P* values. Species name codes in italics indicates actively pollinated fig species.

| Species	Tree	N	Bladders, *B*			Total galls (bladders + wasps), *G*			Proportion of galls that matured to adult wasps, *D*		
			Mean (sem)	*t* (df)	P	Mean (sem)	*t* (df)	P	Mean (sem)	U	P
ins	GB1	12 P- 12 P+	8.42 (2.210) 5.58 (1.438)	1.19 (22)	0.25	184 (12.19) 166.42 (11.10)	*U*: 47.5	0.16	0.9507 (0.0138) 0.9649 (0.00977)	86.5	0.41
max	BS1	20 P- 21 P+	1.35 (0.762) 0.67 (0.222)	*U*: 229	0.56	205.35 (11.4) 208.29 (4.66)	−0.24 (25.18)	0.81	0.9942 (0.00323) 0.9967 (0.00105)	187	0.49
cit	BCI17	3 P- 10 P+	137 (12.124) 36.5 (6.827)	−7.22 (3.34)	**0.004**	143 (11.85) 159.2 (6.45)	*U*: 22.5	0.22	0.0249 (0.00702) 0.7565 (0.0564)	30	**0.007**
nym	BN67	24 P- 15 P+	155.29 (4.799) 32.73 (3.487)	20.66 (36.83)	**<1E-6**	171.08 (5.00) 190.07 (7.65)	−2.17 (37)	**0.036**	0.0927 (0.00646) 0.8163 (0.0278)	360	**<0.001**
	BS1	14 P- 15 P+	145.14 (6.842) 31.13 (2.905)	15.34 (17.58)	**<1E-6**	244.86 (6.80) 254.93 (3.80)	−1.32 (27)	0.20	0.4076 (0.0217) 0.8769 (0.01192)	210	**<0.001**
obt	BN64	10 P- 13 P+	115.40 (17.688) 34.15 (10.568)	5.63 (21)	**1.4E-5**	233.50 (12.50) 299.31 (12.22)	−3.71 (21)	**0.0013**	0.5015 (0.07096) 0.871 (0.05025)	123	**<0.001**
	MIL	34 P- 21 P+	126.68 (17.131) 51.48 (7.170)	4.90 (53)	**9.0E-6**	292.32 (7.99) 272.81 (10.62)	1.48 (53)	0.14	0.5895 (0.04464) 0.8012 (0.02978)	577	**<0.001**
pop	BV11	32 P- 12 P+	37.53 (3.32) 22.67 (2.48)	3.03 (42)	**0.004**	161.88 (6.11) 182.92 (3.97)	−2.037 (42)	**0.048**	0.7496 (0.0268) 0.8761 (0.01296)	306	**0.002**
	JG1	23 P- 15 P+	6.48 (0.984) 2.13 (1.12)	*U*: 50	**<0.001**	139.57 (5.80) 138.67 (6.72)	0.10 (36)	0.92	0.9525 (0.00671) 0.9854 (0.00679)	294	**<0.001**

The selective resource allocation seems to increase with the degree of pollination, although not linearly. The stepwise increases in pollination level in the two-foundress experiment on *F. nymphaeifolia* showed that as the pollination level increases, so does the mass of the fig wall as well as the number of wasp offspring. However, the effects on the symbionts—the proportion of wasp galls developed and the wasp mass—seem to follow asymptotically saturated rather than linear functions. Saturated fitness functions have been theoretically shown to promote the coexistence of mutualists and exploiters when multiple symbiotic partners share a module (Archetti and Scheuring, 2013; Steidinger and Bever, 2016) because it enables exploiters to free-ride on the efforts of the mutualistic partners (Friesen and Mathias, 2010; Jandér et al., 2012). In *F. nymphaeifolia* over 75% of foundresses share a fig in nature (Jandér and Herre, 2010). Because sanctions act on the fig level in this species (Jandér et al., 2012), exploiters (e.g., pollen-free wasps) can thus largely escape sanctions when sharing a fig with a pollinator.

Our previous studies focused on fig abortion and wasp offspring reduction suggested that the passively pollinated fig species of section Pharmacosycea (the phylogenetically most basal section of *Ficus*) do not show any host sanctions (Jandér and Herre, 2010). However, recent findings (Jandér et al., 2016) suggest instead that any differences in host sanctions between passively and actively pollinated species are quantitative rather than qualitative. Although the passively pollinated fig species examined here, *F. insipida* and *F. maxima*, do not show selective abortions of unpollinated figs or any reductions in the number of wasp offspring developed (Jandér and Herre, 2010) or increases in the number of bladders, recent findings indicate that they can have slightly reduced wasp offspring sizes in unpollinated figs. Wasps developing in unpollinated figs of *F. maxima* weighed only 90% of that of wasps developing in pollinated figs (Jandér et al., 2016). The sample size was small, and the experiment would have to be repeated on other trees and species before we can generalize. However, the finding suggests that selective resource allocation to pollinated figs might be universally present in all fig species, just at different degrees. Passively pollinated fig species benefit from the guaranteed pollen dispersal by any wasps developing within a fig (monoecious figs produce not only seeds but also pollen and the pollen-dispersing wasps), making also unpollinated but oviposited figs relatively profitable (male fitness) for passively pollinated monoecious fig trees even if they are seedless (Jandér and Herre, 2010). In the passively pollinated Pharmacosycea species, local availability of resources might be more important than pollination status in determining the resource allocation to a fig; in *F. yoponensis*, the proportion of flowers that developed and the fruit dry mass were directly related to the size of the attendant leaf (Herre, 1996).

Competing hypotheses—The remaining three competing hypotheses were either less or not supported by our data from these New World monoecious fig species:

Gall formation—The first hypothesis (H_1) poses that pollination increases the chance of successful gall formation (Jousselin et al., 2003). It is clear from previous studies of a number of fig species that pollination is not *essential* for successful gall formation because some proportion of wasps are able to develop in completely unpollinated figs (Nefdt, 1989; Jousselin et al., 2003; Tarachai et al., 2008; Jandér and Herre, 2010; Jandér et al., 2012; Jansen-González et al., 2012; Wang et al., 2014, 2015). In addition, our data in this paper show that the total number of *initiated* galls is more or less equal in pollinated and unpollinated figs, but in actively pollinated fig species there are many more bladders in unpollinated figs, i.e., galls were formed but wasp offspring died within them before they fully developed. Although six of our experimental trees showed no difference in the number of initiated galls between unpollinated and pollinated figs, in three of our experimental trees there were slightly fewer initiated galls in unpollinated figs. This result could either mean that for these trees (1) pollination indeed facilitated gall formation, (2) that P- wasps did not lay as many eggs as the P+
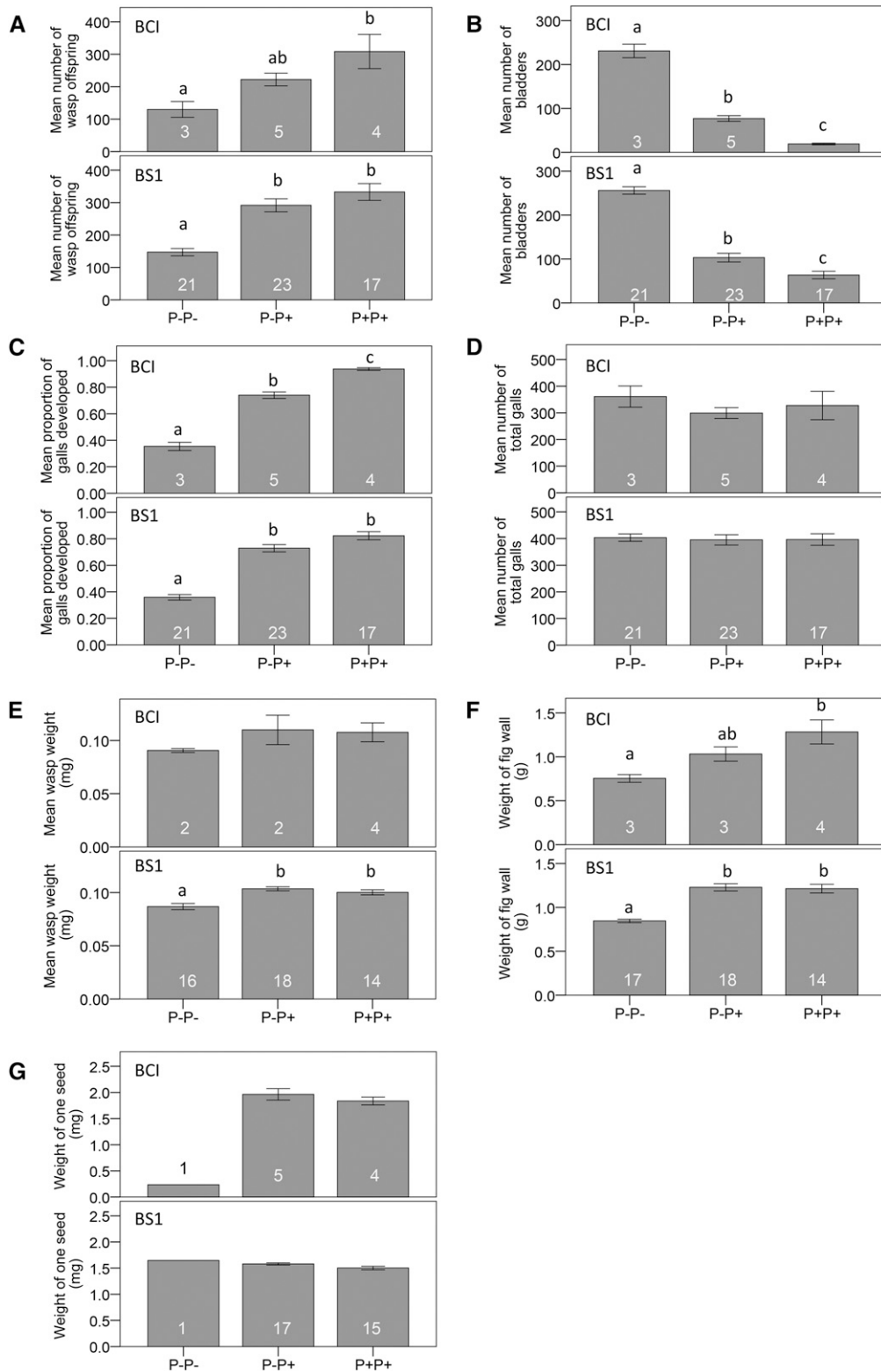
FIGURE 2 Across three experimental treatments of increasing pollination levels in figs with two foundresses (P-P-, P-P+, and P+P+) on two different trees of *F. nymphaeifolia* (BCI and BS1), there were clear differences in (A) the mean number of wasp offspring emerging, (B) mean number of bladders, (C) mean proportion of wasp galls that develop to release adult wasp offspring, (E) mean mass of each emerging wasp offspring, and (F) the mean mass of the fig wall. There were no significant differences across the treatments in (D) the number of initiated wasp galls or in (G) the mean mass of each seed. Sample sizes (number of figs) are indicated inside the bars (panel B has identical sample sizes to panel D). Error bars represent 1 standard error of the mean. Letters represent significantly different subsets within each tree.
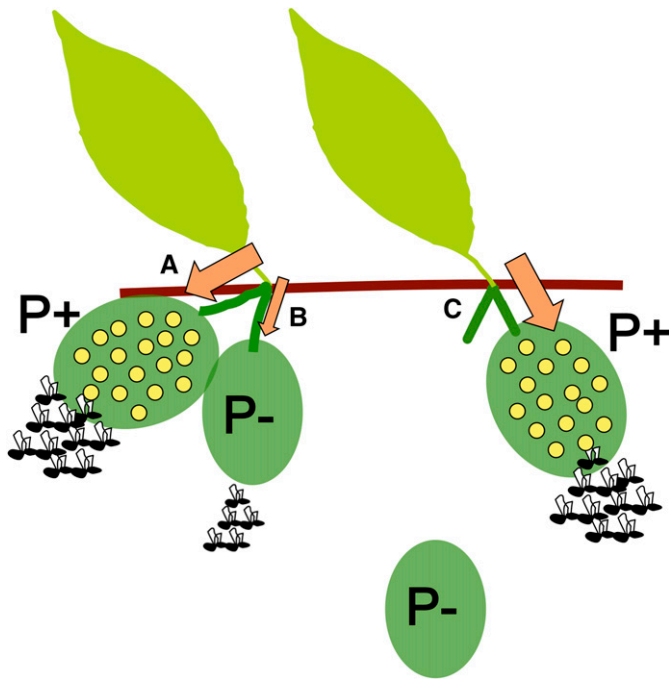
FIGURE 3 Simplified illustration of how selective resource allocation results in *Ficus* host sanctions. (A) Pollinated (P+) figs contain developing seeds (small circles) and are allocated relatively more resources (arrows). More resources allow more wasp offspring to develop, and those that develop are larger. (B) Unpollinated figs contain no seeds and are allocated relatively fewer resources. A smaller proportion of the initiated wasp galls develop, resulting in fewer adult wasp offspring. Wasp offspring are also smaller. (C) Some unpollinated figs are allocated no further resources and abort, thus killing all wasp larvae within.

wasps (although that would contradict our earlier tests [Jandér and Herre, 2010]), or (3) that some wasp larvae died so young that their gall had not fully developed and was not recognized as a bladder, but was considered an undeveloped flower during dissections. We find the latter explanation most likely, especially as G_R is lowest in the species with the known strongest sanctions (Jandér and Herre, 2010). Either way, the difference in total initiated gall number is miniscule compared with the difference in wasp offspring numbers (Jandér and Herre, 2010) across treatments, so even if some galls failed to form, or P- wasps unexpectedly laid fewer eggs than P+ wasps, that would only explain a minor part of the reduced wasp numbers. Therefore, in these Panamanian wasp species, we can discard the hypothesis that pollination facilitates gall formation as being unimportant.

P-/P+ experiments on a variety of *Ficus* species have also found increased bladder numbers in unpollinated figs relative to pollinated figs (Nefdt, 1989; Jousselin et al., 2003; Tarachai et al., 2008; Jansen-González et al., 2012; Wang et al., 2014, 2015), although in *F. microcarpa* (Jousselin et al., 2003) the bladder numbers were relatively low and could not fully explain the lower offspring numbers in unpollinated figs, and in *F. condensa* (Jousselin and Kjellberg, 2001), there were no more bladders in unpollinated figs. In the passively pollinated species *F. maxima* and *F. insipida*, where no reductions in offspring numbers have been found (Jandér and Herre, 2010), there were not significantly more bladders in unpollinated figs. Therefore, an almost universal finding among fig species

with host sanctions is that the reduction in offspring numbers in maturing figs is due to increased wasp offspring mortality in already formed galls rather than lack of gall formation in the first place.

Endosperm—The second hypothesis (H_2) poses that wasp offspring require fertilized endosperm for optimal growth and development (Jansen-González et al., 2012). It is clear that fertilized endosperm is not *essential* for larval growth, because wasp offspring are able to develop in unpollinated figs (where no seeds, and therefore no endosperm, is present) in all species examined to date, even if in smaller numbers and sizes (Nefdt, 1989; Jousselin and Kjellberg, 2001; Jousselin et al., 2003; Tarachai et al., 2008; Jandér and Herre, 2010; Jandér et al., 2012, 2016; Wang et al., 2014, 2015). If lack of endosperm causes suboptimal development and growth, we would expect to see the reduction in the proportion of wasps developed and in the wasp size, acting on the level of individual fig flowers because a single wasp larva develops within the confines of a single flower. In contrast, earlier experiments on *F. nymphaeifolia* have shown that the reduction in offspring numbers and offspring size both act on the level of the entire fig, rather than on individual flowers within the fig (in P-P+ figs, P- and P+ foundresses on average have the same number and size of offspring: Jandér et al., 2012, 2016). These findings are inconsistent with H_2. Although for practical reasons we have only tested the precision of reduction in offspring number and size in one fig species, it seems likely that the closely related fig species in section *Urostigma Americana* examined here (*F. citrifolia*, *F. obtusifolia*, *F. popenoei*) also have fig level sanctions and, hence, that this conclusion applies also to them.

Wasp-specific toxin—The remaining hypothesis, that the tree emits wasp-specific toxins to unpollinated figs (H_3) (Wang et al., 2014) is consistent with available data on wasp numbers and sizes for these Panamanian species, but is less parsimonious than the resource allocation hypothesis. As discussed already, pollinated figs are allocated more resources from the tree. The question is whether the selective resource allocation in itself is sufficient to explain the reduced number of developing wasp galls and the reduced wasp offspring sizes, or whether those effects are caused by a wasp-specific toxin emitted by the tree in response to lack of pollination. A good test to distinguish between the two would be to measure plant-produced antiherbivore compounds in P- and P+ figs, expecting higher levels in P- figs. In the absence of these data, a potentially useful test might be to compare seed size in P- (occasionally a few rare seeds might be available) and P+ figs, as antiherbivore toxins presumably would not affect seeds. However, we did not have data on (rare) P- seed mass available for more than two figs, and they give conflicting results. Furthermore, complicating the matter is the distinct possibility that seeds might be able to selectively pull in sufficient resources to grow to their maximum mass irrespective of the general resource allocation to the fig they are in (in contrast with other fig structures, and wasps). If that is the case, then we could not expect to see a difference in mass between P- and P+ seeds even if wasp numbers and sizes are reduced due to selective resource allocation.

Ultimately, the toxin hypothesis is possible, but it seems less parsimonious than the simple resource allocation hypothesis. For the toxin hypothesis to work, figs would selectively need to produce toxins in response to lack of pollination. If toxins increase with increasing larval damage, we would expect higher larval mortality with more offspring, and in *F. racemosa* this relationship is true for

P- foundresses but not P+ (Wang et al., 2014), consistent with the toxin hypothesis. However, this finding is also consistent with a limited (and reduced) pool of resources in P- figs being split among an increasing number of initiated wasp galls as foundress numbers increase, causing more larvae to die of lack of resources as the number of initiated wasp galls increase. Although possible, it seems complicated to have toxins that activate at some threshold of larval damage, but only if seeds are lacking. In *Trollius*, another nursery pollination system, toxins increase with more larval damage (Ibanez et al., 2009), but in contrast with *Trollius*, fig trees benefit from producing female wasps because they are the pollen dispersers, so a chemical defense seems less likely (Jandér, 2015). In addition, producing toxins is likely to be costly for individual hosts, making it hard to see how it would be promoted by natural selection. In contrast, selective resource allocation to beneficial tissues leads to more or less immediate fitness benefits and would therefore be promoted by natural selection.

Selective resource allocation in other systems—If we look more broadly, selective resource allocation to more profitable symbiont supporting modules (e.g., fruits, nodules, arbuscules) is consistent with what we know other plants do: pollinated flowers are allocated resources and mature into seed-containing fruits, whereas unpollinated flowers are aborted (Frederickson, 2013). Selective resource allocation is also thought to be the basis of host sanctions in a number of mutualistic systems. For example, legume nodules infected with efficient nitrogen-fixing bacteria grow larger and can generally support more rhizobial cells than do nodules infected with strains that fix nitrogen poorly (Kiers et al., 2003; Simms et al., 2006; Sachs et al., 2010; but see Gubry-Rangin et al., 2010). Beneficial rhizobial cells also contain higher levels of the storage lipid PHB than do less beneficial rhizobial cells (Ratcliff et al., 2008; Oono et al., 2011). Similarly, plants allocate more resources to root portions associated with beneficial mycorrhizae than those associated with less beneficial mycorrhizae (Bever et al., 2009; Kiers et al., 2011; Bever, 2015; Werner and Kiers, 2015). Importantly, selective resource allocation to more profitable tissues increases the fitness of the host (for a theoretical model, see West et al., 2002). That we here find selective resource allocation the most parsimonious explanation for host sanctions also in the fig mutualism is therefore not surprising. Although the host most likely allocates resources to more profitable tissues for purely selfish reasons, the effect on the symbiont is often that more cooperative symbionts have higher fitness. Therefore, a purely selfish and efficient resource allocation by the host can have the side effect of promoting cooperation in its symbionts. Such selective resource allocation can help explain the puzzle of how mutualism stability is maintained.

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