

Fitness reduction for uncooperative fig wasps through reduced offspring size: a third component of host sanctions

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Abstract. Mutually beneficial interactions between two species—mutualisms—are ancient, diverse, and of fundamental ecological importance. Nonetheless, factors that prevent one partner from reaping the benefits of the interaction without paying the cost are still poorly understood. Fig trees and their unique pollinators, fig wasps, present a powerful model system for studying mutualism stability. Both partners depend completely 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 impose two types of host sanctions that reduce the fitness of wasps that do not pollinate: (1) fig abortion, which kills all developing larvae, and (2) reduced number of wasp offspring in figs that are not aborted. Here we demonstrate a third component of host sanctions. Through manipulative field experiments, we show that for four of five studied species, offspring of pollen-free foundresses are only 50–90% the size of offspring of pollinating foundresses. We further show that in all four studied species, smaller wasps are less likely to reach and enter a flowering fig to become foundresses themselves. Therefore, the experimentally determined size reduction of offspring is estimated to cause an additional reduction of up to 80% in fitness for a pollen-free foundress. We determine that the size reduction of pollen-free offspring acts on the level of the entire fig fruit rather than on individual flowers. These results show that estimates of the fitness effect of host sanctions on uncooperative symbionts should consider not only offspring quantity but also offspring quality. We discuss implications beyond the fig tree—fig wasp mutualism.

Key words: *Agonidae*; *co-evolution*; *cooperation*; *Ficus*; *fig wasp*; *insect size*; *mutualism*; *partner choice*; *pollination*; *resource allocation*; *sanctions*; *species interaction*.

INTRODUCTION

Mutualisms are ecologically important and widespread—examples include the mycorrhizal fungi that help forest trees take up soil nutrients, gut microbes that help many animals, including humans, take up nutrients, and pollinators that help flowering plants reproduce (Herre et al. 1999, Bäckhed et al. 2005, Douglas 2010, Ollerton et al. 2011). Despite their ecological importance, the evolutionary maintenance of mutualisms presents a puzzle (Sachs et al. 2004, Leigh 2010). How are partners in the mutualism prevented from taking the benefits of the interaction without paying the cost? Mechanisms such as vertical transmission of symbionts and repeated interactions with automatic fitness benefits (partner fidelity) can align the interests of the two partners (Herre et al. 1999, Sachs et al. 2004, Leigh 2010). However, in systems where symbiont transmission is horizontal and partners do not repeatedly interact, other mechanisms are needed to prevent mutualism breakdown. In many mutualisms, host sanctions

(the reduction of fitness of uncooperative symbionts) have been reported (Kiers et al. 2003, Leigh 2010). Host sanctions often involve the co-opting of existing host mechanisms that shift resource allocation away from tissues associated with less cooperative symbionts or groups of symbionts, effectively rewarding more cooperative symbionts (see Jandér and Herre 2010, Kiers et al. 2011, Frederickson 2013).

Host sanctions have so far been documented in a number of ecologically important mutualisms, such as in the plant—mycorrhizae, legume—rhizobia, and fig tree—fig wasp mutualisms (Kiers et al. 2003, 2011, Simms et al. 2006, Bever et al. 2009, Jandér and Herre 2010, Wang et al. 2014). There are two documented components of host sanctions that both reduce the fitness of uncooperative symbionts: (1) completely ceasing interaction with uncooperative symbionts, or (2) retaining the interaction but reducing the resource allocation to uncooperative symbionts. When hosts completely cease interaction with symbionts, symbionts either die (e.g., when figs or yucca fruits are aborted) or symbionts are forced to live in a less productive free-living state (e.g., when legume nodules senesce; Pellmyr and Huth 1994, Denison and Kiers 2004, Jandér and Herre 2010). When

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hosts retain the interaction but reduce the resource allocation, this often reduces the number of offspring from uncooperative symbionts, such as reduced offspring numbers for fig wasps that do not pollinate (Jandér and Herre 2010), or reduced number of rhizobia in nodules that do not fix nitrogen (Kiers et al. 2003). Because resources are required for offspring development, it seems logical that not only the offspring quantity but also the offspring quality can be affected by host sanctions. Offspring quality is expected to be an important fitness component. For example, an earlier study suggests that within species, larger fig wasp individuals are more likely to successfully disperse to receptive trees where they can reproduce (Herre 1989). Despite the expected importance, the potential effect of host sanctions on offspring quality has rarely been studied.

Here we experimentally test whether the quality (size) of offspring is affected when symbionts are uncooperative. We test this in the mutualism between fig trees and their pollinating fig wasps, where manipulative experiments preventing wasps from pollinating are straightforward (Jousselin et al. 2003a, Jandér and Herre 2010). Fig wasps are the sole pollinators of fig trees, and fig wasp offspring can only develop in fig flowers. In monoecious fig species, each fig flower can develop into either a seed or a wasp offspring. 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 (Ramirez 1969, Frank 1984, Kjellberg et al. 2001, Cruaud et al. 2012). However, not all wasp individuals in actively pollinating wasp species transport pollen (Jandér and Herre 2010), and some previously actively pollinating fig wasp species have stopped pollinating altogether (Compton et al. 1991, Peng et al. 2008). Although we have not yet been able to quantify the benefit, it seems reasonable that wasps could obtain some benefit from not actively collecting, carrying, and depositing pollen (Jandér 2011, K. C. Jandér and B. S. Steidinger, *unpublished manuscript*). For example, 2–5% of the wasps' time inside a receptive fig is spent actively depositing pollen—time that otherwise likely could be used to lay more eggs (Jandér 2003, Jandér and Herre 2010, Jandér et al. 2012). However, in most actively pollinated fig species studied to date, wasps that do not pollinate face fitness consequences: (1) unpollinated figs abort, which kills all developing wasp offspring within, and (2) fewer wasp offspring emerge from figs that are not aborted but mature on the tree (Herre et al. 2008, Jandér and Herre 2010, Wang et al. 2014). Together, these two types of host sanctions can reduce the fitness of pollen-free wasps to 67–0.001% of that of pollinating wasps (Jandér and Herre 2010). Although the exact mechanism behind host sanctions in figs is not yet clear, a likely explanation is that the tree allocates less resources to fruits that lack a pollination signal, do not produce seeds, and therefore are less likely to be profitable to the tree (Jandér et al. 2012, Frederickson 2013, K. C. Jandér and E. A. Herre, *in press*).

To determine whether *Ficus* host sanctions lead to not only fewer but also lower quality wasp offspring that themselves have lower fitness, we first use single foundress introduction experiments (Experiment A) on five fig species to ask whether offspring from P– (pollen-free) foundresses are smaller than offspring from P+ (pollen-carrying) foundresses. We then use a dataset of thousands of naturally field-collected foundress and emerging wasps from four different fig species to estimate (a major component of) the fitness consequences of the size reduction we found in the experiments: likelihood of reaching and entering a receptive fig. We test whether foundresses are larger than wasps that emerge. We calculate the relative probability of becoming a foundress given a particular head length. We then combine the data from Experiment A with that of the field collection to estimate the fitness consequences of the experimentally observed size reduction. Finally, we use two-foundress introduction experiments in *F. nymphaeifolia* (Experiment B) to ask whether offspring size reduction acts on the level of the entire fig (as does offspring number reduction, see Jandér et al. 2012), or whether it acts on the level of individual flowers, as suggested in Jansen-González et al. (2012). We find that pollen-free wasps have offspring that are 5–50% lighter than pollinating wasps, that this size reduction can cause an additional up to 80% reduction in fitness due to smaller wasps having a decreased chance of becoming foundresses themselves, and that the size reduction acts on the level of the entire fig. We conclude that the third component of host sanctions, reduction in offspring quality, is substantial and may be present also in other mutualistic systems where host sanctions involve reduced resource allocation.

METHODS

Study species and basic assumptions

We studied natural populations of trees and wasps near the Panama Canal, Republic of Panama. The four actively pollinated fig species (*F. citrifolia*, *F. nymphaeifolia*, *F. obtusifolia*, and *F. poponeoi*) all belong to the subsection *Urostigma Americana*, and are respectively pollinated by *Pegoscapus tonduzi*, *P. piceipes*, two cryptic species *P. hoffmeyer* A and B, and two cryptic species *P. gemellus* A and B (Wiebes 1995, Molbo et al. 2003). The passively pollinated fig species *F. maxima* (subsection *Pharmacosycea*) is pollinated by *Tetrapus americanus*. For simplicity, we will here use the fig species name as a proxy for its associated wasp species. At the study site, the proportion of wasps that end up in single foundress figs (and therefore are subjected to full-strength sanctions if they do not pollinate) are in: *F. obtusifolia* 71%, *F. citrifolia* 52%, *F. nymphaeifolia* 24%, and *F. poponeoi* 7% (Jandér and Herre 2010).

Wasp size was measured either by dry weight (experiment A), length of front femur (experiment B), or head length (field collections). These different measurements are all reliable ways to measure wasp size and highly correlated

with each other (Appendix S1: Fig. S1). Further, producing foundresses for the pollen-free treatment (opening the fig before females have emerged in order to remove pollen) does not affect foundress size (Appendix S1).

P-|P+ field experiments (experiments A and B)

The sample origin and experimental setup for single foundress introductions (experiment A: *F. maxima*, *F. popenoei*, *F. obtusifolia*, *F. nymphaeifolia*, and *F. citrifolia*) are described in Jandér and Herre (2010); those for two-foundress introductions (experiment B: *F. nymphaeifolia*) in Jandér et al. (2012; Fig. S2). Briefly, we created P- (pollen-free) female wasps by removing 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. Prereceptive figs on the experimental 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 (experiment A) or two wasps: P-P-, P-P+ or P+P+ (experiment B) (Appendix S1: Fig. S2). Experiment A tested the fitness of a single uncooperative (P-) foundress wasp compared to a pollinating (P+) wasp in five different fig species. Experiment B tested whether fitness reductions act: on the level of the entire fig fruit (whether one pollinator is sufficient to prevent sanctions or whether resource allocation increases with pollination level), on individual flowers within the fig, or on flowers and nearby surroundings (Jandér et al. 2012). Predictions for experiment B are detailed in Appendix S1: Table S1.

Some weeks later, prior to wasp offspring emergence, the experimental figs were collected and brought to the lab, and the wasps allowed to emerge in petri dishes so that they could be counted and measured, then stored in the freezer. For experiment A, 10 female offspring were blindly sampled from the aggregation of dead wasp offspring from each matured fruit, then dried at 50 °C for 48 h and each weighed to the nearest microgram using a Sartorius MC 15 microbalance. To test if P- wasp offspring were smaller than P+ wasp offspring we used the mean weight of the 10 female offspring from each fig as an independent sample in the *t*-tests (Appendix S1: Table S2). Samples were not obtained from all experimental trees included in Jandér and Herre (2010) because some samples were lost due to a faulty freezer. For each experimental tree in Experiment A we then calculated:

S_{P-} = the mean dry weight (Size) for female pollinator wasps emerging from P- figs

S_{P+} = the mean dry weight for female pollinator wasps emerging from P+ figs

S_R = S_{P-}/S_{P+} = the relative dry weight of female pollinator wasps from P- figs compared to P+ figs. Each tree produced a single value of S_R , and those were compared across species using an ANOVA. *F. maxima* and *F. citrifolia* were not included in the ANOVA due to only having data from one tree each.

For experiment B, the length of the front femur was measured to the nearest 0.01 mm in each of 10 blindly sampled female wasp offspring from each P-P- and P+P+ fig, and 20 from each P-P+ fig, using a dissecting scope with a reticle. The female wasp offspring from the P-P+ figs were then identified to maternal lineage using molecular methods (see Jandér et al. 2012). The mean front femur length of the female offspring from each fig (or from each maternal lineage in each P-P+ fig) was used as an independent sample in the *t*-tests when comparing across different fig types. Within P-P+ figs the mean femur lengths from the P- and P+ lineages in each fig was used as paired samples in the paired *t*-tests.

Size distributions in natural populations

To determine whether relatively larger wasps are more likely to become foundresses, we collected nearly mature figs of *F. citrifolia*, *F. nymphaeifolia*, *F. obtusifolia*, and *F. popenoei* (where males had emerged but females were still in their galls) from multiple crops (Appendix S1: Table S3). In these species, the foundresses' dead bodies usually remain inside the developing fig, so the number of foundresses can be counted and their bodies measured (Herre 1989, 1993). In figs with only a single foundress, the foundress was set aside, and the female wasp offspring were allowed to emerge and then were killed by freezing. We measured the head length of the foundress and five female offspring per fig to the nearest 0.01 mm using a dissecting scope, using the mean of three measurements for each individual wasp. In total, we measured over 9,000 wasps.

We then calculated the ratio (foundress head length)/(mean female offspring head length) for each fig. We compared the resulting ratios to 1 (no difference) with a one-sided *t*-test for each species. We here assume that the emerging offspring are representative of the overall emerging wasp population. This seems reasonable, because samples were collected from many trees over a span of 14 yrs (Appendix S1: Table S3).

Linking the size of female offspring to fitness

To calculate the probability of being a foundress given a certain head length we used Bayes' theorem: $P(F|HL) = (P(HL|F) \times P(F))/P(HL)$ and the field-collected data on wasp head length to link offspring wasp size to fitness (likelihood to survive to reproduction). Here, $P(HL)$ is the probability distribution of head length for emerging wasps, $P(HL|F)$ is the probability distribution of head length for wasps that become foundresses, and $P(F)$ is the baseline probability of becoming a foundress for each species. $P(F)$ was calculated from a different dataset from the same populations (Herre 1989) based on figs with naturally occurring foundress numbers for each fig species. We calculated the proportion of emerging wasps that become foundresses by using the equation: $P(F) = (\text{mean foundress number})/(\text{mean number of emerging female pollinator wasps per unmanipulated fig})$. Combining this

equation with the data from Herre (1989), $P(F)$ is for *F. citrifolia*: 0.81%, *F. nymphaeifolia*: 1.22%, *F. obtusifolia* 0.32%, and for *F. popenoei*: 1.09%. We estimated the probability densities $P(HL|F)$ and $P(HL)$ using the kernel density estimator “density” in the R base package, (R Core Team 2015) then calculated $P(F|HL)$. We produced confidence intervals using a nonparametric bootstrap: We repeatedly resampled from the data to produce bootstrapped samples, then reestimated the densities and $P(F|HL)$ for each bootstrapped sample. We plotted the lower 2.5% and upper 97.5% quantile of the estimates of $P(F|HL)$ for each value of head length. For more details, see Appendix S1.

We used the relationship between head length and dry weight to translate the weight reduction documented in P– wasps in experiment A to the equivalent reduction in head length (linear regression: $\log \text{head length [mm]} = 0.40 (\log \text{dry weight [mg]}) + 0.093$; $r^2 = 0.93$, $t = 42.43$, $P = 3.5E-79$; Appendix S1). Using the calculated probability of becoming a foundress given a certain head length, $P(F|HL)$, we determined for each experimental tree how the size reduction of P– offspring would affect their probability of becoming foundresses. For the entire distribution of wasp sizes, we calculated their probability of becoming foundresses when emerging from pollinated figs (F_{P+}), the probability of becoming foundresses when reduced in size due to developing in an unpollinated fig (F_{P-}), and the average relative probability to become a foundress for a P– relative a P+ offspring (F_R), see Appendix S1 for details.

Incorporating size reduction into previous sanction strength measures

We then incorporated this new component of host sanctions, offspring size reduction, into the previously published measures of sanction strength (Jandér and Herre 2010). We calculated a size-included fitness measure of P– foundresses compared to P+ foundresses (W_{SR}) by multiplying F_R with the previously published relative fitness of a P– foundress compared to a P+ foundress (W_R , based on only fig abortions and the reduction of wasp offspring numbers [Jandér and Herre 2010]) for each tree: $W_{SR} = F_R \times W_R$. Each tree produced a single value of W_{SR} , and those were compared across species using a Welch test robust to unequal variances. Statistical analyses were done in SPSS 21 and R version 3.2.2; all tests were two-tailed unless otherwise stated.

RESULTS

Experiment A: documenting reduced offspring size

The experiments with a single P– or P+ foundress in each fig tested whether P– offspring were smaller than P+ offspring. For all experiments on the actively pollinated fig species *F. citrifolia*, *F. nymphaeifolia*, and *F. obtusifolia*,

offspring of P– foundresses were significantly smaller than offspring of P+ foundresses, weighing 50–80% of P+ offspring (Fig. 1; Appendix S1: Table S2). In the actively pollinated *F. popenoei*, P– offspring were slightly lighter but not significantly so (Fig. 1; Appendix S1: Table S2). The relative dry weight of female offspring from P– figs compared to P+ figs, S_R , varied significantly across species (ANOVA, $F_{2,3} = 10.15$, $P = 0.046$; different letters in Fig. 1 represent significantly different subsets [planned contrasts, $P < 0.05$]). In the passively pollinated *F. maxima*, P– offspring were slightly lighter (90% the weight of P+ offspring; Fig. 1; Appendix S1: Table S2), suggesting fitness reductions for P– wasps not detected by previous estimates based only on abortions and reduction of offspring numbers (Jandér and Herre 2010).

Calculating fitness consequences of reduced offspring size

We then compared the size of field-collected emerging and foundress wasps associated with four fig species. In all species, the head length of foundresses was consistently larger than that of their offspring, demonstrating that larger wasps are more likely to become foundresses (one-sample *t*-tests to a ratio of one: *F. citrifolia* $t_{110} = 4.36$, $P = 2.9E-5$; *F. nymphaeifolia* $t_{80} = 5.30$, $P = 1E-6$; *F. obtusifolia* $t_{1173} = 8.90$, $P < 1.0E-6$; *F. popenoei* $t_{178} = 2.96$, $P = 0.003$; Fig. 2).

Using Bayes’ Theorem and the distribution of head lengths of foundresses and emerging wasps for each species (Appendix S1: Fig. S3) we calculated the probability of becoming a foundress given a wasp’s head length. Larger wasp individuals were more likely to be foundresses in all

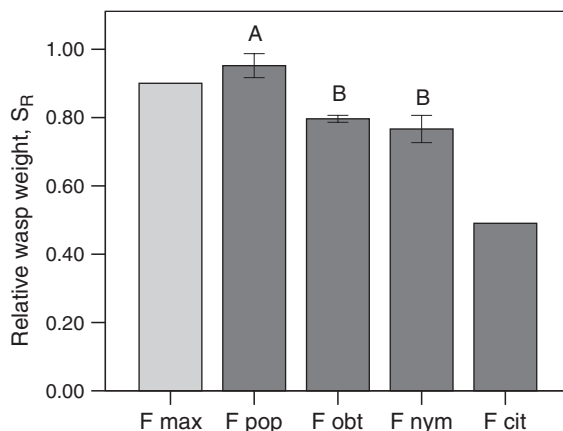


FIG. 1. Wasp offspring that developed in unpollinated figs were significantly lighter than wasps from pollinated figs in all species except *F. popenoei*. The relative dry weight of female pollinator offspring from P– figs compared to P+ figs, S_R , varied significantly across species; letters represent significantly different subsets. Error bars represent 1 SEM for species where experiments are present from more than one tree; source data in Appendix S1: Table S2. Abbreviations in this and the following figures: max = *F. maxima*; pop = *F. popenoei*; obt = *F. obtusifolia*; nym = *F. nymphaeifolia*; cit = *F. citrifolia*.

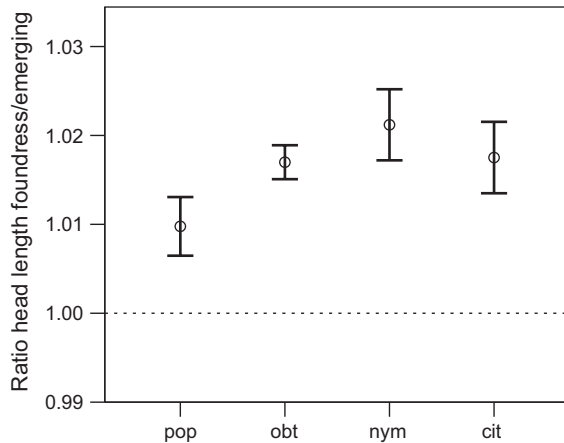


FIG. 2. The head length of the foundress was on average larger than that of her offspring (ratio = [head length of the foundress]/[head length of the emerging offspring]) in pollinator wasps associated with all four studied fig species: *F. popenoei*; *F. obtusifolia*; *F. nymphaeifolia*; *F. citrifolia*. Error bars represent 1 SEM; details on sample sizes in Appendix S1; Table S3. This suggests that there is a dispersal advantage for larger wasp offspring.

wasp species (Fig. 3). The experimentally determined size reduction of P− offspring significantly reduced their probability of becoming foundresses: *F. citrifolia*: F_R (the mean probability of being a foundress for offspring emerging from unpollinated relative those from pollinated figs) = 0.750, $P = 0.002$; *F. nymphaeifolia* tree BN67: $F_R = 0.129$, $P = 2E-5$; *F. nymphaeifolia* tree BS1: $F_R = 0.268$, $P = 1E-5$; *F. obtusifolia* tree BN64: $F_R = 0.636$, $P < 1E-5$; *F. obtusifolia* tree MIL: $F_R = 0.668$, $P < 1E-5$; *F. popenoei* tree BV11: $F_R = 0.980$, $P = 0.0021$; *F. popenoei* tree JG1: $F_R = 0.898$, $P = 0.013$ (Fig. 4a). Therefore, the reduction in offspring size caused by P− foundresses not pollinating a fig reduced the fitness of their offspring to an average of 20% (*F. nymphaeifolia*) to 94% (*F. popenoei*) of that of pollinating foundresses' offspring (Fig. 4a).

As with previously determined host sanction components in these fig species, the strength of the fitness reduction caused by size reduction (measured as F_R) varied across species (ANOVA, $F_{2,5} = 61.42$, $P = 0.004$; sequential Bonferroni corrected Tukey's HSD; Fig. 4a). If this source of fitness reduction is combined with previously documented sanction components causing fitness reduction for P− foundresses (fig abortions and reductions of offspring numbers combined into the relative fitness W_R ; [Jandér and Herre 2010]), the relative fitness of a P− foundress compared to a P+ foundress is reduced even further (W_{SR} ; Fig. 4b). Size-included relative fitness (W_{SR}) varied across species in the same order as the fitness measure in the previous study that did not include size reduction (W_R ; Jandér and Herre 2010; Fig. 4b). Size-included relative fitness (W_{SR}) differed significantly across species (Robust Welch, ANOVA, $df = 3$, 1.67, test statistic = 114.10, $P = 0.017$; Fig. 4b), but pairwise Dunnett's T3 failed to find significantly different subsets after sequential Bonferroni corrections.

Experiment B: precision of size reduction

The experiments with two foundresses, P−P−, P−P+, or P+P+, in each fig addressed whether the size reduction acted on the fig level or flower level. In both experimental trees of *F. nymphaeifolia*, P−P− offspring were smaller than P+P+ offspring, although only significantly so in the tree with the larger sample size (Tree BS#1, t -test unequal variances, $t_{23,2} = -6.29$, $P = 2.0E-6$; Tree BCI#1, t -test, $t_5 = -1.93$, $P = 0.11$; Fig. 5). However, within the P−P+ figs, P− offspring were not smaller than P+ offspring (Tree BS#1, paired t -test, $t_{19} = -0.73$, $P = 0.48$; Tree BCI#1, paired t -test, $t_3 = 0.72$, $P = 0.53$; Fig. 5), a result inconsistent with flower level sanctions. P− offspring were larger in the P−P+ figs than in the P−P− figs, but this was only significant in the tree with the larger sample size (t -tests, tree BS#1: $t_{36} = -6.91$, $P < 1E-6$; tree BCI#1: $t_5 = -1.79$, $P = 0.13$; Fig. 5). This suggests that P− offspring can benefit from the pollination by the P+ foundress with which they share fig. These findings are consistent with the fig-level sanctions detected previously in this species when focusing on offspring number (Jandér et al. 2012). P+ offspring did not differ in size between P−P+ and P+P+ figs on the tree with the smaller sample size (tree BCI#1, t -test $t_5 = 0.38$, $P = 0.72$; Fig. 5), but were slightly larger in P−P+ figs than in P+P+ figs on the tree with larger sample size (tree BS#1, t -test $t_{34} = 2.13$, $P = 0.04$; Fig. 5). Therefore, for wasp offspring size, one pollinator per fig is sufficient to mitigate any sanctions.

DISCUSSION

We document a third component of host sanctions, reduction in offspring quality (here measured as size), and show that it can be substantial in the fig tree—fig wasp mutualism. We establish that in unpollinated single-foundress figs that do not abort, not only are offspring fewer but also smaller, and smaller wasps are less likely to reproduce: (1) Pollen free (P−) foundresses have offspring that are only 50–90% the size of offspring of pollinating foundresses. (2) Smaller wasps are less likely to reach and enter a flowering fig to become foundresses themselves; the experimentally determined size reduction of offspring is estimated to cause an up to 80% reduction in fitness for a P− foundress. (3) The size reduction of P− offspring acts on the level of the entire fig fruit, mirroring the effect on P− offspring numbers (Jandér et al. 2012), and further supporting the hypothesis that host sanctions in these fig species are based on fig-level selective resource allocation (Jandér et al. 2012, K. C. Jandér and E. A. Herre, *in press*). This study shows that both quantity and quality of offspring of uncooperative symbionts can be affected by host sanctions.

We found that foundresses that do not pollinate have smaller offspring. Almost all previous experimental studies of fig sanctions counted offspring of P− wasps but did not measure their size (Jousselin and Kjellberg 2001, Tarachai et al. 2008, Jandér and Herre 2010, Jandér et al.

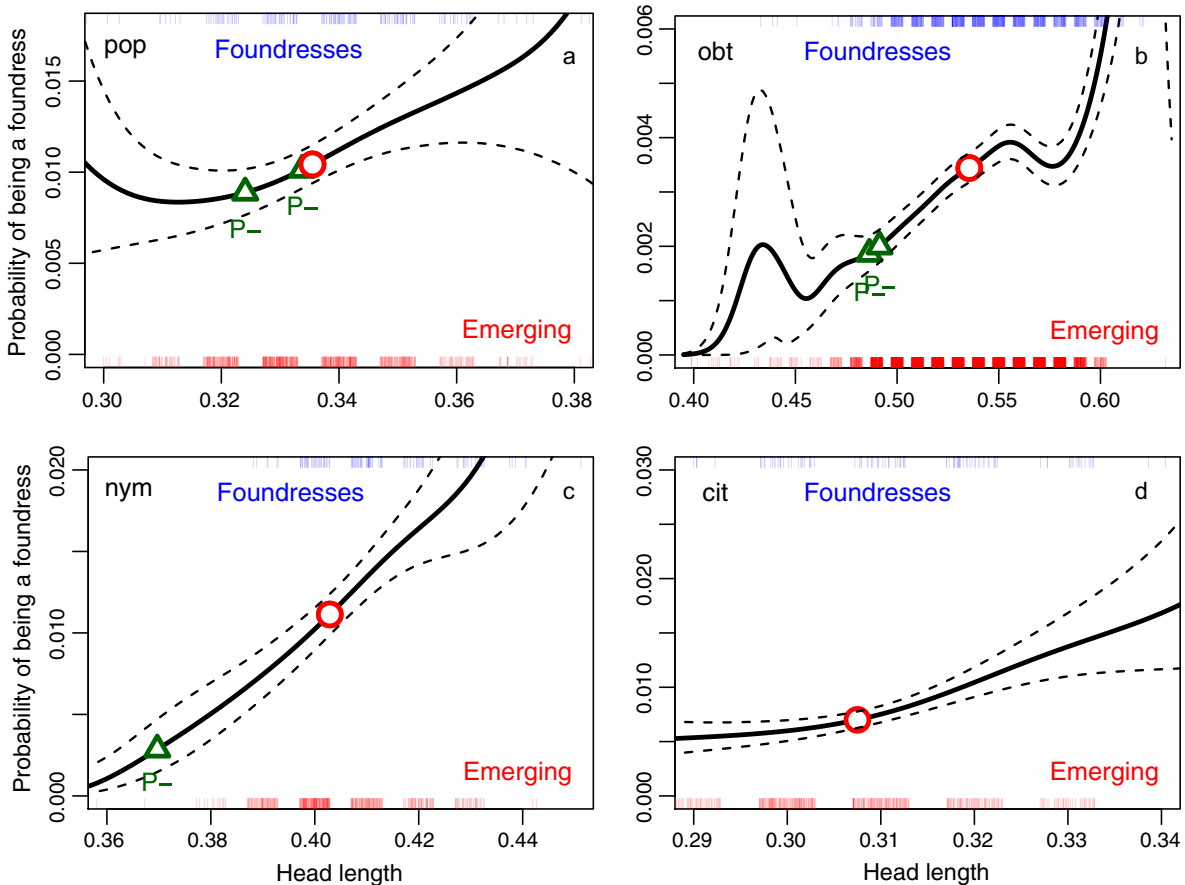


FIG. 3. The probability of being a foundress increases with head length. The four panels show data for pollinator wasps associated with four different fig species: (a) *F. popenoei*, (b) *F. obtusifolia*, (c) *F. nympheifolia*, (d) *F. citrifolia*. Each small vertical line in red (emerging wasps) or blue (foundresses) represents a data point in the field-collected dataset. The dotted lines represent 95% confidence intervals. When the distribution of head lengths of field-collected emerging wasps (mean indicated by the circle) is reduced to that of P⁻ offspring (mean indicated by the triangles; each triangle denotes the estimate from an independent experiment), the probability of being a foundress is reduced in all species. Two experiments produced a mean for P⁻ offspring that fell outside the lower range of the graphs (*F. nympheifolia*: 0.355 mm; *F. citrifolia*: 0.231 mm).

2012, Wang et al. 2014, 2015). The only previous study where the size of P⁻ offspring was compared to P⁺ offspring is Jousselin et al.'s study of *F. microcarpa*, where no significant size difference was found (tibia length P⁺: 0.134 mm, P⁻: 0.132 mm, $F_{1,112} = 0.34$, $P = 0.56$) despite a 50% reduction in offspring numbers (Jousselin et al. 2003). In contrast, here we found a 5–51% reduction in P⁻ offspring dry weight corresponding to a 2–25% reduction in head length in P⁻ offspring in five different fig species. For the four fig species where we have field data on size distributions of foundresses and emerging wasps, the size reduction alone translates to a fitness reduction of up to 80% for foundresses that do not pollinate compared to those that do.

We found that larger fig wasp individuals are more likely to become foundresses. This corroborates an earlier finding where foundresses from 22 figs of *F. obtusifolia* on average were larger than their offspring (Herre 1989). In these species, female fig wasps must disperse to a different

tree in order to become a foundress and reproduce; only 0.3–1.2% of emerging wasps become foundresses (this study; Herre 1989). Our finding suggests that larger individuals are more successful at dispersing. Although fig wasps are typically thought of as aerial plankton where size should not necessarily influence distance traveled (Gislén 1948), larger body size might both protect against desiccation, which is known to shorten wasp lifespan (Dunn et al. 2008), and be beneficial during the last part of dispersal when the wasp drops out of the air stream and actively flies upwind to locate a flowering tree (Ware and Compton 1994a, b), because larger insects can fly farther (Tscharntke and Brandl 2004). In addition, larger wasps might be more likely to escape ant predation as they emerge from their natal fig (Schatz and Hossaert-McKey 2003, Jandér 2015), and are likely at an advantage in female–female aggressive fights prior to entering a receptive fig (Moore and Greef 2003, Zhao et al. 2014, Dunn et al. 2015). Although a large body size seems to

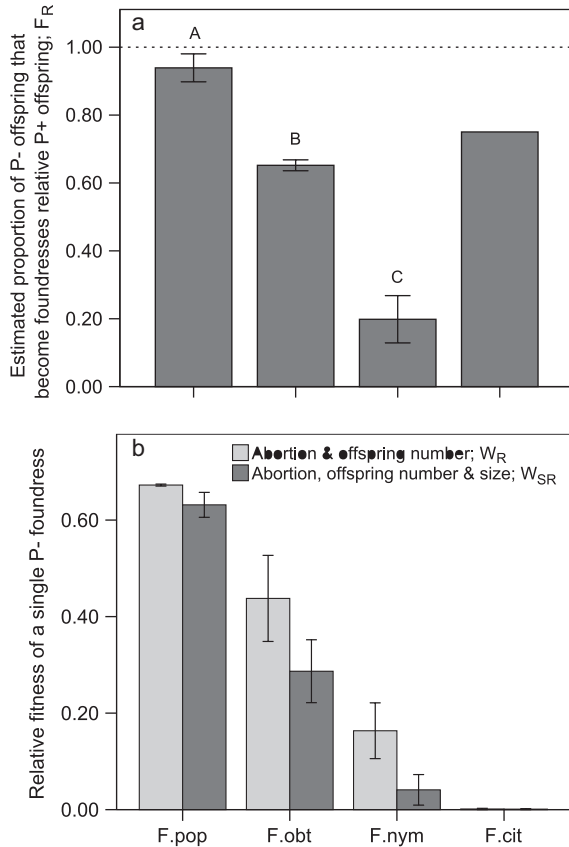


FIG. 4. (a) Wasp offspring from unpollinated figs on average had lower estimated probability of becoming foundresses than offspring from pollinated figs. The estimated proportion of P- offspring compared to P+ offspring that become foundresses, F_R , varied significantly across species. (b) The relative fitness of a single P- foundress compared to a P+ foundress is further reduced when the fitness effect of size reduction is taken into account (W_{SR}). Values of W_R , which only include risk of abortion and reduction of offspring numbers, are from Jandér and Herre (2010). Sample sizes are for (a) two experimental trees for each species except *citrifolia* (one tree), for (b) two experimental trees for each species except *citrifolia* (4) and *nymphaeifolia* W_R (3). Error bars represent 1 SEM.

improve chances of successful dispersal, if taken to the extreme, it could be a hindrance, because fig wasps have to squeeze through a narrow opening (the ostiole) to reach the flowers inside the fig where they lay their eggs. In fact, in Chinese *F. hispida* the pollinator wasps that arrived at receptive trees were larger than those wasps that successfully made it into the figs and became foundresses (Liu et al. 2011). In *F. hispida* over 50% of aspiring foundresses became trapped in the ostioles; the trapped wasps were on average larger than both the wasps that arrived at trees and those that successfully entered the figs (Liu et al. 2011, 2013). In our study species in Panama, the proportion of wasps that become trapped in the ostioles is much lower; we expect that because the monoecious strangler figs studied here grow in much lower densities than the dioecious *F. hispida* (Nason et al. 1998) the

limiting step for becoming a foundress in the species studied here is indeed wasp dispersal.

There are likely also other fitness benefits of having a large body size that take effect once the wasp has entered the fig, and therefore are not investigated here. A previous study of *F. obtusifolia* showed that within single foundress figs, larger foundresses produced more offspring (Herre 1989). This could be due to some combination of larger females (1) living longer and thus laying more eggs; (2) more easily ovipositing in a larger number of flowers; (3) depositing a larger amount of fluid that aids in galling flowers (Martinson et al. 2014); or simply (4) carrying more eggs. On average, larger wasp species carry more eggs than smaller wasp species (Herre 1989), and this is likely also true within species (Compton et al. 1991, Honek 1993). Additionally, larger females are often more successful in female–female fights within the fig, which can have dramatic fitness consequences (Moore and Greef 2003, Dunn et al. 2015). Therefore, the fitness benefits of having large offspring are likely to be even larger than that estimated here.

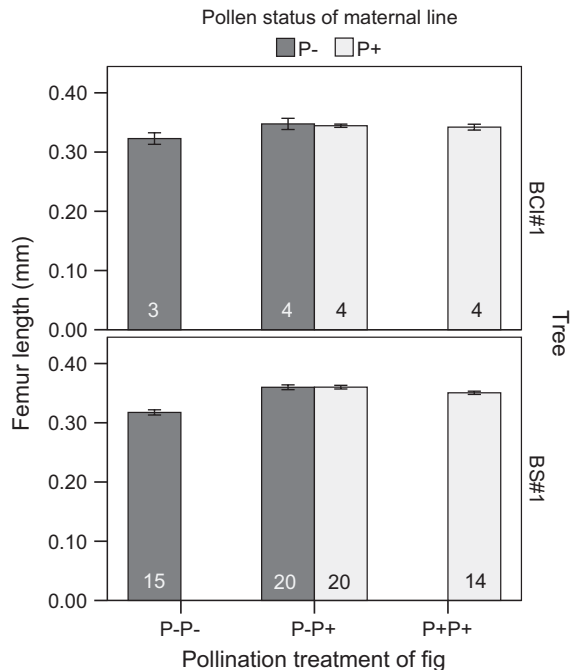


FIG. 5. Wasp offspring size measurements across three pollination treatments on two trees (BCI#1 and BS#1) of *F. nymphaeifolia*. Wasp offspring from figs in which neither foundress carried pollen (P-P-; completely unpollinated figs) were smaller than wasp offspring from figs in which both foundresses carried pollen (P+P+; completely pollinated figs). Within figs where one foundress carried pollen and one did not (P-P+; partly pollinated figs), the offspring of P- foundresses were not smaller than P+ offspring, a result inconsistent with flower-level sanctions. P- offspring were larger in P-P+ figs, where they can free-ride on the pollination of the P+ foundress, than in P-P- figs. The mean femur length from wasps emerging from each fig was used in the analyses; number of figs are indicated inside the bars. Error bars represent 1 SEM.

We suggest that the mechanism for the size reduction of P− offspring in this study is based on differential allocation of host resources to pollinated figs. Indeed, such differential resource allocation could explain all three components of host sanctions in figs: abortion of unpollinated figs, and reduced number and size of wasp offspring (K. C. Jandér and E. A. Herre, *in press*). Such differential resource allocation would result in fewer fig-derived resources available to wasps developing in unpollinated figs, some combination of fewer total wasps supported in galls and/or less resources per gall, and thus likely less nutritious plant material for developing wasp larvae to eat. A less nutritious diet during development is known to result in smaller adult insects (Davidowitz et al. 2004). Our results from experiment B indicate that in the species studied, *F. nymphaeifolia*, increased resources to pollinated figs are allocated on the level of the entire fig rather than to individual pollinated or unpollinated flowers. This finding is not consistent with the suggestion by Jansen-González et al. (2012) that *Pegoscapus* pollinators require fertilized endosperm (a product of pollination) for proper development. Under such a diet requirement, we would expect to see a flower-level effect on size, with P− offspring in P−P+ figs being smaller than P+ offspring, but that was not the case. Although it is theoretically possible that some P− flowers in the P−P+ figs were pollinated by laterally growing pollen tubes (Jousselin and Kjellberg 2001) or imprecise pollination, we do not think this explains our finding. Unless 100% of flowers are pollinated, some P− offspring would develop in unpollinated flowers in the P−P+ figs. If the effect is on the flower level, the wasps developing in unpollinated flowers would be smaller than P+ offspring and therefore lower the mean for P− offspring, but we do not see even a tendency of that. Therefore, as for offspring numbers (Jandér et al. 2012), in *F. nymphaeifolia* the reduction of offspring size seems to act predominantly on the fig level rather than the flower level, and having one pollinator in the fig seems sufficient to prevent it. Wasps that do not carry pollen can therefore avoid also this component of host sanctions by sharing a fig with other, pollinating, foundresses.

If wasps that develop in unpollinated figs have access to less nutritious food during development, we might expect that not only pollen-free individuals of normally pollinating species but also species that do not pollinate would be affected. There is some indication that “cheater species” of fig wasps (species that originate from pollinator lineages but no longer actively pollinate) may have reduced fitness compared to the pollinating species of the same host. On the African host species *Ficus sycomorus*, the cheater wasp species *Ceratosolen galili* is smaller than the pollinator *Ceratosolen arabicus* (Compton et al. 1991). If sanctions act on the fig level also in *F. sycomorus*, we would expect *C. galili* offspring that share a fig with pollinators (and seeds) to be larger than those that only share with other *C. galili* and

therefore develop in an unpollinated fig. The other known cheater species, *Eupristina* sp. of *Ficus altissima*, is not smaller but contains fewer eggs than the pollinator species *Eupristina altissima* (Peng et al. 2008, Zhao et al. 2014). In contrast, the parasitic wasp species (unrelated to pollinators) that oviposit from the outside (and never pollinate) often have a different galling mechanism and can sometimes induce very large galls (West et al. 1996, Elias 2012, Conchou et al. 2014). Some of these parasitic wasps seem to be able to pull sufficient resources into the fig and also prevent abortion, irrespective of the fig’s pollination status.

Because only a small proportion of emerging pollinator wasps succeed in becoming foundresses and thus reproduce, any behavior that increases the offspring’s chances of becoming foundresses will have an enormous effect on their mother’s fitness. Here we have shown that by pollinating, foundresses can increase their female offspring’s size and thereby their chances of becoming foundresses by up to 500%. Another way that foundresses might increase the size of their offspring is to lay their eggs in the flowers closest to the fig lumen (Anstett 2001). Where it has been studied, wasp galls developed from inner flowers are on average 20% larger than those developing from outer flowers, hence wasp offspring developing in these inner flowers are likely to be larger than wasps developing in outer ovules (Anstett 2001, see Martinson et al. 2014). The mechanism of this is not known but might be a result of inner flowers being chemically more suited to wasp development than outer flowers (West and Herre 1994, Martinson et al. 2015). In addition, wasps developing in inner flowers are more likely to be mated, and more likely to successfully emerge from the fig (Anstett 2001, Dunn et al. 2008). A by-product of foundresses preferentially laying eggs in inner flower is that this may prevent over-exploitation of the outer fig flowers that normally develop into seeds (Yu et al. 2004). Therefore, by striving to have as large offspring as possible, foundresses are encouraged both to pollinate and to selectively lay their eggs in the inner flowers, both of which promote stability of this mutualism.

This third component of host sanctions—offspring quality—is likely to be present also in other mutualistic systems. Host sanctions are thought to be based on selective resource allocation by the host to more cooperative symbionts (or modules associated with such symbionts) in as diverse mutualisms as legumes and rhizobia, plants and mycorrhizal fungi, and plants and their nursery pollinators (*Yucca*—*yucca* moth; fig tree—fig wasp). In such intimate mutualisms, reduced resources to developing symbiont offspring are likely to affect not only the number of offspring but also their quality. Offspring quality might be manifested in different ways, such as body size, the number of eggs female offspring carry, or the amount of stored resources such as fat. For example, rhizobia can store energy in the lipid PHB (Poly-3-hydroxybutyrate), and this helps fuel their

survival and reproduction (Ratcliff et al. 2008). Rhizobia in pea-plant nodules that are experimentally forced to not fix nitrogen contain less PHB than rhizobia in nodules that are allowed to fix nitrogen (Oono et al. 2011). Therefore, not only the number of rhizobia but also their quality is affected by pea host sanctions (Oono et al. 2011). Size or quality reduction in the offspring of uncooperative symbionts are likely to be present also in other mutualisms that have host sanctions based on selective resource allocation.

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LITERATURE CITED

- Anstett, M. C. 2001. Unbeatable strategy, constraints and coevolution, or how to resolve evolutionary conflicts: the case of the fig/wasp mutualism. *Oikos* 95:476–484.
- Bäckhed, F., E. L. Ruth, J. L. Sonnenburg, D. A. Peterson, and J. I. Gordon. 2005. Host-bacterial mutualism in the human intestine. *Science* 307:1915–1920.
- Bever, J. D., S. C. Richardson, B. M. Lawrence, J. Holmes, and M. Watson. 2009. Preferential allocation to beneficial symbiont with spatial structure maintains mycorrhizal mutualism. *Ecology Letters* 12:13–21.
- Compton, S. G., K. C. Holton, S. Rashbrook, S. L. van Noort, S. L. Vincent, and A. B. Ware. 1991. Studies of *Ceratostenes galili*, a non-pollinating agaonid fig wasp. *Biotropica* 23:188–194.
- Conchou, L., M. Ciminera, M. Hossaert-McKey, and F. Kjellberg. 2014. The non-pollinating fig wasps associated with *Ficus guianensis*: community structure and impact of the large species on the fig/pollinator mutualism. *Acta Oecologica* 57:28–37.
- Cruaud, A., et al. 2012. An extreme case of plant–insect codiversification: figs and fig-pollinating wasps. *Systematic Biology* 61:1029–1047.
- Davidowitz, G., L. J. D’Amico, and H. F. Nijhout. 2004. The effects of environmental variation on a mechanism that controls insect body size. *Evolutionary Ecology Research* 6:49–62.
- Denison, R. F., and E. T. Kiers. 2004. Lifestyle alternatives for rhizobia: mutualism, parasitism and foregoing symbiosis. *FEMS Microbiology Letters* 237:187–193.
- Douglas, A. E. 2010. *The symbiotic habit*. Princeton University Press, Princeton, New Jersey, USA.
- Dunn, D. W., D. W. Yu, J. Ridley, and J. M. Cook. 2008. Longevity, early emergence and body size in a pollinating fig wasp—implications for stability in a fig-pollinator mutualism. *Journal of Animal Ecology* 77:927–935.
- Dunn, D. W., K. C. Jandér, A. G. Lamas, and R. A. S. Pereira. 2015. Mortal combat and competition for oviposition sites in female pollinating fig wasps. *Behavioral Ecology* 26:262–268.
- Elias, L. G. 2012. Diversification in the use of resources by *Idarnes* species: bypassing functional constraints in the fig–fig wasp interaction. *Biological Journal of the Linnean Society* 106:114–122.
- Frank, S. A. 1984. The behaviour and morphology of the fig wasps *Pegoscapus assuetus* and *P. jimenezii*: descriptions and suggested behavioural characters for phylogenetic studies. *Psyche* 91:289–308.
- Frederickson, M. E. 2013. Rethinking mutualism stability: cheaters and the evolution of sanctions. *Quarterly Review of Biology* 88:269–295.
- Gislén, T. 1948. Aerial plankton and its condition of life. *Biological Reviews* 23:109–126.
- Herre, E. A. 1989. Coevolution of reproductive characteristics in 12 species of New World figs and their pollinator wasps. *Experientia* 45:637–647.
- Herre, E. A. 1993. Population structure and the evolution of virulence in nematode parasites of fig wasps. *Science* 259:1442–1445.
- Herre, E. A., N. Knowlton, U. G. Mueller, and S. A. Rehner. 1999. The evolution of mutualisms: exploring the paths between conflict and cooperation. *Trends in Ecology and Evolution* 14:49–53.
- Herre, E. A., K. C. Jandér, and C. A. Machado. 2008. Evolutionary ecology of figs and their associates: ongoing progress and outstanding puzzles. *Annual Review of Ecology and Systematics* 39:439–458.
- Honek, A. 1993. Intraspecific variation in body size and fecundity in insects: a general relationship. *Oikos* 66:483–492.
- Jandér, K. C. 2003. Fig wasp behaviour and stability of the fig-fig wasp mutualism. Uppsala University, Uppsala, Sweden.
- Jandér, K. C. 2011. Plant sanctions and pollinator behaviour in the fig tree—fig wasp mutualism. Dissertation. Cornell University, Ithaca, NY, USA.
- Jandér, K. C. 2015. Indirect mutualism: ants protect fig seeds and pollen dispersers from parasites. *Ecological Entomology* 40:500–510.
- Jandér, K. C., and E. A. Herre. 2010. Host sanctions and pollinator cheating in the fig tree—fig wasp mutualism. *Proceedings of the Royal Society of London. Series B, Biological Sciences* 277:1481–1488.
- Jandér, K. C., E. A. Herre, and E. L. Simms. 2012. Precision of host sanctions in the fig tree—fig wasp mutualism: consequences for uncooperative symbionts. *Ecology Letters* 15:1362–1369.
- Jandér, K. C., and E. A. Herre. 2016. Host sanctions in Panamanian *Ficus* are likely based on selective resource allocation. *American Journal of Botany* *In press*.
- Jansen-González, S., S. P. Teixeira, and A. S. Pereira. 2012. Mutualism from the inside: coordinated development of plant and insect in an active pollinating fig wasp. *Arthropod-Plant Interactions* 6:601–609.
- Jousselin, E., and F. Kjellberg. 2001. The functional implications of active and passive pollination in dioecious figs. *Ecology Letters* 4:151–158.
- Jousselin, E., M. Hossaert-McKey, E. A. Herre, and F. Kjellberg. 2003a. Why do fig wasps actively pollinate monoecious figs? *Oecologia* 134:381–387.
- Kiers, E. T., R. A. Rousseau, S. A. West, and R. F. Denison. 2003. Host sanctions and the legume–rhizobium mutualism. *Nature* 425:78–81.
- Kiers, E. T., et al. 2011. Reciprocal rewards stabilize cooperation in the mycorrhizal symbiosis. *Science* 333:880–882.
- Kjellberg, F., E. Jousselin, J. L. Bronstein, A. Patel, J. Yokoyama, and J. Y. Rasplus. 2001. Pollination mode in fig wasps: the predictive power of correlated traits. *Proceedings of the Royal Society of London. Series B, Biological Sciences* 268:1113–1121.
- Leigh, E. G. 2010. The evolution of mutualism. *Journal of Evolutionary Biology* 23:2507–2528.
- Liu, C., D. R. Yang, and Y. Q. Peng. 2011. Body size in a pollinating fig wasp and implications for stability in a

- fig–pollinator mutualism. *Entomologia Experimentalis et Applicata* 138:249–255.
- Liu, C., D. R. Yang, S. G. Compton, and Y. Q. Peng. 2013. Larger fig wasps are more careful about which figs to enter—with good reason. *PLoS ONE* 8:e74117.
- Martinson, E. O., K. C. Jandér, Y. Q. Peng, H. H. Chen, C. A. Machado, A. E. Arnold, and E. A. Herre. 2014. Relative investment in egg load and poison sac in fig wasps: implications for physiological mechanisms underlying seed and wasp production in figs. *Acta Oecologica* 57:58–56.
- Martinson, E. O., J. D. Hackett, C. A. Machado, and A. E. Arnold. 2015. Metatranscriptome analysis of fig flowers provides insight into potential mechanisms for mutualism stability and gall induction. *PLoS ONE*. <http://dx.doi.org/10.1371/journal.pone.0130745>
- Molbo, D., C. A. Machado, J. G. Sevenster, L. Keller, and E. A. Herre. 2003. Cryptic species of fig pollinating wasps: implications for sex allocation, precision of adaptation, and the evolution of the fig–wasp mutualism. *Proceedings of the National Academy of Sciences of the United States of America* 100:5867–5872.
- Moore, J. C., and J. M. Greef. 2003. Resource defence in female pollinating fig wasps: two's a contest, three's a crowd. *Animal Behaviour* 66:1101–1107.
- Nason, J. D., E. A. Herre, and J. L. Hamrick. 1998. The breeding structure of a tropical keystone plant resource. *Nature* 391:685–687.
- Ollerton, J., R. Winfree, and S. Tarrant. 2011. How many flowering plants are pollinated by animals? *Oikos* 120:321–326.
- Oono, R., C. G. Anderson, and R. F. Denison. 2011. Failure to fix nitrogen by non-reproductive symbiotic rhizobia triggers host sanctions that reduce fitness of their reproductive clonemates. *Proceedings of the Royal Society of London. Series B, Biological Sciences* 278:2698–2703.
- Pellmyr, O., and C. J. Huth. 1994. Evolutionary stability of mutualism between yuccas and yucca moths. *Nature* 372:257–260.
- Peng, Y. Q., Z. B. Duan, D. R. Yang, and J. Y. Rasplus. 2008. Co-occurrence of two *Eupristina* species on *Ficus altissima* in Xishuangbanna, SW China. *Symbiosis* 45:9–14.
- R Core Team. 2015. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.r-project.org/>
- Ramirez, W. B. 1969. Fig wasps: mechanisms of pollen transfer. *Science* 163:580–581.
- Ratcliff, W. C., S. V. Kadam, and R. F. Denison. 2008. Poly-3-hydroxybutyrate (PHB) supports survival and reproduction in starving rhizobia. *FEMS Microbiology Ecology* 65:391–399.
- Sachs, J. L., U. G. Mueller, T. P. Wilcox, and J. J. Bull. 2004. The evolution of cooperation. *Quarterly Review of Biology* 79:135–160.
- Schatz, B., and M. Hossaert-McKey. 2003. Interactions of the ant *Crematogaster scutellaris* with the fig/fig wasp mutualism. *Ecological Entomology* 28:359–368.
- Simms, E. L., D. L. Taylor, J. Povich, R. P. Shefferson, J. L. Sachs, M. Urbina, and Y. Tausczik. 2006. An empirical test of partner choice mechanisms in a wild legume–rhizobium interaction. *Proceedings of the Royal Society of London. Series B, Biological Sciences* 273:77–81.
- Tarachai, Y., S. G. Compton, and C. Trisornthi. 2008. The benefits of pollination for a fig wasp. *Symbiosis* 45:29–32.
- Tscharntke, T., and R. Brandl. 2004. Plant–insect interactions in fragmented landscapes. *Annual Review of Entomology* 49:405–430.
- Wang, R. W., D. W. Dunn, and B. F. Sun. 2014. Discriminative host sanctions in a fig–wasp mutualism. *Ecology* 95:1384–1393.
- Wang, R. W., B. F. Sun, and Y. Yang. 2015. Discriminative host sanction together with relatedness promote the cooperation in fig/fig wasp mutualism. *Journal of Animal Ecology* 84:1133–1139.
- Ware, A. B., and S. G. Compton. 1994a. Dispersal of adult female fig wasps. 1. Arrivals and departures. *Entomologia Experimentalis et Applicata* 73:221–238.
- Ware, A. B., and S. G. Compton. 1994b. Dispersal of adult female fig wasps. 2. Movements between trees. *Entomologia Experimentalis et Applicata* 73:231–238.
- West, S. A., and E. A. Herre. 1994. The ecology of the New World fig-parasitizing wasps *Idarnes* and implications for the evolution of the fig–pollinator mutualism. *Proceedings of the Royal Society of London. Series B, Biological Sciences* 258:67–72.
- West, S. A., E. A. Herre, D. M. Windsor, and P. R. S. Green. 1996. The ecology and evolution of the New World non-pollinating fig wasp communities. *Journal of Biogeography* 23:447–458.
- Wiebes, J. T. 1995. The New World Agaonidae: pollinators of figs. *Proceedings of the Koninklijke Nederlandse Akademie van Wetenschappen* 98:167–183.
- Yu, D. W., J. Ridley, E. Jouselin, E. A. Herre, S. G. Compton, J. M. Cook, J. G. Moore, G. D. Weiblen. Oviposition strategies, host coercion and the stable exploitation of figs by wasps. *Proceedings of the Royal Society of London. Series B, Biological Sciences* 271:1185–1195.
- Zhao, J. B., Y. Q. Peng, R. J. Quinell, S. G. Compton, and D. R. Yang. 2014. A switch from mutualist to exploiter is reflected in smaller egg loads and increased larval mortalities in a “cheater” wasp. *Acta Oecologica* 57:51–57.

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