

# The evolution of parasitism from mutualism in wasps pollinating the fig, *Ficus microcarpa*, in Yunnan Province, China

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**Theory identifies factors that can undermine the evolutionary stability of mutualisms. However, theory's relevance to mutualism stability in nature is controversial. Detailed comparative studies of parasitic species that are embedded within otherwise mutualistic taxa (e.g., fig pollinator wasps) can identify factors that potentially promote or undermine mutualism stability. We describe results from behavioral, morphological, phylogenetic, and experimental studies of two functionally distinct, but closely related, *Eupristina* wasp species associated with the monoecious host fig, *Ficus microcarpa*, in Yunnan Province, China. One (*Eupristina verticillata*) is a competent pollinator exhibiting morphologies and behaviors consistent with observed seed production. The other (*Eupristina* sp.) lacks these traits, and dramatically reduces both female and male reproductive success of its host. Furthermore, observations and experiments indicate that individuals of this parasitic species exhibit greater relative fitness than the pollinators, in both indirect competition (individual wasps in separate fig inflorescences) and direct competition (wasps of both species within the same fig). Moreover, phylogenetic analyses suggest that these two *Eupristina* species are sister taxa. By the strictest definition, the nonpollinating species represents a "cheater" that has descended from a beneficial pollinating mutualist. In sharp contrast to all 15 existing studies of actively pollinated figs and their wasps, the local *F. microcarpa* exhibit no evidence for host sanctions that effectively reduce the relative fitness of wasps that do not pollinate. We suggest that the lack of sanctions in the local hosts promotes the loss of specialized morphologies and behaviors crucial for pollination and, thereby, the evolution of cheating.**

fig wasps | pollination mutualism | cheating | host sanctions | coevolution

Mutualisms are defined by the net benefits that are usually provided to individuals of each interacting species. These interactions often have influences far beyond the partner species directly interacting, and commonly provide many fundamental ecosystem services (1, 2). For example, in most cases, mycorrhizal fungi provide nutrients to forest trees, pollinators help flowering plants set fruit, intestinal bacteria promote nutrient uptake across diverse animal taxa, bacteria in lucinid clams help detoxify benthic sediments, and photosynthetic algae help maintain the coral reefs that structure nearshore marine environments around the world (3–6).

However, while both partners in a mutualism usually receive net benefits from the interaction, mutualisms also usually impose costs on one or both partners interacting mutualistically. In the absence of fitness-aligning mechanisms between the partners (e.g., vertical transmission of symbionts, or repeated interactions with immediate fitness benefits), theory suggests that other mechanisms are needed to maintain a mutualism's stability. Specifically, it has been proposed that a mutualism's long-term stability often depends on mechanisms that limit the invasion of "cheater" individuals into the populations of either partner species (2, 3, 7–14). Broadly, cheaters can be defined as individuals (or species) that

do not provide a beneficial service to their partners. By not providing a potentially costly service to their partners, cheaters are thought to benefit themselves relative to "cooperating" individuals or species in the short term (12–14). Invasion by such cheaters potentially erodes the net benefits resulting from the interaction, and therefore can lead to a breakdown of the mutualism itself.

Consistent with this viewpoint, data suggest that in many cases the hosts (the larger of the two partners in the mutualism) can effectively promote cooperation by selectively allocating more resources to those symbionts that provide them with greater benefits. For example, some legumes have been shown to selectively allocate more resources to nodules containing rhizobia that are better at providing fixed nitrogen (14–16). In other studies, some host plants allocate more carbon to strains of mycorrhizal fungi that provide their hosts with more phosphorus (17–19).

However, other authors question the biological relevance of much of this experimental evidence to natural species interactions, the direction of cause and effect, and the actual costs for providing benefits. A central question is the degree to which evidence for cheaters, defined as receiving fitness benefits by not providing services (relative to a mutualist that does provide benefits), exist at all (12, 13, 20). Key empirical issues concern whether or not individuals with a cheating phenotype do, in fact, cheat (impose a

## Significance

Both the role of host sanctions (differential resource allocation to more beneficial symbionts) in stabilizing mutualisms, and the existence of "cheaters" (species gaining fitness by not benefiting their hosts) and their role in destabilizing them are controversial. A detailed study of an evolutionary transition from mutualism to parasitism focusing on two functionally distinct *Eupristina* wasp species associated with the fig, *Ficus microcarpa* in Yunnan Province, China, documents both. Within the comparative context of many existing studies of costs and benefits of active fig pollination, the results suggest that the low to nonexistent host sanctions on wasps that do not pollinate in this region promote the loss of specialized morphologies and behaviors crucial for pollination and, thereby, the evolution of cheating.

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reproductive cost on their partner, relative to a cooperating mutualist). In addition, are cheating individuals that fail to benefit their host at least as fit as cooperating (mutualistic) individuals that do? Does the host allocate relatively more resources to more beneficial partners (effectively expressing sanctions against cheaters relative to cooperators)? Ultimately, this becomes a set of specific empirical questions: What is the relative fitness of cooperators and cheaters that interact with the same partner (host)? And, does the host effectively sanction cheaters relative to cooperators, and if so, to what degree (21, 22)? At a fundamental level, the relative fitness of cheaters and cooperators is only measurable and relevant within the context of a given host's responses to them (3, 21, 22).

To resolve these questions, it is useful to study those mutualistic host–symbiont interactions in which it is straightforward to measure and experimentally manipulate both benefits and costs to each partner under natural conditions (22–32). Ideally, we should be able to comparatively assess experimental results across a diversity of host–symbiont mutualisms that differ in what theory suggests should be key metrics (e.g., strength of host sanctions, existence and relative abundance of cheaters, and so forth).

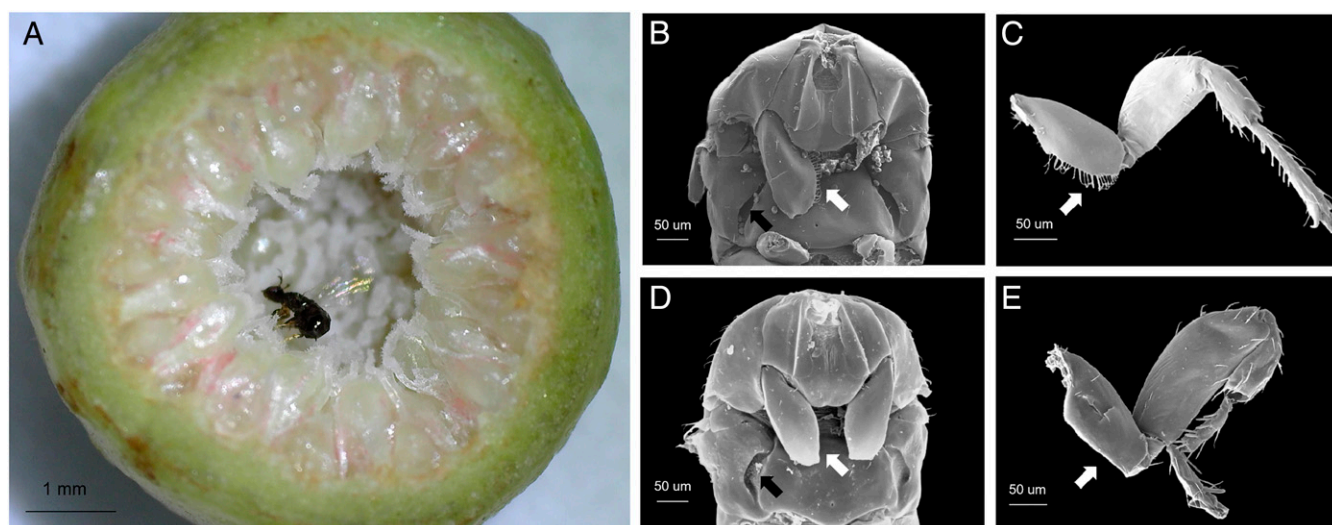
The over 750 species of host figs (*Ficus*: Moraceae) and their obligately pollinating wasps (Agaonidae: Hymenoptera) provide such a range of both experimental and comparative options that can be exploited to address these questions (22–32) (SI Appendix, Supplementary Text and Fig. S1). Ovipositing female fig wasps deposit a drop of fluid from their poison sac into the ovules of flowers into which they lay their eggs. This fluid initiates the formation of gall tissue upon which the developing larvae feed (33) (SI Appendix). At any given site, each fig species is typically pollinated by only one or two fig wasp species (24, 26). Morphological and molecular studies broadly support coevolution between genera of pollinating wasps and their respective sections of figs, while functional studies demonstrate coadaptation between them (33–51).

For example, different groups of figs are characterized by either active or passive pollination (43–45) (SI Appendix). Passive pollination does not require specialized wasp morphologies or behaviors. In contrast, active pollination requires specialized female wasp morphologies and behaviors (44). The wasps collect pollen in their natal fig using coxal combs on their forelegs and store it in pollen pockets on their thoraxes (Fig. 1). After emerging from their natal figs, female wasps use volatile chemical scent cues produced

by receptive figs to identify them (35–37). Dispersal flights from the natal fig are aided by prevailing winds and routinely cover scores of kilometers (38–41). Upon finding and entering a receptive fig of an appropriate host species, the foundress wasps repeatedly remove a few grains of pollen from their pockets and place them on the stigmatic surfaces of the individual flowers on which they attempt to lay eggs. Active pollination provides clear benefits for the host fig. Pollination is more efficient in actively pollinated fig species relative to passively pollinated species. This is reflected in the dramatically lower (~1/10) amounts of pollen that active species typically produce (43–45). Conversely, active pollination appears to be costly for the wasps in terms of specialized body structures, energy, and time (22, 42, 45).

The most basic mutualistic services (e.g., the wasp's ability to pollinate) can be experimentally manipulated. By allowing or restricting the female pollinator wasps' access to, and ability to actively collect pollen, pollinators that either do (P+) or do not (P–) carry pollen can be produced and then introduced into receptive figs (22). Furthermore, the effects on pollinator wasp fitness (i.e., lifetime reproductive success) of pollinating the host fig (or not) can be quantified by counting their relative number of offspring in naturally occurring figs (22–32). Moreover, the many existing experimental studies using the same methodologies provide context for the findings of any given experiment (22–32). In previous experiments on actively pollinated fig species, wasps that do not pollinate (P–) have lower fitness than wasps that pollinate (P+) due to increased rates of fig abortion (killing all wasp larvae) and increased larval mortality reducing the number of P– offspring that emerge. These “host sanctions” are likely caused by selective resource allocation by the tree to better-pollinated figs (28). Although pollination typically leads to a higher number of wasp offspring, pollination is not an absolute requirement for wasp offspring to develop (28). Finally, there are at least two known cases of cheating wasp species, in which species of wasps that lack both morphologies and behaviors that permit efficient, active pollination of their host co-occur with a congeneric pollinator possessing these traits. Importantly, the species that lack these traits have clearly evolved within lineages of wasps that otherwise possess these apparently costly traits that permit them to actively pollinate their host (52, 53) (SI Appendix).

Here, we exploit the opportunity provided by a third case (54, 55), in which a mutualistic active pollinator and a congeneric cheater



**Fig. 1.** Receptive *F. microcarpa* fig and pollinating structures of *E. verticillata* compared with *Eupristina* sp. (A) A cheater wasp (*Eupristina* sp.) laying eggs in a receptive fig of her host *F. microcarpa*. Pollinator wasps (*E. verticillata*) (B and C) have specialized morphological structures such as pollen pockets (black arrow) on the underside of their thorax and coxal combs on their forelegs (white arrows) that facilitate pollination. Pollen is stored in the pockets and coxal combs facilitate pollen transfer (43, 44). Cheater wasps (*Eupristina* sp.) (D and E) retain pollen pockets (black arrow) but lack coxal combs (white arrow).

species co-occur on the same monoecious host fig. Specifically, we conducted a combination of behavioral, morphological, phylogenetic, and experimental studies to compare these wasps and the outcomes of their interactions with their shared host fig, *Ficus microcarpa* (subgenus *Urostigma*: section *Urostigma*: subsection *Conosycea*), in and near the Xishuangbanna Tropical Botanical Garden (XTBG), China. *Eupristina verticillata* is the described active pollinator of *F. microcarpa* at this location, while an undescribed coexisting wasp species (*Eupristina* sp.) lacks the necessary adaptation for active pollination and appears to be a cheater (54, 55).

In this study, we address and answer the following questions: 1) Does the undescribed *Eupristina* sp. wasp associated with *F. microcarpa* impose a reproductive cost on its host? We find that it does, and that the cost for host reproductive success is large. 2) Does the cheater exhibit significantly higher levels of reproductive success than the pollinator in their host? Yes, in both direct and indirect competition. Combined with the reproductive loss it imposes on the host, this species meets the strictest definition of cheater. 3) Is this cheater closely related (possibly a sister species) to the mutualist pollinator of their shared host? We find that within the context of other sympatric *Eupristina* species associated with seven fig hosts in this area, it is. Furthermore, it represents an independent loss of pollination structures from another case previously reported in this genus. 4) Does the host (*F. microcarpa*) locally exhibit detectable host sanctions against wasps that do not pollinate it? In sharp contrast with all 15 other cases of actively pollinated *Ficus* species that have been reported (22, 29–32), we find that it does not. 5) Given that cheaters exhibit equal or greater fitness than the pollinator, how do they coexist? Although deserving further study, we suggest that regular seasonal fluctuations in the relative abundances of the two wasp species facilitate their coexistence at this site (54, 55). Seasonal changes in the prevalence of westerly winds cause regional spatial heterogeneity in source pools of pollinators and cheaters that immigrate to the local host, *F. microcarpa*.

## Results

### Morphology and Behavior of the Cheater and Pollinator Wasp Species.

The pollinator species, *E. verticillata*, and the congeneric cheater species, *Eupristina* sp., are morphologically similar. Naturally emerged pollinators (*E. verticillata*) and cheaters (*Eupristina* sp.) showed similar head widths, which is a good proxy for body size [pollinator:  $0.32 \pm 0.0019$  mm,  $n = 242$ ; cheater:  $0.32 \pm 0.0013$  mm,  $n = 138$ ;  $t_{(378)} = 2.46$ ,  $P = 0.12$ ], and similar egg loads [pollinator:  $89.86 \pm 1.39$ ,  $n = 242$ ; cheater:  $91.69 \pm 1.20$ ,  $n = 138$ ;  $t_{(378)} = 0.96$ ,  $P = 0.34$ ]. Therefore, the differences in fitness that we see in the experiments cannot be explained by differences in egg load. However, the two species differ dramatically in morphological and behavioral traits that are directly linked to active pollination. All *E. verticillata* (pollinator) individuals whose morphology and behavior were examined in detail ( $n = 10$ ) possessed a coxal comb (a brush-like structure on the foreleg that is essential for collecting pollen) and all exhibited the pollination behaviors of actively collecting and depositing pollen (SI Appendix, Supplementary Text). In striking contrast, the cheaters (*Eupristina* sp.) lack coxal combs (Fig. 1) and did not exhibit any pollination behaviors ( $n = 24$ ). Thus, wasp morphological traits (presence or absence of coxal combs) corresponded to the presence or absence of active pollination behavior (Fisher exact test,  $P = 7.6E-9$ ) (Fig. 1).

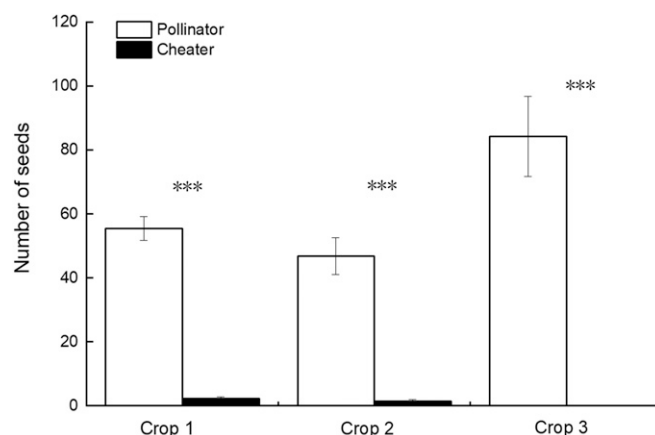
### Seed Production in Figs with Single Pollinator and Cheater Foundresses.

The figs in which a single P+ pollinator or cheater female wasp (foundress) were introduced showed striking and significant differences in seed production. In the three experimental crops, figs with a cheater foundress produced on average only 0 to 4% the number of seeds compared to that of figs with a P+ foundress (between ~48 and 84 seeds per fig (Materials and Methods, Fig. 2, and SI Appendix, Tables S1 and S3) (see ref. 23 for context). These differences were

large and statistically significant in all three experiments (crop 1:  $n = 30$ ,  $U = 600$ ,  $P = 2.3E-09$ ; crop 2:  $n = 18$ ,  $U = 216$ ,  $P = 2.5E-06$ ; crop 3:  $n = 18$ ,  $U = 315$ ,  $P = 4.7E-08$ ). Thus, the undescribed *Eupristina* sp. is a cheater on *F. microcarpa* in that it fails to efficiently pollinate its host while imposing significant costs on it (the developing wasp offspring consume flowers that otherwise could have developed into seeds, but do not pollinate or disperse pollen in return).

**Fitness Outcomes of Indirect and Direct Competition between Pollinators and Cheaters.** We next compared the relative lifetime reproductive success of pollinator and cheater wasps when either occurs as a single foundress in separate figs (indirect competition) or when both pollinator and cheater foundresses share a fig (direct competition). To do this we followed the developmental sequence from initiation of fig development with estimates of rates of fruit abortion (22), to the estimates of total oviposition (31), to estimates of final, successful development of viable adult offspring in unaborted figs (22–25, 29–32).

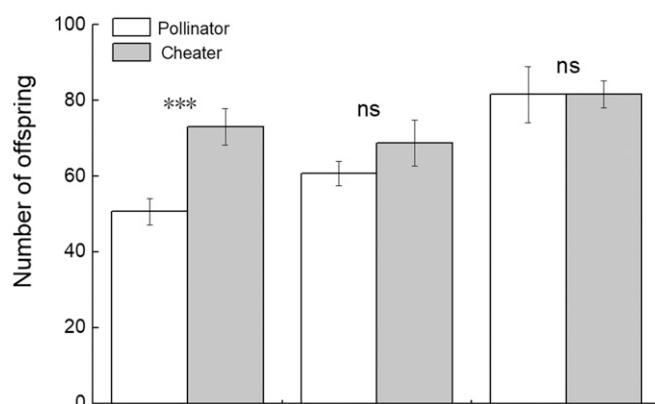
**Indirect competition between pollinator and cheater wasps in separate figs.** Overall, the number of offspring that successfully developed in single-foundress figs was significantly higher for the cheater wasps compared to both P+ (pollen-carrying) and P– (pollen-free) pollinating wasps (~25% more cheaters) (SI Appendix, Fig. S2 and Table S2) [generalized linear models (GLM),  $F_{(2,136)} = 10.53$ ,  $P = 5.6E-5$ ; Bonferroni-corrected post hoc tests C vs. P+,  $P = 1.5E-4$ , C vs. P–,  $P = 0.0027$ ]. This also was either significant or trending when each experiment was analyzed separately [ANCOVAs, crop 1:  $F_{(1,47)} = 21.64$ ,  $P = 2.7E-5$ ; crop 2:  $F_{(1,27)} = 1.40$ ,  $P = 0.25$ ; crop 3:  $F_{(1,33)} = 0.027$ ,  $P = 0.87$ ] (Fig. 3A and SI Appendix, Table S2). Decomposing the effects that potentially contribute to the estimate of relative reproductive success of different kinds of wasps, the abortion rates of figs did not differ between the cheaters and pollinators in any of the experiments, so abortion rate was not included in calculating relative success of cheating and pollen-carrying pollinator wasps (22) (SI Appendix, Table S1). Gall production is roughly equal to the number of successful ovipositions by the wasps and is estimated by successfully developed wasps + bladders (unsuccessfully developed wasps remaining in galls) (31). Across all experiments, there was significantly higher gall production in figs with a single cheater foundress compared to figs with a single pollen-carrying pollinator foundress (cheaters produced ~25% more galls) [GLM,  $F_{(2,136)} = 14.52$ ,  $P = 2.0E-6$ , Bonferroni-corrected post hoc tests C vs. P+,  $P = 9.7E-7$ ] (SI Appendix, Fig. S2). This is consistent with the cheater wasps regularly ovipositing in significantly



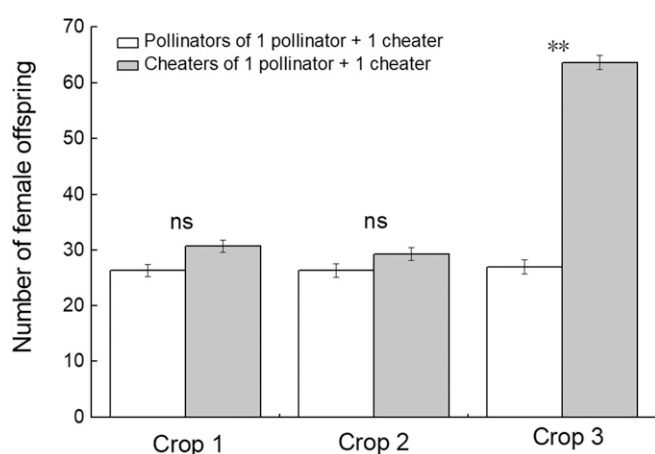
**Fig. 2.** Mean number of seeds produced in figs into which a single pollinator (white bar) or a single cheater (dark bar) were introduced. Figs with introduced cheaters exhibited significantly lower seed set in all three crops. \*\*\* $P < 0.001$  level. Error bars represent 1 SE of mean.



## A Indirect competition (each wasp in different figs)



## B Direct competition (two wasps in the same fig)



**Fig. 3.** Numbers of offspring (mean  $\pm$  SE) produced by pollinator or cheaters introduced into receptive figs. (A) Given indirect competition (a single pollinator or a single cheater introduced into each receptive fig), cheaters exhibited similar or greater numbers of offspring with single foundresses in different figs compared to pollinators. (B) Given direct competition (both pollinator and cheater introduced into the same receptive fig), cheaters also produced similar or greater numbers of offspring compared to pollinators,  $^{**}P < 0.01$ ,  $^{***}P < 0.001$ , "ns" indicates no significant difference (SI Appendix, Tables S2 and S3). Error bars represent 1 SE of mean.

more flowers than the pollen-carrying pollinators. The cheater was thus better able to exploit the host as a single foundress than either the pollen-carrying or pollen-free pollinator (SI Appendix, Fig. S2 and Table S2). We note that the single foundress situation is common in nature, as it represents roughly 65% of all figs in *F. microcarpa* at this locale (55).

**Direct competition with P+ pollinators and cheaters sharing a fig.** Under direct competition in two-foundress figs (a P+ pollinator and a cheater introduced into the same fig), randomization tests indicated that the cheater produced significantly more female offspring than the pollinator (the mean difference = 12.75,  $P = 0.009$ ) across the three experiments (Fig. 3B). In individual experiments, the number of female offspring of cheater individuals either trended higher than those of the pollinator (crop 1: mean difference = 4.4,  $P = 0.15$ ; crop 2: mean difference = 2.93,  $P = 0.35$ ) or was significantly higher (crop 3: mean difference = 36.64,  $P = 0.0097$ ) (Fig. 3B and SI Appendix, Table S3). Therefore, as with indirect competition, under direct competition in the same fig, the cheater showed similar or

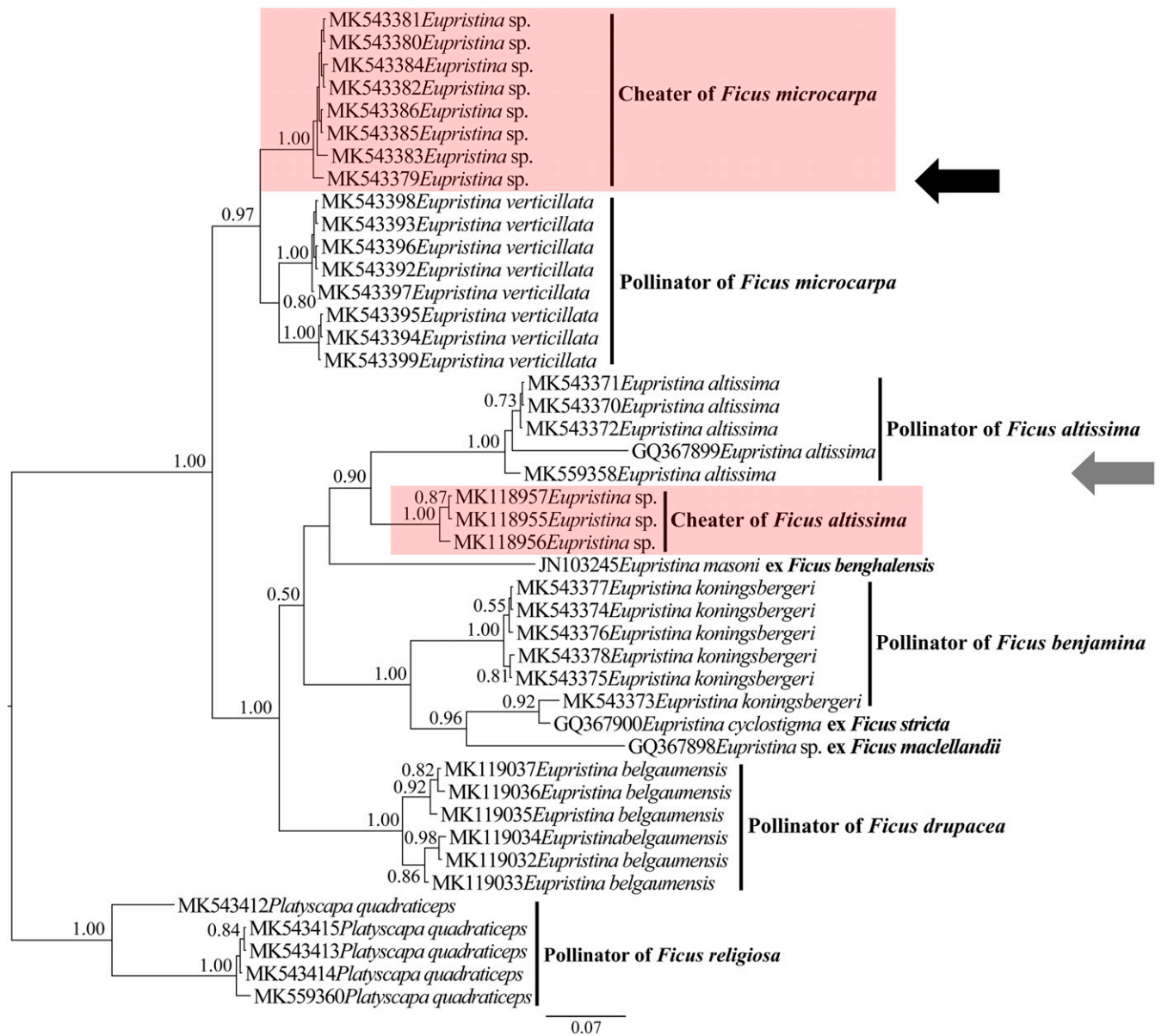
higher reproductive success than the pollinator. Combined, the experimental data show that, by dramatically reducing both seed production and pollen-dispersal components of its host's reproductive success, as well as by realizing significantly higher fitness than the mutualistic pollinator, *Eupristina* sp. fulfills the most stringent definition of cheater (12, 13).

**Phylogenetic Relationship between Cheater and Pollinator.** Analyses of the molecular evidence (670 bp of mitochondrial cytochrome *c* oxidase [COI] sequence) showed that coexisting *E. verticillata* (the pollinator) and *Eupristina* sp. (the cheater) were genetically distinct (4.5%, Kimura two-parameter distance) and were clearly sister species, relative to the other *Eupristina* species from XTBG (Fig. 4 and SI Appendix, Fig. S3). Individual wasps sampled from seven different fig species formed monophyletic lineages with moderate to strong support (Fig. 4). Furthermore, the phylogenetic analysis indicates that the loss of coxal combs and pollination behavior (i.e., evolution of cheating) has evolved independently at least twice in this group; once in wasps associated with *F. microcarpa*, and once in wasps associated with *Ficus altissima* (47, 53, 56). Additional analyses conducted with publicly available COI sequences from other *Eupristina* species collected throughout Southeast Asia are consistent with the cheater species being phylogenetically embedded within the radiation of mutualistic pollinator species associated with *F. microcarpa*, and representing an independent evolution of cheating from that observed in *F. altissima* (SI Appendix, Fig. S3).

**Estimation of Strength of Host Sanctions in *F. microcarpa*.** Following established protocols used in several previous studies, we estimated the strength of sanctions (relative fitness disadvantage of not pollinating) in the local host *F. microcarpa* by comparing: 1) the fig abortion rates and 2) the relative production of wasp offspring in unaborted figs in cases where single P+ and P− pollinating foundresses had been introduced (22, 26–32). Overall, the number of offspring produced in unaborted figs did not differ significantly between figs with P+ and P− pollinators when all four experiments were analyzed together [GLM,  $F_{(1,136)} = 0.54$ ,  $P = 0.47$ ] (Fig. 5 and SI Appendix, Tables S1 and S2). Furthermore, the number of offspring of P− (pollen-free) pollinator foundresses was similar or greater than that of P+ pollinator foundresses in each experimental crop individually [ANCOVAs, crop 2:  $F_{(1,26)} = 0.76$ ,  $P = 0.39$ ; crop 3:  $F_{(1,30)} = 23.82$ ,  $P = 0.19$ ; crop 4:  $F_{(1,29)} = 0.09$ ,  $P = 0.77$ ; crop 5:  $F_{(1,46)} = 6.70$ ,  $P = 0.013$ ].

Decomposing the effects that potentially contribute to the estimate of sanction strength, the abortion rates did not differ between the two groups (P+ versus P− pollinators) in any of the experiments (Fisher exact test: crop 2:  $P = 0.36$ ; crop 3:  $P = 0.47$ ; crop 4:  $P = 1.00$ ; crop 5:  $P = 0.44$ ) (SI Appendix, Table S1). The total number of oviposition attempts (estimated by successfully developed wasps + bladders (unsuccessfully developed wasps remaining in galls) (31) did not differ significantly between the P+ and P− pollinators. When all four experiments were analyzed together there was a nonsignificant trend for P− pollinating wasps to oviposit more [GLM,  $F_{(1,136)} = 3.71$ ,  $P = 0.056$ ] (SI Appendix, Fig. S4). When the four experiments were analyzed separately, only in crop 5 did pollen-free wasps show significantly increased oviposition compared to pollen carrying wasps [ANCOVAs, crop 2:  $F_{(1,26)} = 0.41$ ,  $P = 0.53$ ; crop 3:  $F_{(1,30)} = 0.56$ ,  $P = 0.46$ ; crop 4:  $F_{(1,29)} = 0.44$ ,  $P = 0.51$ ; crop 5:  $F_{(1,46)} = 7.20$ ,  $P < 0.01$ ].

Calculating host sanction strength as  $1 - W_R$ , where  $W_R$  is the relative fitness of a P− pollinator wasp relative to a P+ pollinator (22), we find the average host sanction strength for *F. microcarpa* at this location to be 0.02, with the estimated sanction strength of individual crops 2 through 5 being 0.12, 0.09, −0.16, and 0.02, respectively. These findings show that this Chinese population of *F. microcarpa* has low or nonexistent sanctions, with effectively no

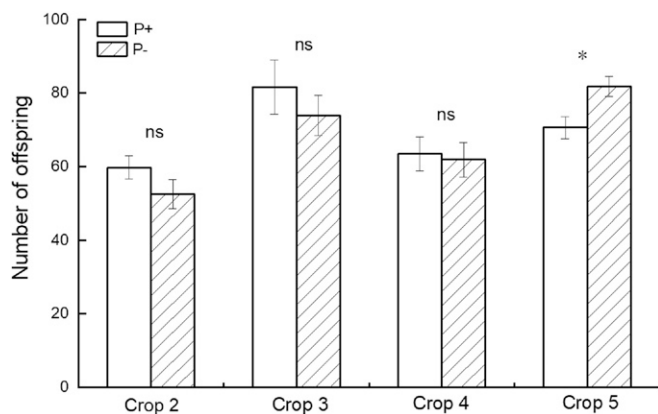


**Fig. 4.** Consensus phylogenetic tree of *Eupristina* pollinator species associated with *Ficus* hosts near XTBG constructed using Bayesian inference of 670 bp of the mitochondrial COI region. Bayesian posterior probabilities ( $\geq 0.50$ ) are shown beside the branches. GenBank accessions of the sequences are indicated before the species name. The cheater species associated with *F. microcarpa* in XTBG is most closely related to pollinators of the same host (SI Appendix, Fig. S3). Cheating species occurring in the vicinity of XTBG (pink highlight with arrows) have evolved from pollinator lineages independently at least twice, once in association with *F. microcarpa* hosts and once with *F. altissima*. More detailed analyses using additional samples of *Eupristina* from across Asia support these inferences (SI Appendix, Fig. S3).

significant fitness costs for wasps that do not pollinate. This contrasts with all 15 previous reports of actively pollinated fig species, in which host sanction strengths were estimated to range from 0.33 to 0.99, with a mean of 0.54 (22, 29–32). Finally, if we limit the comparisons to only the four fig species in which estimates from multiple experiments have been reported (as with the *F. microcarpa* population studied here), *F. microcarpa* still has significantly weaker sanctions than the other species [Welch ANOVA, statistic = 12, 234, df = 4, 3.45,  $P = 1.9\text{E-}7$ , planned contrasts  $t_{(3,96)} = 9.65$ ,  $P = 6.8\text{E-}4$ ].

**Seasonal Patterns of Relative Abundance of Pollinators and Cheaters.** The relatively higher reproductive success of the cheater found

in both indirect and direct competition with the pollinator (~25%) in exploiting the host fig as a resource for rearing their young raises the question of why does the pollinator exist at this site at all. Censuses conducted from August 2012 to March 2013, and then again from October 2015 to March 2017, were consistent in suggesting a seasonal pattern in the relative abundances of pollinators and cheaters (Fig. 6). Specifically, during the two nonmonsoon seasons occurring in 2016 and 2017, a majority of the total *Eupristina* offspring that emerged from D-phase figs were cheaters ( $71.45\% \pm 2.49\%$ , SE,  $n = 307$ ), and significantly exceeded that of pollinators ( $\chi^2 = 17.64$ , df = 1,  $P = 2.7\text{E-}5$ ). In contrast, during the monsoon season from June to November 2016, pollinators (mean =  $63.65 \pm 3.08\%$ , SE,  $n = 219$ ) significantly exceeded



**Fig. 5.** The mean offspring number of single pollen-carrying (P+, white bar) and pollen-free (P-, hatched bar) *E. verticillata* foundress wasps realized in four introduction experiments on *F. microcarpa* crops in Yunnan Province, China. Offspring numbers did not differ between P+ and P- treatments when all four experiments were analyzed together (GLM, see text); when experimental crops were analyzed separately there was a significant difference only in one in which P- wasps produced significantly more offspring (ANCOVAs, see text; "ns" indicates no significant difference, \* $P < 0.05$ ). There is no evidence for fitness costs for P- wasps in *F. microcarpa* at this location. Error bars represent 1 SE of mean.

cheaters ( $\chi^2 = 7.84$ ,  $df = 1$ ,  $P = 0.005$ ) (Fig. 6 and *SI Appendix*, Fig. S5). We found that the percentage of pollinators emerging from D-phase figs from each of the 14 observed crops from December 2015 to January 2017 for which we also had accurate dates of their receptivity correlated with the incidence of the west winds that prevailed during the 3-d period of the receptive B-phase for these same crops (Spearman rank,  $P < 0.0025$ ,  $n = 14$ ) (*SI Appendix*, Figs. S5 and S6). This receptive phase corresponds to the period when the adult wasps (pollinator or cheater) arrived to pollinate and oviposit on these same crops (*SI Appendix*, Figs. S5 and S6). Furthermore, the relative monthly change in the proportion of pollinators emerging from D-phase fruits also correlated significantly with relative monthly change of incidence of westerly winds during the corresponding periods of the receptive B-phases (Spearman rank, 0.01,  $P < 0.025$ ,  $n = 13$ ). These observations are consistent with cheaters and pollinators being preferentially derived from different source pools arriving at these local trees, with the pollinator mainly coming from the west, when winds from that direction prevailed during most of the monsoon period (*SI Appendix*, Figs. S5 and S6).

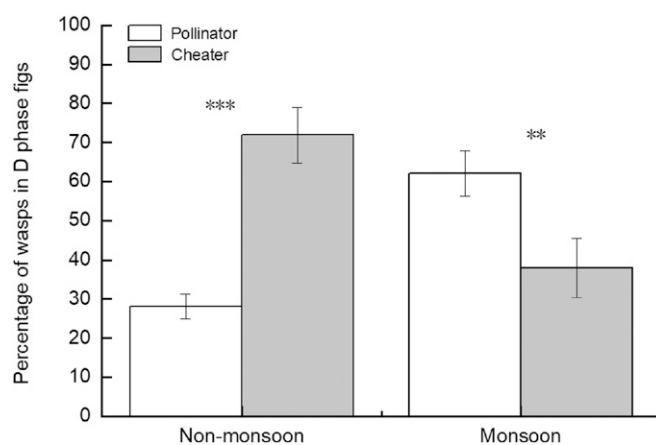
## Discussion

Here, we compare the reproductive biology of *E. verticillata*, the primary pollinator of *F. microcarpa* in and near XTBG in Yunnan Province, China, with that of the closely related species (*Eupristina* sp.) that co-occurs on their shared host. *E. verticillata* is a competent pollinator that is associated with high seed set. In sharp contrast, *Eupristina* sp. is not. The adults of the cheater *Eupristina* sp. do not pollinate the host, and thereby inflict a cost in lost seed production (reduced host female fitness). The developing cheater offspring add insult to injury by costing the host in lost pollen dispersal (reduced male fitness). Despite not benefiting their host in any way, the developing cheater larvae consume flowers that could have developed into seeds; typically about 32% of flowers in single-foundress figs are consumed by developing wasp larvae. Furthermore, we find that the cheater *Eupristina* sp. consistently produces significantly more offspring than the pollinator, apparently as a result of higher oviposition. Moreover, in the context of other sympatric *Eupristina* wasp species, we find that these two wasp species appear to be sister taxa, and that this evolution of

cheating has occurred independently from the case that has been previously described in wasps associated with the closely related fig, *F. altissima* (53). The combination of large costs imposed on the host (through not pollinating) and increased fitness relative to the mutualistic pollinator is consistent with *Eupristina* sp. fulfilling the strictest definition of a cheater (12, 13). Furthermore, this cheater has evolved from a mutualist ancestor to become a parasite on this host. Finally, in stark contrast to 15 previous studies of actively pollinated fig species, we found no evidence for detectable host sanctions (lower fitness of wasps that do not pollinate) (22, 29–32). This is consistent with theory suggesting that the evolution of cheating is promoted when benefiting a host is costly and host sanctions are absent (2, 7–18, 21, 22, 26–32).

The pollinator enables seed set in its host, the cheater does not. We find that the pollinator, *E. verticillata*, exhibits active pollination behavior and the morphology (e.g., coxal combs) that are essential for seed set (Fig. 1). Experiments show that when this wasp species enters a syconium (fig inflorescence) as a foundress, there is high seed production, reflecting efficient pollination: between ~48 and 84 seeds per fig, representing 30 to 40% of all female flowers (Fig. 2 and *SI Appendix*, Tables S1 and S3) (see ref. 23 for context). In contrast, the cheater associated with this host (*Eupristina* sp.) lacks pollination behavior and coxal combs (Fig. 1). Experiments show that corresponding seed production is extremely low or non-existent (zero to two seeds per fig), and that this species is effectively a parasite on the system (i.e., a cheater). Specifically, relative to the pollinating species, the cheater foundresses reduce both the fig's female function (seed set), and the fig's male function (pollen dispersal). The latter is reduced by the production of wasp offspring that will neither collect nor disperse nor distribute its pollen. Thus, relative to the pollinator, the cheater imposes a very real cost to both major components for the host fig's potential reproductive success.

There is no apparent fitness reduction associated with a cheater lifestyle in this system. If anything, there is a benefit (3, 8–20). In a natural setting, our experiments indicate that cheater foundresses exhibit equal or greater reproductive success (in this case, total lifetime fitness) than a pollinator, as measured by total number of offspring. The cheater offspring exhibited similar body sizes, equal or greater egg loads, and a greater capacity to oviposit, compared



**Fig. 6.** Mean percentage of pollinator and cheater wasps in different seasons. The proportion of pollinator (white bar) and cheater (gray bar) emerging from D-phase figs during monsoon or nonmonsoon seasons. During nonmonsoon season (from December to May in D-phase figs), the proportion of cheater ( $71.45 \pm 2.49\%$ , SE,  $n = 307$ ), significantly exceeded that of pollinators ( $\chi^2 = 17.64$ ,  $df = 1$ ,  $P = 2.67 \times 10^{-5}$ ). In contrast, the proportion of pollinators ( $63.65 \pm 3.08\%$ , SE,  $n = 219$ ) was significantly greater during the Monsoon season (June to November,  $\chi^2 = 7.84$ ,  $df = 1$ ,  $P = 0.0051$ ). \*\* $P < 0.01$ , \*\*\* $P < 0.001$ . Error bars represent 1 SE of mean.



to pollinator offspring (22, 23, 27, 42) (*SI Appendix, Fig. S2 and Table S2*). Here, there are two lines of experimental evidence. One compares the reproductive success of single foundresses of either the pollinator or cheater when introduced into different receptive fruit on the same tree (indirect competition, a situation that naturally occurs in ~65% of the figs). The other compares the reproductive success when each type of wasp is introduced into the same fig (direct competition, roughly two-thirds of the remainder). In both direct and indirect competition, the cheater produces significantly greater numbers of offspring, apparently as a result of laying more eggs. The cheater's ability to lay more eggs presumably reflects time saved by not performing the pollination movements, which can be used to lay more eggs (22, 27, 42). This finding emphasizes that not only is there no loss of fitness associated with being a cheater in this fig system, there is an advantage, fulfilling the strictest definition of cheater (12, 13).

Within the context of other *Eupristina* wasp species associated with the seven closely related *Ficus* hosts in this region (all fig hosts belong to the subgenus *Urostigma*: section *Urostigma*: subsection *Conosycea*), we find that these two *Eupristina* wasp species are closely related taxa (Fig. 4 and *SI Appendix, Fig. S3*). Analyses of 670 bp of mitochondrial COI sequences produced well-supported phylogenetic relationships of all of these wasps. The results suggest that, despite dramatically different effects on their host, *E. verticillata* (a mutualistic pollinator) and *Eupristina* sp. (a nonpollinating cheater) are very closely related, probably sister taxa. Furthermore, we find that pollen-manipulating coxal combs (Fig. 1) and pollination behavior, two characters essential for pollination, were lost twice in this group of wasps, once in the taxon discussed here and once independently in a previously described, apparent cheater associated with *F. altissima* (53). We conclude that cheating has evolved independently at least twice in this genus of wasps that otherwise pollinate this subsection of fig hosts. Additional analyses including publicly available COI sequences from other *Eupristina* species are consistent with both principal conclusions based on XTBG samples (*SI Appendix, Fig. S3*).

Currently, no studies estimating host sanctions have been yet reported in either of the other fig hosts (the sympatric *F. altissima* or the African *Ficus sycomorus*) for which cheater species that are phylogenetically embedded in otherwise actively pollinating lineages. However, it is clear that these three cases of loss of behaviors and morphologies necessary for pollination (evolution of cheater syndrome) show different characteristics. In the African *F. sycomorus* system, the cheater (*Ceratosolen galili*) has lost its active pollinating behavior, but still retains partially developed coxal combs (52, 56). In the Asian *F. altissima* and *F. microcarpa* systems, the loss of pollination appears more advanced, as both cheaters have lost both the behavior (as is true in the African case), as well as the morphological adaptations for pollination (this study and ref. 53). The loss, first of behavior then followed by a loss of relevant morphology, was predicted and follows a general trend seen in other systems (22). In the African case, the cheating *Ceratosolen* species appears to have shifted its host (56). In contrast, in both *F. microcarpa* and *F. altissima*, the differentiation of the two species of *Eupristina* leading to cheating in one of them appears to have taken place while in association with the same host.

Interestingly in the *F. microcarpa* trees examined for this study, no negative fitness consequences were detected for wasps that did not pollinate (i.e., no host sanctions) (22). This finding contrasts with the findings of all 15 published experiments using similar methodologies to conduct P+ and P− experiments in actively pollinated host figs. The interpretation across the previous studies has been that these host sanctions effectively reward a wasp that provides a beneficial mutualistic service (active pollination), and effectively impose fitness costs for cheating (i.e., not pollinating). Ecologically, host sanctions are thought to provide a selective

incentive that contributes to the ecological and evolutionary stability of the fig-pollinating mutualism. Evolutionarily, host sanctions are likely derived from preexisting adaptations in the host to allocate more resources to more productive tissues (22, 28).

This reported case of no (or very low) host sanctions in an actively pollinated fig species is unique. We suggest that it is no coincidence that this also corresponds to a case in which a cheater appears to have descended from the co-occurring pollinator of that shared host. We interpret this set of findings as suggesting that lack of sanctions on the part of the fig host actually promotes the evolution of cheating (7–19, 22, 28–32). We suggest that studies of the hosts of other known cheating species that coexist with congeneric pollinators (i.e., *F. altissima* and *F. sycomorus*) are likely to reveal low or nonexistent host sanctions. We further suggest that it also will be instructive to determine the degree to which measurements of host sanction strength in this narrow pollinator-related context correlate with other aspects of the host's physiology (e.g., host's tendency to allocate resources to more or less productive leaf or root tissues). The degree to which there are correlations might give clues to the degree to which host sanctions can be selected independently from generalized resource allocation responses (22, 28). We note that selection could favor increased strength of host sanctions, given sufficient benefits of increased pollen dispersal relative to costs of detection and sanctioning of cheaters (22, 26–28).

We find that the cheater consistently produces significantly larger numbers of similarly sized offspring that carry as many eggs as the pollinator. This raises the question of why do we see the pollinator in this system at this locale at all? We found that the relative abundances of the different types of wasps varied dramatically throughout the seasons over two different, nonsequential census periods. Neither the seasonal variation nor its magnitude is consistent with, or easily explainable by, the relative reproductive success that we observed in our experiments (Fig. 3 and *SI Appendix, Figs. S5 and S6 and Tables S1 and S2*). Specifically, the relative percentage of pollinators produced in D-phase figs consistently exceeds that of cheaters when the west wind prevailed during the receptive B-phase of the corresponding crops (Fig. 5 and *SI Appendix, Figs. S5 and S6 and Table S2*). This observation is consistent with regional heterogeneity in fig and wasp populations that contribute to the pool of pollinator and cheater wasps arriving at the receptive fig trees in and near XTBG. We note that these sites are located close to the northern edge of the geographic range of *Ficus* (55, 57–59) (*SI Appendix, Fig. S5*).

A testable set of hypotheses that are consistent with these observations is that the pollinators are more characteristic of populations to the south and west, whereas the cheaters are more characteristic of populations to the north and east. We also expect that the fig populations that produce more pollinators will exhibit detectable sanctions against wasps that do not pollinate, while the populations that harbor higher densities of cheaters will largely or entirely lack evidence for these sanctions. Consistent with this conjecture, we note that one of the previous P+ and P− experiments was conducted on a different (more southern) population of *F. microcarpa* in which evidence for sanction was detected (29). Taken together, these studies are consistent with the concept of a geographical mosaic (57, 59). Pending further detailed studies, we suggest that different populations of *F. microcarpa* will exhibit predictably different sanction strengths against cheating wasps at local and regional scales (55, 57–59).

Finally, we note that of the more than 500 species of pollinating fig wasps that are described, this case of the cheater, *Eupristina* sp., associated with *F. microcarpa* in southern China is only the third-documented cheating species that has clearly been derived from an otherwise actively pollinating lineage (54, 55). There appears to be little doubt that the evolution and retention of a cheating species in actively pollinated fig–fig wasp mutualisms is still very

rare (22, 29, 30, 52–54, 56). However, we suggest that the reason that cheating in fig wasps appears to be rare is that most host fig species exhibit what are effectively host sanctions. In these cases in which host figs express detectable sanctions, individual wasps that provide the basic mutualistic service of pollination routinely experience higher relative fitness compared to the wasps that do not (22). We also suggest that it is within the prism of the host's response (i.e., the presence and strength of host sanctions) that the rarity of the loss of pollination behaviors and morphologies that lead to the evolution of cheating should be viewed.

## Materials and Methods

**Study Sites.** The study sites were located at XTBG (101°15' E, 21°55' N, altitude 555 m), Jinghong city (100°45' E, 22°01' N, 552 m), and Puer city (100°58' E, 22°46' N, 1,305 m), Yunnan province, southwest China, near the northern range limits of the fig–wasp system (SI Appendix, Fig. S5). All sites are within the documented range of wind-borne fig wasp pollination, which is routinely >10, up to 160 km (38–41). The Xishuangbanna region has a typical monsoon climate dominated by tropical southwest monsoon from the Indian Ocean roughly between May and October, which delivers about 80% of the annual rainfall with predominately moist, warm south and west winds. In contrast, the subtropical jet streams dominate the climate roughly between November and April with drier, colder north and east winds. This regular variation produces a pronounced dry season between November and April and a wet monsoon season between May and October (60).

**Study Species.** *F. microcarpa* is a monoecious species, with individual figs typically producing both seeds and pollinator fig wasps. It grows as a hemiepiphytic strangler or free-standing medium to large sized tree with a wide natural distribution extending from Australia northwards to Japan and westwards to India (61) (SI Appendix, Fig. S5). Within its natural range, *F. microcarpa* figs are small with ~200 female flowers, and an average anther-to-ovule ratio of ~0.09, characteristic of actively pollinated fig species (44). The figs ripen red and are mainly dispersed by birds (62), with secondary seed dispersal by ants (63).

In Xishuangbanna, in the southwest of China, discrete crops of *F. microcarpa* are produced year-round, often with thousands of individual figs per tree (54, 55). Usually, individual figs are pollinated by one to three foundress wasps (~65% with one foundress) (55). The tree's pollinator is recorded as *E. verticillata* Waterston, which appears to constitute a complex of multiple closely related wasp species across the range of the tree (Fig. 4 and SI Appendix, Fig. S3). Additionally, an undescribed *Eupristina* species that lacks the coxal combs (a critical morphological adaptation required for active pollination) coexists with *E. verticillata* in the figs of *F. microcarpa* (54, 55). This lack of essential morphology and the high rate of seed-free fruit suggest that this wasp might not be able to pollinate its shared host at this site (54, 55). We refer to this undescribed *Eupristina* sp. as the cheater.

**Measuring Wasp Sizes and Egg Loads of the Pollinator and Cheater.** Pollinator offspring numbers are limited by the number of eggs that they carry. Egg numbers are directly correlated with fig wasp size (23, 33). To test whether the fitness differences between the two species could be caused by differences in wasp size or egg numbers we randomly selected 5 to 10 female wasps from each of 69 D-phase figs from two trees (tree 1,  $n = 219$  wasps; tree 2,  $n = 161$  wasps), and identified the individual wasps to species based on presence of coxal combs (242 pollinators, 138 cheaters). We measured each wasp's head width under the microscope (LEICA S8AP0) with a micrometer (64) and counted her eggs by dissecting her abdomen (65).

**Ventral View of Pollinator and Cheater by Scanning Electron Microscope.** We were able to detect whether or not individual wasp species possessed coxal combs under a binocular microscope (LEICA S8AP0). After confirming that this was a diagnostic character (see below), we used coxal combs to distinguish the two *Eupristina* species as pollinator and cheater. We took scanning electron microscope (SEM) photographs of the ventral thorax and the fore coxae to demonstrate the presence of coxal combs and pollen pockets. SEM samples were prepared for observation following protocols described in Li et al. (66), using a Quorum K850 drier (Quorum Technologies), and then gold-coated in a Quorum Q150R sputter device (Quorum Technologies). Finally, we took SEM photos (using EVO LS10) of the pollination structures of two *Eupristina* species.

**Behavioral and Morphological Observation of the Pollinator and Cheater.** Prereceptive *F. microcarpa* figs were bagged to prevent fig wasps from entering or ovipositing in them. When figs of target trees were observed to be receptive (B-phase), different trees with male phase [D-phase, in the sense of Galil and Eiskowitch (34)] (SI Appendix, Fig. S1) figs were selected to collect the pollinator and the cheater foundresses used in the experiments. We placed the D-phase figs in individual nylon bags to let the wasps emerge naturally. We then carefully introduced the recently emerged live female wasps into the receptive (B-phase) figs. After cutting these figs open, we observed the foundress wasp's behavior in the fig cavity under the microscope for 30 min for each wasp. If the foundress characteristically waved the coxae of her front legs to disperse pollen grains (10 cases), this was recorded to be active pollination behavior (44). If there was no pollination behavior (24 cases), this was scored as "no pollination behavior." We then checked to determine whether the wasps with active pollination behavior also exhibited coxal combs.

**The Relative Fitness of the Pollinator and Cheater, and Their Effects on Seed Production.** We first bagged prereceptive figs on experimental trees to prevent disturbance by other wasps (67). We then acquired experimental wasps that had emerged from D-phase figs on different trees. We identified each live, recently emerged wasp to species (pollinator or cheater) by quickly visually assessing the presence of coxal combs under a microscope (LEICA S8AP0). Experimental pollinator wasps were of two types: either P+ pollen-carrying wasps that were allowed to collect pollen or P– pollen-free wasps, where the male flowers in the natal fig cavity were removed so that there was no chance for the wasps to collect pollen grains (22). The pollinator or cheater wasps were then placed carefully onto the surface of the bagged receptive (B-phase) figs that they could then enter.

We performed the following introductions into figs: 1) one pollinator with pollen (P+), 2) one pollen-free pollinator (P–), 3) one cheater (C), or 4) one pollinator with pollen (P+) plus one cheater (C). After the wasps entered figs, each fig was clearly labeled and experimental figs were rebagged. Figs were allowed to mature (taking 4 to 10 wk to mature to D-phase, depending on season), and all wasps that developed in each fig were collected and stored in 75% ethanol. Finally, the wasps were identified to species (based on presence of combs) and counted under microscope. In the direct competition experiments, only female wasps were identified to species in the figs that received both a pollinator and a cheater, as the males are difficult to identify to species (SI Appendix, Table S3). In addition to counting the wasps, the numbers of undeveloped female flowers (23–25), bladders [=incompletely developed wasps (32, 33)], and seeds in each experimental fig were also counted. We estimated total oviposition attempts in each fig as the number of successfully developed wasp offspring + the number of incompletely developed wasp larvae that did not emerge from their galls ("bladders") (31–33).

With these data we compared cheater and pollinator (both P+ and P–) individuals in terms of their effects on host seed production and their own relative fitness. Comparisons of the fitness of wasps when they are alone in a fig, either a P+ pollinator or a cheater, allowed the estimation of fitness effects under indirect competition (among wasps in different figs). Comparison of the relative fitness of cheater and P+ pollinators when they shared a fig allowed the estimation of the outcome of direct competition between individuals of these species. Furthermore, we used these data to estimate the strength of "host sanctions" [host sanction strength =  $1 - (\text{fitness of P– pollinators} / \text{fitness of P+ pollinators})$ ] (22), exhibited by these *F. microcarpa* trees. The experimental introductions were performed on five crops on three trees. The date of experiments, identity of the tree and crop, and sample sizes are shown in SI Appendix, Tables S1 and S3.

**The Phylogenetic Relationships of *Eupristina* Pollinators and Cheaters.** Eight individual wasps of both the pollinator (with combs) and the cheater (without combs) associated with *F. microcarpa* near XTBG were randomly collected from different trees for phylogenetic analyses. We also included the congeneric actively pollinating *Eupristina* wasps associated with six other fig hosts (*Eupristina altissima* from *F. altissima*, *Eupristina belgaumensis* from *Ficus drupacea*, *Eupristina cyclostigma* from *Ficus stricta*, *Eupristina koningsbergeri* from *Ficus benjamina*, *Eupristina masoni* from *Ficus benghalensis*, and the undescribed *Eupristina* pollinator associated with *Ficus maclellandii*), as well as other publicly available COI sequences from *Eupristina* (68). We also included samples of the cheater wasp species that is associated with local populations of *F. altissima* (53). The pollinator of *Ficus religiosa*, *Platyscapa quadraticeps*, was selected as a relatively closely related outgroup (48). The mitochondrial COI regions of these species was sequenced following protocols outlined in Wang et al. (68) or downloaded from GenBank in order to estimate



the phylogenetic relationships among these species. Sequences were aligned and adjusted by eye using BioEdit 7.0.9.0, and 670 aligned base pairs of data in the COI sequences were analyzed for the phylogeny (69–72).

**The Relative Abundances of Pollinators and Cheaters in Different Seasons.** We conducted two separate, year-long censuses of pollinator and cheater wasps associated with *F. microcarpa*. First, from August 2012 to May 2013, we sampled a total of 183 D-phase figs (34) (SI Appendix, Fig. S1) from 6 crops produced by different trees (30 to 32 figs per tree) across 3 different sites: XTBG (2 crops), Jinghong (3 crops), and Puer (1 crop). Second, we conducted weekly phenological censuses on 83 trees of *F. microcarpa* in XTBG and Jinghong. Here, we collected 349 D-phase figs from 14 crops produced by 13 trees (12 to 30 figs per crop) between October 2015 and March 2017. For each fig, we identified and counted the female pollinators and cheaters emerging from each D-phase fig. We used data on wind speed and direction, recorded automatically for each 10 min by the XTBG Ecological Station. This allowed us to associate the relative abundances of cheater and pollinator individuals in the D-phase fruit crops to wind pattern that corresponded to the date that each D crop was pollinated (i.e., when its B-phase was receptive). To do this, we identified the dominant wind direction that corresponds to the period of peak arrival of potential foundress wasps (during the 3-d period leading up to arrival and pollination) in order to identify likely directions of the source populations for the foundress wasps that pollinated any given tree's crop.

**Data Analysis.** Two-sample *t* tests were used to compare the difference of head widths and egg loads between pollinators and cheaters. We used a Fisher exact test to determine whether there was a significant relationship between the presence of active pollination and coxal combs, so that presence of coxal combs could serve as a diagnostic character between pollinators and cheaters. To address the question “Do cheaters actually cheat the host by reducing seed set?” we used nonparametric Mann–Whitney *U* tests to compare the numbers of seeds produced by single foundress pollinators and cheaters in three crops.

Phylogenetic analyses of the COI dataset were carried out by Bayesian inference using the program MrBayes 3.1.2 (69, 70). Modeltest 3.7 (71, 72) was used to select the best-fit evolutionary model. The Bayesian inference was conducted over 2,000,000 generations with sampling every 200 generations. Examination of the log-likelihood values suggested that stationarity was reached in ~200,000 generations. Thus, the first 1,000 trees were discarded and the remaining 9,000 trees were used to construct the 50% majority rule consensus tree, with the proportion of bifurcations found in this consensus tree given as posterior probabilities.

To estimate the host sanction strength expressed by *F. microcarpa* in and near XTBG, we compared the total number of wasp offspring produced by P+ (pollen-carrying) and P– (pollen-free) pollinator wasps (22). We also compared the number of galls (bladders + wasp offspring) produced by P+ and P– wasps, respectively. We used a GLM where the dependent was either offspring number or gall number (log-transformed for the analyses), fixed effect was foundress type (P+ or P–), random effect was crop, and covariate was number of flowers. The number of flowers in a fig is known to be able to affect the total number of wasps produced in a fig (23–25), and was a significant covariate here also. We additionally analyzed the individual crops with ANCOVAs with flower number as a covariate (results in SI Appendix). The proportion of figs that aborted after being entered by pollen-carrying (P+) or pollen-free (P–) pollinators was analyzed by Fisher exact tests. Host sanction strength was calculated (following protocols from ref. 22): We first calculated the relative proportion of P– figs that matured compared to P+

figs ( $M_R$ ) and the relative number of wasp offspring in unaborted P– figs compared to P+ figs ( $O_R$ ). If the proportion aborted did not significantly differ between the groups, we set  $M_R = 1$ . These two fitness components were then combined ( $M_R \times O_R$ ) to calculate the relative fitness of a single foundress P– wasp compared to P+ wasp ( $W_R$ ) for each crop. Sanction strength is calculated as  $1 - W_R$ .

To assess whether the fitness of single foundress cheaters differed from that of pollinators (P+), we compared the total number of wasp offspring produced by single foundress cheaters and P+ (pollen-carrying) pollinator wasps. We used a GLM, where the dependent was either offspring number or gall number (log-transformed for the analyses), fixed effect was foundress type (P+ or P– or C), random effect was crop, and covariate was number of flowers. We additionally analyzed the individual crops with ANCOVAs with flower number as a covariate (results in SI Appendix). The proportion of figs that aborted after being entered by cheaters (C), or pollen-carrying pollinators (P+) was analyzed by Fisher exact tests.

To determine if the fitness of cheaters differed from that of pollinators when sharing the same fig, we tested whether the number of pollinator and cheater offspring was different in three crops. We used R 3.6.2 to perform a randomization test as described in Jandér et al. (26). First, we compared the number of cheaters and pollinators among the three crops. We performed a hierarchical random sample for each crop; we then merged the data and calculated the mean difference (mean number of cheater minus mean number of pollinator) for each merged random sample. The process was replicated 9,999 times to create a distribution of the mean difference between pollinators and cheaters. The created distribution was then compared with the observed data to obtain a *P* value.

We collected a total of 20 crops of D-phase figs (6 in 2012 to 2013 and 14 in 2015 to 2016) during periods that corresponded to the warm, wet monsoon season (roughly May to October) and the cool, dry nonmonsoon season (roughly November to April). Crops collected from December to May were dominated by the cheaters (Fig. 6 and SI Appendix, Fig. S4), while crops collected from June to November were mostly dominated by the pollinators. Therefore, we separated all crops into two groups, corresponding to season. To compare the relative abundances of cheaters and pollinators encountered in crops ripening in these seasons, we calculated the percentage of pollinators and cheaters in each D-phase fig. A  $\chi^2$  test was used to compare the percentage difference of cheaters and pollinators with months and monsoon types. All analyses were conducted using R 3.6.2.

**Note.** A recent article has been published providing additional evidence for host sanctions in a 16th actively pollinated host fig species (73).

**Data Availability.** All study data are included in the article and SI Appendix.

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