

Original Article

Mortal combat and competition for oviposition sites in female pollinating fig wasps

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Aggressive contests between animals are common but rarely result in death because the benefits of winning a fight rarely exceed the cost of losing. Lethal combat can evolve, however, when the contested resource translates to much of the future reproductive success of each combatant. Female agaonid fig wasps pollinate and lay their eggs in the flowers within the enclosed inflorescences ("figs") of fig trees (*Ficus* spp.). Wasps rarely leave the first fig entered so the reproductive success of each "foundress" usually depends on the availability of flowers within a single fig. We report for the first time lethal combat between female agaonids, in the undescribed *Pegoscapus* sp. that pollinates *Ficus citrifolia* in southeastern Brazil. In staged dyadic contests, wasps showed no aggression outside or inside the fig until one foundress oviposited. The first wasp to oviposit then became aggressive, which usually resulted in the death of its competitor. Examination of dead foundresses in naturally occurring figs showed that injuring competitors, particularly through decapitation, was effective at reducing their oviposition rates. In a Panamanian *F. citrifolia* population, pollinated by another wasp species, *Pegoscapus tonduzi*, there was little aggression between foundresses in similar contests. We suggest that reduced aggression in *P. tonduzi* reflects less competition for resources essential for successful reproduction due to on average fewer foundresses per flower in Panama.

Key words: Agaonidae, contest behavior, female–female, *Ficus*, intraspecific competition, lethal combat.

INTRODUCTION

Aggressive fights between animals are common and occur in a wide range of taxa. Fights are costly, occur most often between evenly matched individuals over limited resources, and are usually won by those with the highest resource holding potential (Parker 1974; reviews in Hardy and Briffa 2013). High resource holding potential is often determined by relatively large body size and a preferential status regarding resource usage, for example being the occupier of a territory rather than an intruder attempting a take-over. Although the literature has tended to focus on male–male aggression associated with mate competition, female–female fights are also widespread, for example, in birds (Owens et al. 1994), fishes (Draud et al. 2004), arachnids (Elias et al. 2010), and insects (De Vita 1979; Roulston et al. 2003; Hardy et al. 2013). The factors influencing the occurrence and outcome of female–female fights can be similar to those affecting male–male encounters (Humphries et al. 2006;

Clutton-Brock 2009; Rosvall 2011). However, in addition to direct competition for mates, associated resources important to fecundity and/or the survival of individual offspring such as territories, food, and shelter are often important factors affecting and predicting female–female fights (Clutton-Brock 2009). Thus, females will fight over high-value resources essential to offspring production, with the individual to whom the resource has greatest value being most likely to win (Draud et al. 2004; Elias et al. 2010).

Lethal aggression is generally uncommon because the benefits obtained from winning a fight rarely outweigh the cost of losing. It is predicted to evolve when the value of the contested resource equates largely to the potential total lifetime reproductive success of each combatant (Enquist and Leimar 1990). Female–female lethal aggression has been recorded in mammals (McGraw et al. 2002), birds (Piper et al. 2008), and insects (Humphries et al. 2006). Although much lethal female–female aggression in vertebrates and solitary insects appears occasional, it is common in social hymenopterans, such as bees, ants, and wasps (De Vita 1979; Roulston et al. 2003; Hardy et al. 2013). Indeed, lethal aggression is most well known in honeybee queens, who fight to the death over

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the high reproductive opportunities provided by future monopolization of a colony (Gilley 2001; Tarpay et al. 2004).

Fig wasps consist of several families of small hymenopterans whose larvae only develop within the specialist enclosed inflorescences (syconia, colloquially “figs”) of fig trees (*Ficus* spp.; Weiblen 2002; Herre et al. 2008). The wasps consist of several families of nonpollinating species and the pollinators (Agaonidae; Weiblen 2002). Each of the 750+ species of *Ficus* has its own species (sometimes more than one) of pollinating wasp (Weiblen 2002; Cook and Rasplus 2003; Herre et al. 2008). The already mated females (foundresses) enter a receptive fig and spread pollen from their natal tree whilst laying their eggs individually into some of the flowers. In most species, foundresses do not leave the first fig they enter and have short life spans (<48 h; Dunn et al. 2008) so their total lifetime reproductive success relies on a single brief period of oviposition. Depending on the tree species, each fig can contain from approximately 100 to several thousand flowers and from one to several tens of foundresses (Herre 1989; Cook and Rasplus 2003). The intensity of competition between foundresses for oviposition sites is thus likely to vary but will be highest when individual wasp potential fitness is limited by more eggs (foundress number \times foundress egg load) than available flowers being present in a single fig. Prior to dispersal to the fig in which they themselves will reproduce, female agaonids mate within their natal fig and do not compete for males so precopulatory sexual selection or previous fighting experience is unlikely to influence female–female aggression. The biology of agaonids thus sets the stage for aggression to occur between foundresses, if an individual wasp can prevent a competitor from using flowers that can otherwise be used for its own offspring. This makes agaonids ideal for investigating how limited resources essential for offspring production (i.e., fig flowers) affect the occurrence and outcome of female–female agonistic interactions.

Compared with the well recorded and often fatal fighting between male fig wasps over access to females (Hamilton 1979; Cook 2005), little is known about aggression between female fig wasps. To our knowledge, there are 2 published records of overt aggression between female pollinating fig wasps. Ramirez (1970) reported anecdotally that New World *Pegoscapus* (previously *Blastophaga*) show intraspecific aggression (biting) during oviposition. Moore and Greff (2003) found that in ovipositing foundress pairs of the African species *Platyscapa awekei*, the larger individual would lift its smaller opponent to prevent it from ovipositing. This resulted in increased reproductive success for the larger wasp due to more oviposition sites (flowers) becoming available. Additionally, there is interference competition during oviposition in 4 species of Asian pollinating fig wasps from 2 genera, *Ceratosolen* and *Eupristina* (Wang et al. 2009, Wang RW, Sun BF, Dunn DW, unpublished data), although the precise nature of the physical interactions between individuals is unknown. No previous study has described aggression between pollinating fig wasp foundresses resulting in death and/or physical damage inflicted by the contest winner onto the loser(s).

We report for the first time overt lethal combat between foundresses in a pollinating fig wasp, an undescribed species of the genus *Pegoscapus* that pollinates *Ficus citrifolia* in southern Brazil. We attempted to answer 3 questions regarding the nature of this behavior. 1) Does aggression resulting in mortality occur only in the presence of a limited resource, in this case receptive *F. citrifolia* flowers? 2) Do injuries resulting from aggression—decapitation, limb loss, and ovipositor loss—result in reduced fitness via disrupted oviposition? 3) Does *Pegoscapus tonduzi*, the pollinator species of *F. citrifolia* in a more northerly part of the range of this tree species, exhibit the same aggressive behavior as the pollinator in the Brazilian population?

METHODS

Pollinating fig wasp life cycle

Pollinating fig wasps are pro-ovigenic, in that they emerge from their galls fully laden with mature eggs and do not produce further clutches during their brief lifetimes (Dunn et al. 2011). After mating, female wasps laden with pollen disperse from their natal tree and are attracted by olfaction to other conspecific trees that have a crop of receptive figs. “Foundress” wasps enter a single receptive fig through a specialist bract-lined tunnel, the ostiole. During this process, each foundress will usually lose both wings and often also some of its antennal segments. When inside the fig, foundresses will spread pollen as they oviposit individually into some of the flowers. They do this by inserting their ovipositors through the style of a flower to reach its ovary, into which they lay a single egg, before moving onto other flowers to repeat the process. Each pollinated ovary containing an egg that develops successfully becomes a gall in which a single wasp larva will reach maturity. In most *Ficus* species, after they have finished ovipositing, foundresses usually die within the first fig they enter (but see Moore et al. 2003).

Study system

Ficus citrifolia resides in the *Americana* section of *Ficus* and is monocious, meaning that both pollen-dispersing wasps and seeds are produced in each of its figs (Cook and Rasplus 2003). It is a medium-sized tree (up to 15 m in height) and has a wide Neotropical distribution from Florida to southern Brazil–northern Argentina. The pollinating wasp species of *F. citrifolia* differs between geographical locations. For example in Central America, including Barro Colorado National Monument (BCNM), Panama, where part of this study was performed, the pollinator of *F. citrifolia* is *P. tonduzi* (Herre 1989), whereas in Florida, it is *P. franki* (Wiebes 1995). In southeastern Brazil at Ribeirão Preto, where most of this study was performed, *F. citrifolia* is pollinated by an undescribed *Pegoscapus* species (Rasplus JY, unpublished data).

Do foundresses show aggression in the presence of a common resource?

Male flower phase figs (Galil and Eisikowich 1968) were collected from a single *F. citrifolia* tree that was growing in the campus grounds of the University of Sao Paulo, Ribeirão Preto, Brazil. These figs were placed collectively into a fine mesh bag for 24 h to capture the emerging wasps.

Experiment 1: aggression outside of the fig

Three trees that had crops of receptive (B-phase; Galil and Eisikowich 1968) figs that had been previously covered with fine mesh bags to prevent natural wasp infestation were used for this experiment. All 3 trees were growing in the university grounds at Ribeirão Preto. An arbitrarily selected pair of female wasps that had been collected as described previously were removed from the mesh bag, and then carefully placed onto the surface of a receptive fig with a fine paintbrush and allowed to enter naturally. Wasp behavior prior to entry was recorded. The maximum time for both wasps to enter a fig was 20 min. A total of 46 figs and wasp pairs were used for this experiment.

Experiment 2: aggression within the fig

Freshly emerged wasps, and receptive figs from 2 receptive trees (neither tree was used for experiment 1), were collected using the methods previously described. All figs had previously been covered with fine mesh

bags to prevent the natural influx of any pollinating or nonpollinating fig wasps, and after removal from the tree were taken to the laboratory. In the laboratory 2, arbitrarily selected wasps were allowed to enter sequentially 1 fig. After the wasps had entered, the fig was transversally bisected with the aid of a razor blade and 2 pairs of fine forceps. Because a *Pegoscapus* sp. foundress takes approximately 30 min. to crawl the entire length of the ostiole into the fig cavity, the second foundress had not reached the fig cavity when the fig was cut open. Passing through the narrow ostiole damages the antennae and wings of foundresses. Patterns of this damage differ between foundresses so it was thus possible to follow over time and identify each foundress within the fig cavity. The fig half containing both wasps was then sealed with a coverslip and placed under a binocular microscope at $\times 20$ magnification in order to score wasp oviposition behavior and aggression. Each pair of wasps was observed for 90 min. Wasps were then removed from their fig half, killed by immersion in 70% alcohol, and their body sizes estimated by the measuring of 1 hind tibia. This was achieved by using a digital camera connected to a Leica MZ16 stereomicroscope equipped with measurement software (LAS version 3.6). A total of 28 figs and wasps pairs were used for this part of the experiment, totaling 42 h of observation.

The receptivity of the flowers in each fig was confirmed by the use of a sterase test (Peroxtesmo KO kit) following the methods of Dafni and Maués (1998).

Do injuries disrupt oviposition?

Experiment 3: do injuries reduce oviposition?

Twenty-eight figs from 2 *F. citrifolia* trees, on the campus grounds at Ribeirão Preto, were collected. Foundress presence was confirmed by the presence of detached wasp wings on the outer ostiole bracts of each fig. After collection, all figs were returned to the laboratory for processing. Initially, each fig was bisected. If it contained any wasps that were still alive, the fig was discarded and the wasps it contained were not included in the study. If the fig contained all dead foundresses, they were carefully removed with a moistened paintbrush and placed in insect Ringer's solution. All figs contained foundress wasps ($N = 99$; range 1–13 per fig; mean \pm standard error = 3.93 ± 0.75) that had entered naturally. A total of 84 wasps were present in the 15 sampled figs that contained multiple foundresses (≥ 2 ; mean \pm standard error = 5.33 ± 1.03).

Because we knew that female–female aggression can result in physical damage, we assessed the injury status of each wasp by recording the number of missing leg segments, ovipositor damage, and any decapitations, using a system similar to that used by Murray (1987) for male nonpollinating fig wasps (Table 1).

Table 1
Injury scoring criteria used for dead female *Pegoscapus* sp. at Ribeirão Preto

Injury	Score
Missing tarsus	1
Missing tibia (including tarsus)	2
Missing femur (including tarsus and tibia)	3
Missing ovipositor	10
Decapitation	10

The total injury score is the sum of that for all legs and other body parts shown in the table. Total scores thus ranged from 0 (undamaged) to 38 (a wasp with no legs that had also suffered decapitation and ovipositor loss). Scores at 10 or above typically indicate loss of reproductive ability (such as more than 3 legs lost, ovipositor loss, or decapitation).

We did not record missing wings or antennal segments because both structures are damaged during entry to the fig through the narrow ostiole (Weiblen 2002). Furthermore, because female *Pegoscapus* fig wasps are black or dark brown, we could not record any bruising to their thoraxes or abdomens (*sensu* Murray 1987).

Each foundress then had the eggs remaining in its body counted. Briefly, the abdomen of each wasp was carefully split with 2 fine entomological pins mounted on metal handles. Each ovary was then removed and placed into a drop of insect Ringer's solution. The ovarioles were then carefully spread, again using the 2 fine pins, and counted. Because each egg is connected to an ovariole, ovariole counts equate to egg counts (Dunn et al. 2011).

Are the foundresses of *F. citrifolia* at BCNM aggressive?

Experiment 4: quantifying wasp aggression at BCNM

Wasp aggression at BCNM was quantified using similar methods as at Ribeirão Preto. For behavioral recording of live wasps, we used 3 *F. citrifolia* trees to produce the necessary material. To obtain multifoundress wasps to record injury data, we used a single tree not used for the production of other material. Because we knew that multifoundress figs in *F. citrifolia* at BCNM are uncommon (Herre 1989; Jandér and Herre 2010), we first prevented wasp access to a randomly chosen subset of pre-receptive (A-stage) figs by covering them with fine mesh bags. When the untreated figs on the same tree had been entered by pollinators, the bags were removed and figs were exposed to free-living pollinators for 24–48 h. This increased the probability of multifoundress entry to individual figs, which were processed as previously described.

Statistical analyses

For experiment 1 at Ribeirão Preto, we used a chi-square test to test the hypothesis that wasps exhibited competition avoidance behavior prior to entry into a fig. We did this by comparing the frequencies of replicates in which the pairs of wasps exhibited different categories of behaviors prior to entering their given fig.

For our second experiment, we used a generalized linear model (with binomial errors and a logit link function) to test if the size difference between the wasps (winner size – loser size), affected the outcome of a fight (first wasp to oviposit won the fight = 1, otherwise = 0; Briffa et al. 2013). Data for the 6 pairs of wasps that did not fight were excluded from this analysis.

For the data collected to examine if injuries affected oviposition, we first used a generalized linear model, which assumed a quasi-Poisson error structure (to correct for data over-dispersion), to best fit our data. Initially, the explanatory variables consisted of a single factor relating to foundress number (single foundress or multiple foundresses), flower number, and the interaction between the terms. The number of eggs remaining within each foundress was the response variable. Because *Pegoscapus* egg-loads vary little (coefficients of variation 11–19%; Martinson et al. 2014), we thus assumed that each wasp had once contained an average number of eggs, and that the more eggs remaining in a wasp was the result of fewer eggs laid.

For data that only consisted of wasps from figs containing multiple foundresses, we first categorized figs into those that contained only 2 competing wasps and those that contained 3 or more wasps. We then tested the hypotheses that these foundress numbers were associated with 1) the frequency of occurrence of foundresses that had laid all of their eggs and 2) the likelihood that wasps remained

injury free. To do this, we used 2 separate Fisher's Exact tests because the expected values for some components of each 2×2 contingency table were less than 5 (Zar 1984). For the same reason, we also used this test to examine the relationship between injury and total egg depletion. Because decapitations were common, we used a generalized linear model (with a binomial error function) to test how competition via foundress and flowers numbers influence this behavior. We also used this method to examine how the level of leg injury related to the likelihood of a wasp being decapitated.

Finally, we used 2 additional generalized linear models, with either a Poisson or a quasi-Poisson error function to measure how injury affected oviposition. Our first model used total injury scores as an explanatory variable of the number of eggs remaining in each wasp. We then repeated the analysis with injury partitioned into 3 separate components—decapitation, ovipositor loss, and leg damage—as 2 categorical and 1 continuous explanatory variables, respectively.

Each generalized linear model also included where appropriate foundress and flower numbers, and all biologically relevant interactions, as explanatory variables. Models that initially contained more than a single predictor were simplified by stepwise deletion of non-significant terms ($P > 0.10$; Crawley 1993).

RESULTS

Do foundresses show aggression in the presence of a common resource?

Experiment 1: before entering a fig

On the outer surface of figs, none of the wasps showed any overt aggression except for pushing each other if they came into contact whilst searching for the ostiole. Out of 46 wasp pairs, both wasps in 3 pairs failed to enter the ostiole and flew away so were not used for statistical testing. Of the remaining 43 pairs, in 11 (25.6%), both wasps simultaneously found the ostiole and entered it by crawling under the same bract, and in a further 11, both wasps simultaneously found the ostiole but entered it via different bracts. In 5 pairs (11.6%), 1 wasp found the ostiole before the other and both wasps entered it through different bracts, and in the remaining 16 pairs (37.2%), only 1 wasp found and entered the ostiole, whereas the other individual flew away. The frequencies of all groups of observations were not significantly different to those expected by chance (10.75 – the number of all replicates [$N = 43$] divided by the number of observational categories (4); $\chi^2_3 = 5.65$, $P > 0.10$) and suggests that wasps neither avoid nor aggregate on the outer surface of a fig prior to entry.

Experiment 2: inside the fig

Out of the 28 pairs of wasps observed within a fig, 22 (79%) pairs had a fight in which each wasp attempted to bite and push the other. In 20 of these fighting pairs (91%), 1 wasp killed the other, whereas in the remaining 2 pairs, 1 wasp immobilized the other. In 6 of the 22 pairs (27%), there was only a single fight, whereas in the remaining 16 (73%), there were multiple fights. In all 22 fighting pairs, aggression only began after 1 wasp began ovipositing. This can be determined because an ovipositing wasp adopts a characteristic posture when it inserts its ovipositor down a flower style, and then remains stationary for up to several minutes. After 1 wasp (the contest winner) had successfully killed or immobilized the other (the loser), it always immediately resumed oviposition. Until oviposition by 1 wasp commenced, no aggression was exhibited by either wasp,

even when both wasps came into direct contact when searching for their first oviposition site.

In 18 of the 22 fighting wasp pairs (82%), the first wasp to begin oviposition was the winner. The size difference between the 2 wasps did not significantly predict the outcome of fights (generalized linear model, binomial errors: $\beta \pm$ standard error [SE] = -15.88 ± 30.59 , $G_1 = 0.28$, $P = 0.59$).

Do injuries reduce oviposition?

As expected, none of the wasps in the figs that each only contained a single foundress had any injuries, apart from the detached antennal segments and wings that could be attributed to their entering the fig. Wasps from single foundress figs (mean \pm SE = 23.83 ± 15.33) had significantly fewer eggs remaining in their bodies than wasps from figs containing multiple foundresses ($N \geq 2$; mean \pm SE = 78.95 ± 5.86 ; generalized linear model, Poisson errors: $G_1 = 11.32$, $P < 0.001$).

Only 7 out of the 84 wasps (8.33%) from figs that contained multiple foundresses had laid all of their eggs. Out of the 84 wasps, 51 (60.7%) had sustained injury. In both cases, the frequencies of occurrence were not significantly associated with figs that contained only 2 foundresses or those that contained 3 or more foundresses (both Fisher's Exact tests, $P = 0.14$). Lack of injury was also not significantly associated with total egg depletion (Fisher's Exact test, $P = 0.70$). Of the 51 injured wasps, all of which had sustained leg injuries, only 2 (3.9%) had lost their ovipositors, with 31 (60.8%) having been decapitated. Both wasps that had lost their ovipositors had also been decapitated. The likelihood of decapitation was not significantly associated with the number of foundresses present in a fig (generalized linear model, binomial errors: $\beta \pm$ SE = -0.02 ± 0.09 , $G_1 = 0.03$, $P = 0.85$). Moreover, there was no significant association between the likelihood of decapitation between figs that contained 2 foundresses and those that contained 3 or more foundresses (Fisher's Exact test, $P = 0.46$). Wasps that had been decapitated (mean \pm SE = 11.06 ± 0.70) tended to have fewer leg injuries than those that had retained their heads (mean \pm SE = 12.95 ± 0.87 ; generalized linear model, Poisson errors: $G_1 = 3.63$, $P = 0.06$).

Wasps with higher levels of total injury retained most eggs (generalized linear model, quasi-Poisson errors: $\beta \pm$ SE = 0.04 ± 0.01 , % deviance explained = 12.68; $F_{1,47} = 8.12$, $P = 0.006$; Figure 1). When the 3 measures of injury (leg damage, decapitation, and ovipositor loss) were used as separate predictors, only decapitation significantly predicted the number of eggs retained. As expected, wasp bodies with heads (mean \pm SE = 43.37 ± 10.06) contained fewer eggs than those that had been decapitated (mean \pm SE = 93.77 ± 17.21 ; generalized linear model, quasi-Poisson errors: $F_{1,47} = 13.71$, $P < 0.001$).

Are the foundresses of *F. citrifolia* at BCNM aggressive?

At BCNM, the pollinators of *F. citrifolia*, *P. tonduzi*, showed much less aggression than the wasps at Ribeirão Preto.

In the direct behavioral observations, aggression was only observed in 1 out of 9 pairs of wasps, significantly fewer than what we observed at Ribeirão Preto (Fisher's Exact test, $P < 0.001$; Figure 2). In the 1 BCNM fig in which wasps exhibited aggression, 1 foundress was notably larger and more aggressive than the other. This large wasp repeatedly turned and bit in the air towards the smaller wasp if the smaller wasp moved close to the ovipositing

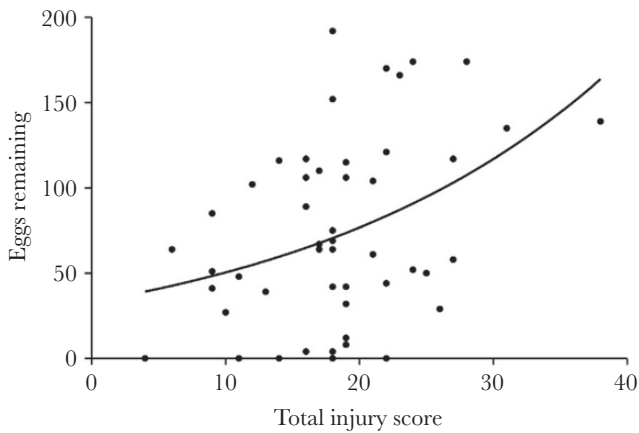


Figure 1
Relationship between total injury score and eggs left in wasps from multifoundress figs at Ribeirão Preto. The line shows the joined fitted values from a generalized linear model.

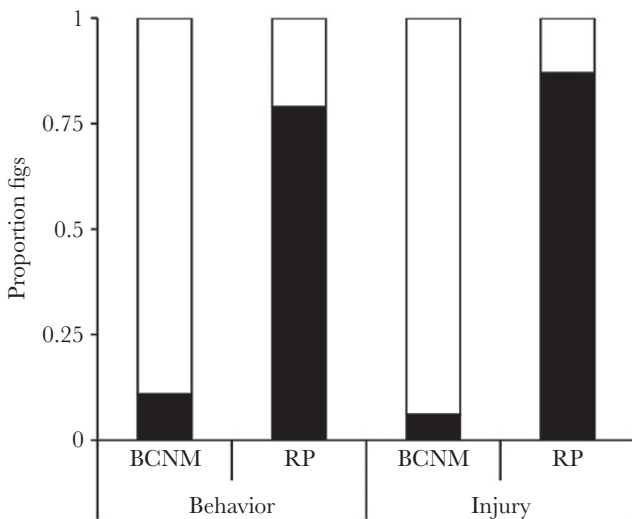


Figure 2
The proportion of the total number of figs at either BCNM or Ribeirão Preto (RP) used to measure the occurrence of aggression (black bars) or absence of aggression (white bars) between experimental live wasps (behavior) or natural foundresses (injury). The numbers of figs used (N) at BCNM were behavior = 9, injury = 48, and at RP behavior = 28, injury = 15.

larger wasp. There was no physical contact during this biting and no signs of injury. In the remaining 8 wasp pairs, there was no sign of aggression or injury; these wasps seemed to ignore each other despite periodic, apparently random body contact.

In the figs in which dead foundresses were screened for injuries, injuries only occurred in 3 of the 48 multifoundress figs, significantly fewer than what was observed at Ribeirão Preto (Fisher's Exact test, $P < 0.001$; Figure 2). The wasp injuries present at BCNM were similar to those at Ribeirão Preto but consisted only of leg injuries. Due to the small sample size, we cannot assess whether injured BCNM wasps laid fewer eggs than unharmed wasps.

The figs produced by *F. citrifolia* at BCNM had on average more flowers (mean \pm SE = 400 ± 6.51) than those at Ribeirão Preto (mean \pm SE = 376 ± 9.26 ; t -test: $t_{65} = 2.12$, $P = 0.04$). Moreover, on average, fewer foundresses enter each fig at BCNM (mean \pm

SE = 1.23 ± 0.09 ; Herre 1989) than at Ribeirão Preto (mean \pm SE = 3.93 ± 0.75 ; t -test: $t_{57} = -3.76$, $P < 0.001$). This results in more flowers per foundress and hence less intense competition for ovipositing sites in the BCNM population (BCNM = 325.37 vs. Ribeirão Preto = 95.67).

DISCUSSION

Our results enable 3 main conclusions to be drawn regarding variation in female–female aggression in the pollinator wasps of *F. citrifolia*. 1) In the Ribeirão Preto population, aggression is only exhibited in the direct presence of a limiting resource that immediately and directly contributes to the total lifetime reproductive success of each combatant—the receptive flowers within a fig. 2) Killing or incapacitating competitors directly reduces their ability to lay eggs and thus increases the resources available to the contest winner. 3) Aggression was common in *Pegoscapus* sp. at Ribeirão Preto but rare in Panamanian *P. tonduzi*, consistent with this behavior being associated with a higher level of competition for oviposition sites.

Aggression in *Pegoscapus* sp. at Ribeirão Preto concurred with theoretical predictions that individuals are only likely to fight over a limited resource, and that fatal fighting may evolve if the value of the resource contributes to a high proportion of future fitness (Enquist and Leimar 1990). More specifically, our findings also confirm the prediction of Moore and Greeff (2003), that fatal fighting may exist in female pollinating fig wasps when competition for flowers between foundresses is high. We found that aggression was absent prior to entry to a fig, and within the fig until 1 of the 2 wasps present began oviposition. Extreme aggression, which could result in the decapitation of 1 competitor, was then likely to ensue after being initiated by the first wasp to oviposit. This suggests that the first wasp to oviposit gains an information advantage over its competitor, for example that the resources (fig flowers) are of high value due to their receptivity to wasp eggs. Having this information may thus increase the likelihood of a wasp launching an immediate attack on a competitor, even if the potential costs of fighting were high, due to the high benefits of winning the fight. Because fig wasp behavior is highly stereotypical (Frank 1984; Jandér KC, unpublished data), it may be that aggression is not “switched on” until after oviposition has begun, thus ensuring that costly fighting only takes place in the presence of a valuable resource. Fighting costs may also be asymmetric if the foundress that attacks does so with an element of surprise, which may reduce the likelihood that the other wasp could injure the attacker by retaliation.

Wasps with higher levels of injury retained more eggs. However, the only injury incurred by individual wasps that significantly affected the ability to lay eggs was decapitation, after which oviposition would have abruptly ceased. The effects of leg injuries on oviposition showed no distinct patterns, other than decapitated wasps tending to have slightly fewer leg injuries than those that retained their heads. Some individuals may have been able to withstand a series of attacks and/or prolonged attacks that resulted in the removal of several leg segments prior to decapitation, although leg damage per se did not appear to affect oviposition. Alternatively, within the confines of a fig containing multiple foundresses, the aggression exhibited by individuals may take on a different dynamic to the patterns recorded in our experiment. Our data regarding foundress numbers, injury, and eggs remaining unlayed do not support an increase in polydactic contests (with a single winner) (*sensu* Sherratt and Mesterton-Gibbons 2013) in multifoundress figs. This is because the frequency of decapitations did

not increase with increasing foundress number. The first wasp to oviposit may launch a lethal attack on its nearest competitor, with other foundresses being subject to increased leg injuries from more nonlethal forms of aggression taking place amongst the remaining foundresses. Some wasps in multiple foundress figs were also able to remain injury free, suggesting that multiple foundresses did not automatically increase the likelihood of more than a single lethal dyadic contest occurring within a single fig. Further experiments exploring aggression involving figs that contain multiple competitors (≥ 3) are required.

Body size has been shown to influence the occurrence and outcomes of aggressive dyadic contests in a variety of taxa. For instance, evenly sized male red deer *Cervus elephas* are often more likely to fight over females than when one is clearly larger than the other (Clutton-Brock et al. 1982) because smaller individuals often avoid potentially costly contests they are unlikely to win. Thus, recognition of competitive asymmetry (e.g., through assessment displays) is likely to evolve when the accurate prediction of future reproductive success elsewhere is higher than the probability of losing a lethal contest (Enquist and Leimar 1990; Pereira and Prado 2005). Within the dark confines of a fig, wasps cannot visually assess the body sizes of potential opponents prior to physical contact. Moreover, selection for additional mechanisms to assess body size is probably low for pollinating fig wasps because any future reproductive success outside of the first fig entered for each combatant is limited. Foundresses are unlikely to exit the first fig entered, find and enter another, and then successfully oviposit (Herre 1989; Moore et al. 2003). Our data from Ribeirão Preto support this because wasp size did not significantly affect the likelihood of fight occurrence. More surprisingly, we found no evidence that wasp size affected the outcome of the fights that did occur, which conflicts with previous reports of aggression between females of another species of pollinating fig wasp, *Platyscapa awekei* (Moore and Greeff 2003). In *Pl. awekei*, the pollinator of the African fig species *F. salicifolia*, larger foundresses win most fights, especially in dyadic contests. The behavior of *Pl. awekei* foundresses during aggressive encounters differs markedly to that of *Pegoscapus* sp. in Ribeirão Preto. In *Pl. awekei*, large foundresses prevent smaller competitors from ovipositing by physically lifting them up in their jaws, but physical injuries have not been reported (Moore and Greeff 2003). Body size probably correlates with strength so larger, stronger individuals can physically prevent smaller competitors from ovipositing. Aggression in *Pegoscapus* sp. is more extreme, with decapitation of an opponent the most effective tactic to prevent competitors from ovipositing. Large size per se may thus not be as important if strength is not correlated with the ability to fatally injure or incapacitate an opponent. Additionally, large size may even be a hindrance within the confines of the fig cavity if maneuverability facilitates quick decapitation of a competitor, and/or other morphological components may be important in enabling decapitation of competitors. For example, in nonpollinating fig wasps, the males of some species have evolved enlarged mandibles to facilitate combat, which includes decapitating opponents (Hamilton 1979; Moore et al. 2009). More detailed comparative studies into the size and shape of the morphological components of female pollinating fig wasps, especially the mandibles, coupled with behavioral work into the occurrence of lethal and non-lethal aggression, are thus likely to be productive in the future.

Aggression levels differed dramatically between the 2 studied wasp species. Although aggression was common and often extreme in *Pegoscapus* sp. at Ribeirão Preto, it was much reduced in *P. tonduzi* at BCNM (Figure 2). We suggest that at the species level, aggression is driven by the average level of competition for oviposition

sites, that is fig flowers. At Ribeirão Preto, high average foundress numbers and fewer flowers per fig combine to increase competition during oviposition relative to BCNM. Moreover, in Florida, where *F. citrifolia* is pollinated by *P. franki*, competition levels are also lower than at Ribeirão Preto due to low average foundress numbers (mean foundress number = 1.89; Frank 1983, 1985). Previous behavioral observations of ovipositing *P. franki* foundress pairs ($N \approx 50$) did not report any aggression (Frank 1984). This supports our hypothesis that competition for fig flowers drives aggression in these wasps and concurs with theoretical predictions that the costs of aggressive interactions will increase when the resource value increases (Maynard Smith and Price 1973; Maynard Smith and Parker 1976; Enquist and Leimar 1987). For example, individuals may fight harder for increased payoffs. There are numerous empirical examples of this phenomenon within species (reviewed by Enquist and Leimar 1987), such as *Polistes* wasp queens being more likely to fight, and fighting for longer and harder, when resource value (nest size) is high (Tibbetts and Shorter 2009). Similarly, soil-nesting wasps are more likely to fight over existing nests as soil hardness increases because the value of already dug nests increases due to the high costs of digging a new nest in hard soil (Ghazoul 2001). Comparative studies are uncommon but include male nonpollinating fig wasps that fight for mating opportunities, with males being more likely to incur severe injuries in species in which there are few available females (West et al. 2001). Fighting in scelionid wasps is also more common in those species in which competition over resources essential to reproduction is most likely to occur (Waage 1982). Thus, the idea that intrasexual competition can drive variation in aggression among species is supported in the literature.

At the species level, although foundress and flower numbers correlate positively with fig size (Herre 1989), the increase in flower numbers in larger figs is much higher than for foundress numbers (Dunn DW, unpublished data). Because fig size varies considerably amongst *Ficus* (this ranges from the size of a pea to that of a tennis ball; Cook and Rasplus 2003), competition for flowers should be weakest in larger figs because the number of flowers available to each foundress is high. We therefore suggest for the future a comparative test of the hypothesis that aggression in pollinating fig wasps is negatively correlated with fig size.

Finally, current consensus leans towards the definition of sexual selection as “competition for mates” (Shuker 2010) so female traits that increase fecundity and/or offspring survival are “naturally” selected (Rosvall 2011; but see Clutton-Brock 2009). The extreme aggression we report in *Pegoscapus* fig wasps only occurs after mating has first taken place in the natal fig prior to dispersal (Weiblen 2002; Dunn et al. 2008) and is strongly linked to resources associated with fecundity and offspring survival (Dunn et al. 2011). The behavior we report is thus unlikely to be the product of sexual selection. However, the partitioning of mating and extreme intrasexual competition over resources between females in both space and time, makes agoonids a previously uncited model example to support definitions as to what is and what is not sexual selection in female animals.

In summary, we report for the first time lethal aggression between female pollinating fig wasps. As predicted, this occurs directly in the presence of a limited resource essential to the total future reproductive success of each combatant, receptive fig flowers. In staged dyadic contests, 1 wasp, the first to oviposit, was likely to kill or incapacitate the other, a tactic shown in nature to be effective in reducing competitor oviposition and hence reproduction. In *P. tonduzi*, the pollinator species of a Panamanian population of the same species of fig tree, aggression was low or absent, consistent

with lower levels of competition between ovipositing wasps having a reduced selective influence on aggressive behavior.

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