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Specialist fig-consuming lepidopterans can inflict costs to plant reproductive success that are mitigated by ant bodyguards

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A B S T R A C T

Insect herbivores can inflict substantial costs on plant reproductive success. Seed herbivory impacts directly by reducing the number of seeds and therefore the dispersal and reproductive potential of the plant. Fig trees, *Ficus*, provide keystone resources for tropical forests. The pollinating fig wasps develop inside figs, so consumption of unripe figs results in trees not only losing seeds but also their pollen dispersers. Selection to defend figs should therefore be strong. Seed herbivory is understudied in tropical forests and most data has been collected from fallen fruits. Here we use canopy sampling to identify fig-consuming larvae in central Panama and quantify both their consequences for the fig trees' reproductive success and the defensive value that ants provide against these larvae. Field surveys of 46 crops from nine fig species revealed that larvae could destroy up to 80% of figs on a tree. From seven *Ficus* species we barcoded (using COI) 51 individual fig consuming larvae (mainly Lepidoptera) that grouped into seven molecular operational taxonomic units. Lepidopteran larvae formed two feeding strategies, either stationary within a fig or tunneling between figs. Within the context of our study, stationary larvae were specialists whereas tunneling larvae were either specialists or generalists found on different *Ficus* species. Trees with ants had significantly fewer figs consumed by larvae (9% ± 17% (mean ± SD) for trees with azteca ants and 16% ± 24% for trees with other ants) than did trees without ants (51% ± 27%). Our results corroborate earlier findings that hosting ants can be an effective defensive mechanism for trees against seed herbivores or other antagonistic insects. Our study contributes to a wider body of research around the networks of insects associated with figs that highlights the importance of a multitrophic approach for understanding mutualism stability and persistence in the face of antagonism.

1. Introduction

Plant-insect interactions can drive major processes in plant ecology. Insects pollinate the majority of flowering plant species (Ollerton et al., 2011), insects can protect plants against unwanted visitors (Turlings et al., 1995; Offenberg and Damgaard 2019) and insect herbivores are a dominant mover of energy and matter through the terrestrial ecosystem (Seastedt and Crossley, 1984). Not only is herbivory very common, it is an interaction that can cost plants considerable resources and cause correspondingly large indirect effects on reproductive success (Marquis 1984; Coley and Barone 1996; Ramos and Schiestl 2019). A more direct effect on the plant's reproductive success derives from herbivores predating seeds and thereby directly reducing the female reproductive output (Collin and Shykoff 2010; Lecomte et al., 2017; Stachurska-Swakoń et al., 2018; Rodriguez-García et al., 2019). The effect of seed predation can be very high, reducing recruitment and plant density significantly (Janzen 1970; Connell 1970; Borchert and Jain 1978; Louda 1982). In the species rich tropics (Wilson et al., 2012) seed predation is not well studied (but see (Robertson et al., 1990; Herrerías-Diego et al., 2008; Basset et al., 2018; Gripenberg et al., 2019)).

Fig trees (Moraceae: *Ficus*) are keystone species in tropical forests (Mackay et al., 2018). They produce fruit year-round and up to 70% of local vertebrates can consume these fruits (Shanahan et al., 2001; Harrison 2003). Fig trees are in an intimate mutualistic relationship with their pollinators: fig trees are exclusively pollinated by agaonid fig wasps, and fig wasps can only develop inside figs (formally syconia, hereafter figs) (Galil and Eisikowitch 1968). Each species of fig tree is typically locally pollinated by only one species of fig wasp (Cruaud et al.,

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2012), however exceptions exist (e.g. Molbo et al., 2004; Su et al., 2008, Satler et al., 2022, 2023). Depending on the tree species, and sometimes the temperature, the figs containing the offspring of the pollinating wasps need a few weeks to several months to develop (e.g. Figueiredo and Sazima, 1997; Pereira et al., 2007). Fig trees and fig wasps have been interacting for around 75 MYA (Cruaud et al., 2012) and have radiated to include over 750 species (Berg and Corner, 2005). Much work has focused on mutualism stability from the perspective of fig trees and pollinator fig wasps (Jousselin et al., 2003; Jandér and Herre 2010; Dunn 2020; Zhang et al., 2021) but third parties such as predators (e.g., ants) and other antagonists of the mutualism (e.g., non-pollinating fig wasps, gall midges) can have an important effect on this mutualism (Schatz et al., 2006; Bai et al., 2008; Dunn et al., 2008; Segar and Cook 2012; Segar et al., 2014; Jandér 2015; Wang et al., 2018). An important group of fig tree antagonists are insect herbivores.

Fig trees can host both generalist and specialist herbivores that occupy a range of trophic guilds (Novotny et al., 2010; Volf et al., 2018). Fig trees and their associates (depending on their host-specificity) therefore make a considerable and distinct contribution to tropical diversity. Fig-consuming larvae have been found in multiple fig species, and are known to predate on the seeds and the developing wasps by foraging on unripe figs (Bronstein 1988; Sugiura and Yamazaki 2004; Piatscheck et al., 2018; Palmieri and Pereira 2018; Gripenberg et al., 2019). In comparison to leaf feeding insects, they are, however, understudied. Fig trees host pollinator wasps that develop in galls within the developing figs. Therefore, when a larva consumes a developing fig, it not only destroys the seeds and thereby decreases the fig tree's direct female reproductive success, but in monoecious species (that contain both male and female flowers within each fig) it additionally destroys the pollinator brood that would disperse the pollen to the next fig tree. Fig consuming larvae can therefore directly decrease the male as well the female reproductive success of the fig tree. Lepidopteran larvae can have a particularly devastating effect on crops, causing destruction of up to 100% of the fruits (Piatscheck et al., 2018). Although several studies have highlighted the presence of fig consuming lepidopteran larvae on different continents such as the Americas (Janzen 1979; Bronstein 1988; Jandér 2015; Palmieri and Pereira 2018; Piatscheck et al., 2018; Gripenberg et al., 2019), Africa (Compton 1993), and Asia (Sugiura and Yamazaki 2004; Yang et al., 2008), the primary focus of research on fig tree reproduction has been on the wasp groups; to date only two studies have focused entirely on fig consuming lepidopteran larvae (Sugiura and Yamazaki 2004; Piatscheck et al., 2018). Other types of fig consuming larvae (e.g. Diptera, Coleoptera, Hemiptera) typically have a less devastating effect on crops (reviewed in Palmieri and Pereira 2018). When seed and fruit predation is studied by collecting only fallen fruit, as is often done for practical reasons, data about losses in developing fruits that remain in the canopy are lacking, which can partly explain the knowledge gap concerning the effect of fig consuming lepidopteran larvae.

If fig consuming larvae are as destructive as previous studies suggested (Sugiura and Yamazaki 2004; Piatscheck et al., 2018), fig trees will benefit from defending their developing figs against these crop destroyers. Fig trees protect themselves against herbivores and parasites in different ways. Fig trees produce latex (Janzen et al., 1984; Farrell et al., 1991; Basset and Novotny 1999; Volf et al., 2018; Ramos et al., 2019) of which the sticky content hinders the performance of the herbivores' mouthparts (Ramos and Schiestl 2019). Some types of latex also contain toxic compounds like phenanthroindolizidine alkaloids and cysteine proteases, that reduce growth or kill non-specialist herbivores (Damu et al., 2005; Konno et al., 2004). As an indirect form of defense, fig trees can also host predatory ants (Thomas 1988; Bain et al., 2014; Harrison 2014) which can protect against herbivores and other antagonists such as non-pollinating fig wasps (Janzen 1966; Compton and Robertson 1988; Schatz et al., 2006; Bain et al., 2012; Wang et al., 2014; Jandér 2015).

This study focuses on fig consuming lepidopteran larvae in central Panama. The fig mutualisms (fig trees and pollinator fig wasps) of central Panama are among the most well-studied in the world (e.g. Herre 1985, 1987, 1989; Nason et al., 1996; Herre et al., 2008; Jandér and Herre 2010; Satler et al., 2022, 2023), yet other fig-associated organisms that can dramatically reduce the reproductive success of fig trees and their pollinators have been less well studied in this location (although there are some studies of non-pollinating fig wasps and of fig-specific nematodes (Herre 1993; West and Herre 1994; Marussich and Machado 2007; Jandér 2015, Van Goor et al., 2022)). A few species of fig consuming lepidopteran larvae have been reported in central Panama (Jandér 2015; Gripenberg et al., 2019), but little is known about their species diversity, ecology, and their effects on the reproductive success of the mutualistic partners. Through surveys, collections, and barcoding, we here identify the genera of fig consuming larvae, compare their host specificity, quantify their ecological effect on the fig mutualism, and investigate whether the presence of ants protects against the losses of reproductive success that fig-consuming lepidopteran larvae cause. Our study reveals that this seldom studied group of herbivores can have very large consequences for the reproductive success of this keystone mutualism. Furthermore, we contribute to a wider understanding of how fig trees and their pollinators persist in the face of antagonistic interactions.

2. Material and methods

2.1. Fieldsite and study species

Data was collected at the Barro Colorado Nature Monument (BCNM) in central Panama (9°09′ N, 79°51' W). Using small boats to access the canopies of fig trees growing by the shorelines, we observed, collected (2004–2021), and surveyed (2005, 2015–2021), fig consuming larvae from nine of the most common fig species, all monoecious. The fig species were of the subgenus *Urostigma* section *Americanae*: *F. americana* subsp. *americana: eugeniifolia*-form (also referred to as *F. perforata* in other publications), *F. bullenei, F. citrifolia, F. crocata* (also referred to as *F. trigonata* in other publications), *F.* aff. *crocata* (also referred to as "F. triangle" or *F.* near *trigonata* in other publications), *F. nymphaeifolia, F. obtusifolia, F. paraensis,* and *F. popenoei* (Berg 2007; Croat 1978; Herre 1989). We also opportunistically sequenced fig consuming larvae from *F. costaricana* and *F. dugandii* (both section *Americanae*) as well as *Ficus maxima* (section *Pharmacosycea*), but did not study these fig species in a quantitative way. This study only includes larvae consuming developing figs, not leaves or twigs.

From our observations we determined that the fig-consuming lepidopteran larvae present at BCNM, Panama, can be divided into two different feeding strategies: stationary larvae and tunneling larvae. Stationary larvae develop within a single fig (Fig. 1a) whereas tunneling larvae feed on multiple figs on a twig which they connect by a tunnel that they construct (Fig. 1b). These two groups are very easy to tell apart in the field once they are past their earliest development; all our surveys for determining infestation rate were at times when the larvae were at their later stages of development: stationary larvae filled their home fig, and tunneling larvae had eaten through several figs, having made holes in the sides of figs and connected them with frass and silk tunnels. Infestation rates were calculated differently for the different feeding strategies. The tunneling structures of the tunneling larvae are externally visible on the twig. We haphazardly chose numerous twigs, and for each fig on those twigs visually determined whether it was included in a tunnel or not. On trees with visible tunneling structures, we surveyed and counted 100–300 (in one case 87; see Fig. S1) figs per tree. We calculated the infestation rate for tunneling larvae as the number of figs included in a tunnel structure divided by the total number of figs surveyed on each tree. In contrast, figs containing stationary larvae do not look different than uninfested figs; on all trees we therefore haphazardly collected 50 figs (at times fewer were locally available, or more were collected) and opened them in the lab. We calculated the infestation rate

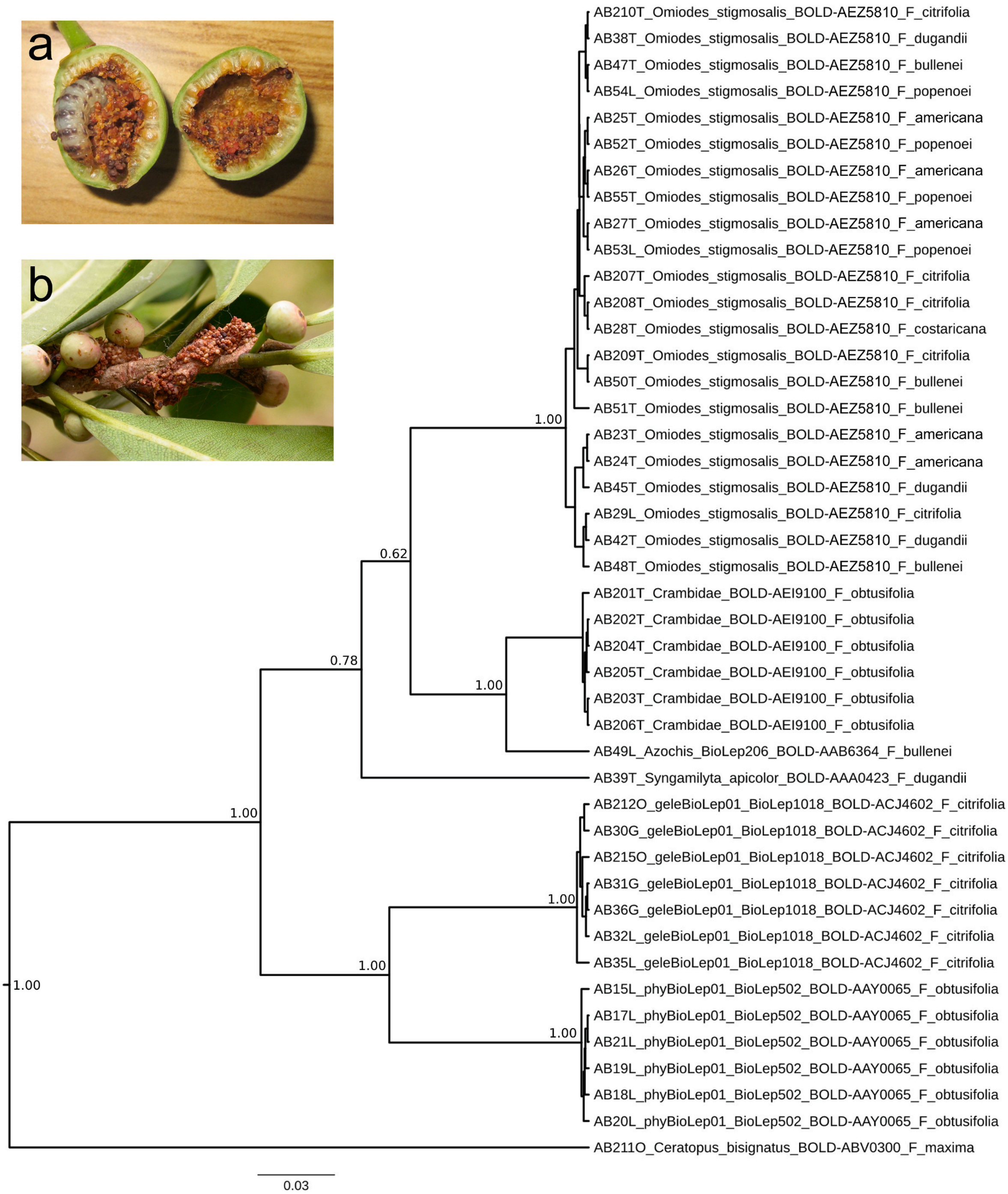

Fig. 1. a) Each stationary larva consumes a single fig – this larva (ACJ4602) is about to emerge from *F. citrifolia*. b) Each tunneling larva consumes multiple figs, connecting them with a tunnel of silk and frass. Here a tunnel of *Omiodes* sp. on *F. americana* subsp. *americana*. c) Phylogenetic tree with maximum clade credibility consensus topologies for COI. Posterior clade credibility given for major clades within each phylogeny. Tip labels denote individual voucher code, taxon assignment according to closest match in the BOLD database, BOLD Barcode Index Number (BIN) and *Ficus* host. Note that all moth species level identifications are tentative and the information provided here is intended to serve as context, please refer to the BOLD data set dx.doi.org/10.5883/DS-KOLF22 for the most current classifications.

for stationary larvae as the number of infested figs (each with a single larva) divided by the total number of figs opened from each tree. In total we determined the larval infestation rate for 46 trees. Additionally, the presence or absence of ants on the tree was visually determined for 42 out of 46 samples (Figs. S1 and S2 detail the sample sizes for each study species and where ant presence was assessed; Tables S1–S4 detail the sample sizes of each subset of the main data set used for the primary analysis and contingency tables). We recorded whether ants belonged to the genus *Azteca* (easily recognizable in the field; workers run with their gaster raised, and mud nests are usually present on the tree), or whether they were of a different genus: "other"; see Jandér (2015) for a list of common ant species found on fig trees in the area. Out of the nine fig species included in the survey, larvae were collected for barcoding from seven fig species of section *Ameriacanae* (*Ficus citrifolia*, *F. obtusifolia*, *F. americana*, *F. bullenei*, *F. costaricana*, *F. dugandi* and *F. popenoei*), and from *Ficus maxima* (section *Pharmacosycea*).

2.2. Barcoding to identify larval species

We sampled tissue from 51 individual larvae (stored in absolute ethanol at −20 °C) in order to obtain cytochrome oxidase I (COI) barcode sequences (primers and protocols are detailed in (Hebert et al., 2004; Wilson, 2012)). We sent samples as extracted and amplified DNA for sequencing at Macrogen Korea. We uploaded the sequences to BOLD (Barcode of Life Data System; Ratnasingham and Hebert, 2007) which assigned them to Barcoding Index Numbers (BINs) that we used as corroborating evidence, alongside photographs, to further improve our field-based identifications. We use BINs as proxy taxonomic units (Ratnasingham and Hebert 2013). For a subset of 29 samples (selected to span the greatest phylogenetic distance and most diverse clades), we also generated sequences for a fragment of CAD (Carbamoyl-Phosphate Synthetase 2, Aspartate Transcarbamylase, and Dihydroorotase) using primers and conditions outlined in Wahlberg and Wheat (2008). This nuclear gene was used to confirm the monophyly of the major MOTUs (Molecular Operational Taxonomic Units) and the overall topology of the phylogeny. Whilst COI is generally good for species delimitation in Lepidoptera, nuclear insertion of mitochondrial genes or incomplete lineage sorting can occur, and congruence between the nuclear and mitochondrial genomes provides more robust support for molecular operational taxonomic units. Our 51 sequences sorted into eight BINs which can be used as interim species level groupings (Table 1). We note that the BOLD database is dynamic, while this is a huge advantage (because species identifications improve with the accumulation of data) it does make our species labels liable to change. The species boundaries themselves are less likely to do so and we consider BINs to represent robust entities.

Bayesian molecular phylogenies were estimated using BEAST v2.6.3 (Bouckaert et al., 2019) as implemented on the CIPRES Science Gateway (Miller et al., 2011). For the COI matrix we selected a single partition grouping all codon positions. We set the substitution rate to 1.0 by using a clock rate of the same value and modeled substitutions at each site using an HKY + I + G model because the TIM2+I + G model selected in jModelTest2 (Guindon and Gascuel 2003; Darriba et al., 2012) faced convergence issues. Initial priors for the substitution model were selected according to those estimated in jModelTest2. Finally, we retained the default priors for a Yule model and ran two MCMC chains of 8 million generations (Yule 1924; Bouckaert et al., 2019). The combined COI and CAD matrix was partitioned into two loci both modeled with GTR + I + G substitution models, again the initial priors for substitution rates and other parameters were derived from jModelTest2. As with the COI only data set, we ran two MCMC chains for 8 million generations. Both analyses assumed a relaxed log normal molecular clock. Log files were analyzed in Tracer v.1.7.1 to ensure adequate Effective Sample Sizes (ESS values over 200) and convergence between chains. Trees were combined across runs and summarized as maximum clade credibility trees after excluding the first 10% of generations as 'burnin' using TreeAnnotator 2.6.3 (Drummond and Rambaut 2007).

Table 1

Taxonomic information of the fig consuming larvae in this study deriving from comparisons with sequences in the BOLD database. Each barcode sequence was assigned a Barcode Index Number (BIN). Unique BINs were created when accessions had no matches in the existing BOLD database. Along with the BIN we also present order and family level information, the nearest species match in BOLD, the ecological guild, and the number of individuals per BIN in our sample. Note that family level IDs are here derived from the closest BIN in BOLD and not using morphological features. However, according to BLAST the most similar sequence to AE19100 is from *Azochis* sp. BioLep206 (Crambidae). Morphological appraisal and phylogenetic monophyly also suggest Crambidae for this BIN.

BIN	Order	Family	Nearest Species	Max Distance within BIN	Distance to Nearest Neighbor	N	Status	Guild	Host in previous studies	Host in this study	References
AEI9100	Lepidoptera	Crambidae	Azochis Biolep206	0%	4.74%	6	Unique	Tunneling	N/A; genus *Azochis* associated with *F. carica*, *F. prinoides* and *F. stahlii*	*F obtusifolia*	dx.doi.org/10.5883/DS-KOLF22
AAA0423	Lepidoptera	Crambidae	*Syngamilyta apicolor*	1.12%	7.94%	1	Non-Unique	Tunneling	Unknown (Light)	*F. dugandii*	dx.doi.org/10.5883/BOLD:AAA0423
AAB6364	Lepidoptera	Crambidae	Azochis BioLep206	0.71%	2.88%	1	Non-Unique	Tunneling	Unknown but genus *Azochis* associated with *F. carica*, *F. prinoides* and *F. stahlii*	*F. bullenei*	dx.doi.org/10.5883/BOLD:AAB6364 and NHM HOSTS, Robinson et al., 2010
AAY0065	Lepidoptera	Pyralidae	phyBioLep01 BioLep502	0.68%	7.37%	6	Non-Unique	Stationary	Unknown (Light)	*F. obtusifolia*	dx.doi.org/10.5883/BOLD:AAY0065
ABV0300	Coleoptera	Curculionidae	*Ceratopus bisignatus*	1.61%	11.22%	1	Non-Unique	Stationary	*F. insipida* and *F. yoponensis*	*F. maxima*	dx.doi.org/10.5883/BOLD:ABV0300
ACJ4602	Lepidoptera	Pyralidae	geleBioLep01 BioLep1018	0.34%	7.61%	7	Non-Unique	Stationary	*F. citrifolia* and *F. colubrinae*	*F citrifolia*	Gripenberg et al., (2019), dx.doi.org/10.5883/BOLD:ACJ4602
AEZ5810	Lepidoptera	Crambidae	*Omiodes stigmosalis*	2.46%	1.16%	22	Non-Unique	Tunneling	*F. petiolaris* and Neotropical *Ficus* (unspecified species) in Florida and Costa Rica	*F. citrifolia, F. costaricana, F. popenoei, F. americana* subsp. *americana, F. bullenei* and *F. dugandii*	Piatscheck et al., (2018), dx.doi.org/10.5883/BOLD:AEZ5810

2.3. Statistical analyses ecological data

Some fig species are much more common than others, and fig trees fruit asynchronously. We collected data from as many crop-producing trees as we could in the time available, but some species are not well sampled in this dataset. We assessed the independence between fig tree species and the frequency of fig crops with ant presence using the Fisher's Exact Test for Count Data. We employed the same test to examine the independence between fig tree species and the frequency of fig crops infested by moth larvae. We found that the frequency of fig crops with ant presence was independent of the *Ficus* species ($p = 0.931$) (please refer to Tables S2–S4 for the contingency tables used and results from each test). We found that the frequency of fig crops infested by moth larvae was not independent of fig tree species ($p = 0.029$), but this result was driven by larvae in *F. citrifolia* ($p = 0.543$ with *F. citrifolia* excluded). Because of this, and due to a limited sample size ($n = 42$ fig crops where presence of ants was assessed) with unbalanced sample sizes across species, subsequent statistical analyses were conducted on the entire dataset without including *Ficus* species as a formal variable in the model.

To determine the effect that ant presence has on the reproductive success of trees we performed a Kruskal-Wallis test in R ver. 4.1.0 (R Core Team 2021) where the response variable was the proportion of infested figs and the explanatory variable was a single factor with three levels: (1) "no ants" (2) "other ants" and (3) "Azteca ants". We used pair wise Wilcoxon signed rank tests with a Bonferroni correction to determine the statistical significance of any differences in infestation rates across each level of the explanatory variable. We also performed an equivalent generalized linear model with a quasibinomial error structure. The response variable in this model was infestation (the bound values of infested and non-infested figs) and the explanatory variable was as above. A summary Analysis of Deviance table was produced using an F-test and the statistical significance of any differences in infestation rates across each level of the explanatory variable was explored with pair-wise comparisons using the 'emmeans' function of the R package 'emmeans' (Lenth, 2023). These tests used a data set of 42 crops and 3616 figs. Food webs were summarized using the R package 'bipartite' (Dormann et al., 2008).

3. Results

3.1. Natural history

The fig-consuming lepidopteran larvae were observed to be of two distinct feeding strategies: stationary larvae and tunneling larvae. Stationary larvae spent most of their larval period (from newly hatched to larva ready to pupate) each inside a single maturing fig, consuming both seeds and wasp galls within, until the remaining fig was essentially a shell filled with a larva (Fig. 1a). Stationary larvae chewed a hole and exited the fig at the phenological stage D (Galil and Eisikowitch 1968) when the rest of the figs on the tree were mature and adult wasps emerged from the figs.

Tunneling larvae instead moved from fig to fig inside a self-constructed silk and frass tunnel (Fig. 1b), consuming as few or as many figs as needed to complete growth. Both developing seeds and wasp galls were consumed, but sometimes only part of the fig contents were consumed before moving on to the next fig. Damaged figs invariably ceased development and dried up, and would have fallen from the tree if not held in place by the frass tunnel – these damaged figs could remain attached to the tree for many months past the maturation of the fig crop.

3.2. Larval infestation reduces the reproductive success of fig trees

We found fig consuming larvae on all fig tree species included in the study except on *F. nymphaeifolia* and *F. paraensis*; both of those had only one crop surveyed. We quantified larval presence on nine fig species (Figure S1 and Table S2). We found lepidopteran larvae on 61% of crops: of 46 trees, 20 contained stationary larvae, eight contained tunneling larvae, and 18 were not infested by larvae. Where fig consuming larvae were present, they could destroy a large proportion of the crop, but the level of larval infestation was highly variable across crops: larvae with stationary feeding strategy destroyed 2–84% of figs in a crop (without ants: 53% ± 27%, $n = 4$ crops, (mean ± SD), with ants: 24% ± 25%, $n = 13$ crops); larvae with a tunneling feeding strategy destroyed 9–73% of the figs in a crop (without ants: 62% ± 15%, $n = 4$ crops, with ants: 25% ± 20%, $n = 4$ crops) (Fig. 2a).

3.3. Presence of ants reduces larval damage

The vast majority (33 out of 42 trees where ant presence was assessed; 79%) of fig trees had ants visible on the tree: 17 trees had azteca ant (*Azteca* sp.) populations, 16 were populated by other ants, and nine trees had no visible ants. In our sample, ants were equally likely on all *Ficus* species (Fisher's exact test: $p = 0.931$; see Fig. S2). Fig trees hosting ants had dramatically fewer figs destroyed by lepidopteran larvae. The mean proportion of figs destroyed in trees with azteca ants was (mean ± SD) 9% ± 17% compared to trees with other ant genera 16% ± 24% and trees without any ants 51% ± 27% (Fig. 2b). There was a significant difference in infestation rates across categories of ant presence (Kruskal-Wallis $\chi^2_{2,39} = 11.626$, $p = 0.003$, GLM: $F_{2,39} = 9.950$, $p < 0.001$). The proportion of figs destroyed in trees without ants was significantly higher than the proportion of figs destroyed in trees with azteca ants (Wilcoxon: adjusted $p = 0.004$, GLM: adjusted $p < 0.001$) and other ants (Wilcoxon: adjusted $p = 0.029$, GLM adjusted $p = 0.001$), but there was no significant difference between azteca ants and other ants (Wilcoxon: adjusted $p = 1.000$, GLM: $p = 0.993$).

3.4. Phylogenies and BIN assignment

Lepidopteran larvae of six different species were found consuming maturing figs on ten different fig tree species in Panama. Our BOLD dataset 'DS-KOLF22' contained 51 individuals grouped into six non-unique (existing) BINs and two unique (newly established) BINs (Table 1; the unique BIN AEH9333 includes leaf feeding Lepidoptera sampled incidentally; this BIN is included in the BOLD dataset but is not otherwise part of this study). The majority of larvae sampled were Lepidoptera, but we also found one beetle larva (the larva collected from *F. maxima*). Almost all fig consuming Lepidoptera were in the superfamily Pyraloidea. The most numerously sampled species ($N = 22$) were close relatives to *Omiodes stigmosalis*, a tunneling crambid moth previously found associated with *Ficus petiolaris* in Mexico (Piatscheck et al., 2018), further sampling and examination is required for species assignation. Other crambids, including *Syngamilyta* sp. and *Azochis,* were rare in our sample. Of stationary larvae, moths of BIN ACJ4602 had previously been reared by Gripenberg et al., (2019) from *Ficus colubrinae* and *F. citrifolia* in Panama. Previous records of moths from most other BINs were from individuals caught at light by Janzen et al. (Table 1; Ratnasingham and Hebert, 2007) in Costa Rica, and the host plant was not determined. Our unique BIN AE19100, a tunneling larva on *F. obtusifolia,* matched most closely to the crambid Azochis BioLep206. We note that BOLD BINs are reliable for determining Operational Taxonomic Units (OTUs) and clustering based on genetic similarity, but that like all barcode-based approaches they rely on a well populated data base (Solé-Cava and Wörheide 2007). In the case of BIN AE19100 we await more comprehensive sampling before speculating further on identification.

The single and multigene phylogenies were both well supported and well resolved and are displayed in Fig. 1c and Fig. S3. Molecular data allowed us to assign individuals a Barcode Index Number (BIN), these are reliable provisional taxonomic units and approximate species level groupings (Ratnasingham and Hebert 2007, 2013). Associating these

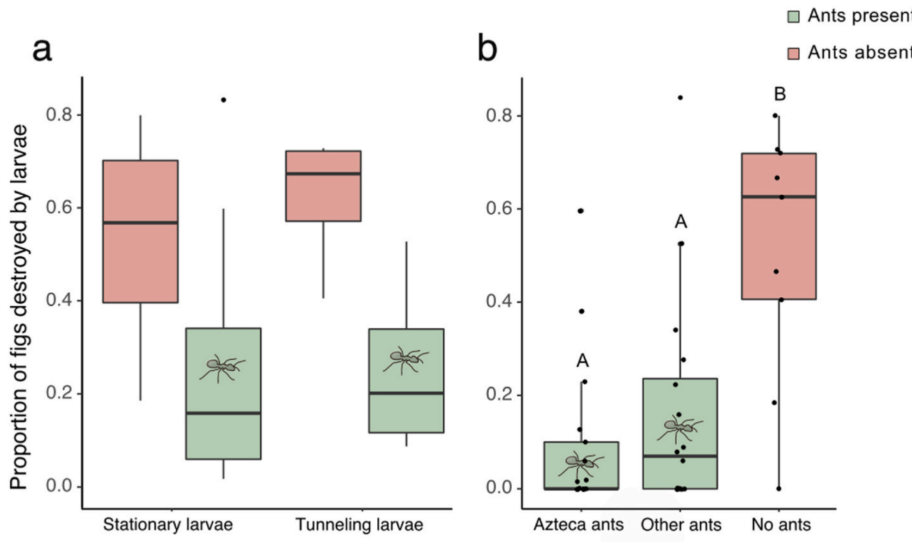

Fig. 2. a) Stationary larvae and tunneling larvae destroyed a similar proportion of the fig crop on the subset of trees that were infested. b) Trees with ants present had a much lower proportion of figs destroyed compared to trees without ants (including both infested and uninfested trees). Letters in figure b show significant differences. The sample size of each group is given in table S1.

OTUs with Latin binomials requires the sequencing of additional genes and taxa as well as expert taxonomic evaluation. At a minimum we can use these groupings to define monophyletic clades for downstream analysis.

3.5. Food webs

At the guild level stationary larvae were largely associated with a distinct set of hosts when compared to tunneling larvae, with the former being more specialized (Fig. 3a). Most species of moth were specialists attacking only one fig species, but most fig species hosted more than one moth species. Indeed, only the *Omiodes* sp. (AEZ5810) larva was found to attack more than one host *Ficus* species and thus connect modules (Fig. 3b).

4. Discussion

The results of this study show that the effect of Lepidopteran predation on the reproductive success of fig trees and pollinators in Panama can be extensive. Across nine surveyed fig tree species, 54% of the crops had fig consuming larvae present. The proportion of infested figs ranged between 0 and 84% of the crop; this large variation could partly be explained by the presence or absence of ants. Fig consuming lepidopteran larvae can thus have a large impact on the reproductive success of fig trees, both the female component (seeds) as well as the male component (pollen-dispersing wasps). Larval fig consumption resulted

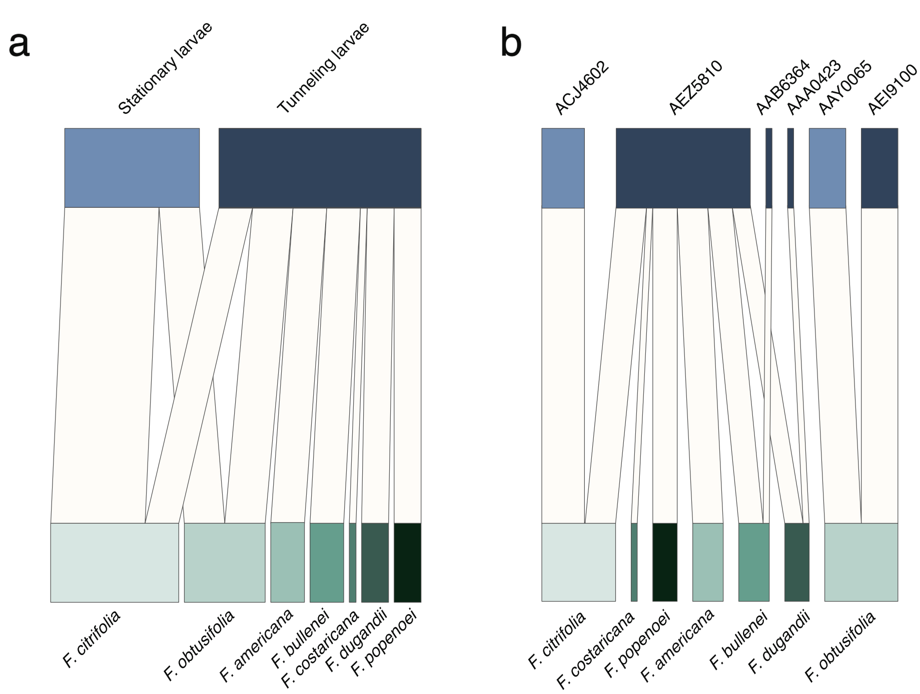

Fig. 3. Guild level (a) and species level (b) bipartite food webs. The lower level represents the host and the upper level the herbivore. The width of each block is proportional to the number of individuals included.

in up to 84% of a tree's resources invested in reproduction being lost. Barcoding of fig-consuming larvae from ten different fig tree species revealed that the fig-consuming larvae belonged to six different species of moth, and one beetle.

The infestation rates in this study were higher than that of fig consuming moths studied in Japan (Sugiura and Yamazaki 2004) and Mexico (Piatscheck et al., 2018). In the Japanese study 0–38.5% of the figs on trees from six different *Ficus* species (*F. superba, F. variegata, F. virgata, F. irisana, F. bengutensis* and *F. septica*) were infested with moth larvae compared to 0–84% in our study. In the study of Mexican *F. petiolaris* there was one tree with 100% larval herbivory, but the mean infestation rate across the different sites was between 0 and 40%, compared to 54% in our study. Taken together, these three studies show that the infestation rates of fig consuming moth larvae are highly variable across trees, times, and sites. In a similar range, Bronstein (1988) found that up to 20% of *Ficus pertusa* figs in Costa Rica were infested with weevils and stationary moth larvae. When comparing the reproductive consequences of the larval infestation with the consequences of infestations by non-pollinating fig wasps or nematodes (Bronstein 1991; Herre 1993; Van Goor et al., 2018, 2021; Shi et al., 2019; Zhang et al., 2021), it is important to emphasize that infestation by non-pollinating fig wasps or nematodes often does not lead to fig abortion, meaning that even with a decrease in pollinator number or pollinator lifespan, there will nevertheless be some seeds and pollinators produced by the infested figs (although exceptions are known: Silva and Pereira, 2018; Segar et al., 2014).

Our study highlights previously unrecognized diversity in neotropical fig feeding moths, and links larval species to fig host species. However, an increased barcoding effort is needed to confirm species concepts and patterns of host-specificity across regions (Mally et al., 2019). The three most abundant fig consuming larvae of our study was the tunneling larva *Omiodes* sp. and the two stationary larvae ACJ4602 and AAY0065. The tunneler *Omiodes* sp. has previously been found on unspecified *Ficus* species in Costa Rica and Florida, on *F. petiolaris* in Mexico (Piatscheck et al., 2018), and in this study on six different fig species. In contrast, the two species of stationary larvae, ACJ4602 and AAY0065, formed interactions with only one *Ficus* species each (Fig. 3b). We barcoded only a subset of all the stationary larvae that we found, but stationary Lepidopteran larvae are very common in *F. citrifolia* and *F. obtusifolia*, yet we have not encountered them in any other of the fig species in the area that we have studied here (this study, and KCJ pers. obs.; although Gripenberg et al., 2019 found ACJ4602 also in *F. colubrinae* that we did not study due to its rarity). We speculate that these stationary larvae are indeed relatively species-specific, but suggest that a more comprehensive sampling strategy is needed to confirm this. We hypothesize that any potential specialization may be related to adaptation to the nutritional content of a single fig (Kalko et al., 1996), the duration of fig development (time from pollination to maturation vary across the studied species (KCJ unpublished)), or to the chemical defenses of the fig (Villard et al., 2019). Stationary larvae develop within a single fig (syconium) until crop maturation and are therefore limited by the resources, both in size and time, that a single fig provides. Tunneling larvae, on the other hand, are not limited by the resources of a single fig, and they also seem to leave the figs well before crop maturation (KCJ and LD pers. obs.). Investment into leaf secondary metabolite concentration and diversity varies across species, for example some fig species produce a wide range of alkaloids while others do not (Volf et al., 2018; Villard et al., 2019). It is not unreasonable to expect similar levels of variation with respect to the syconium wall, and a correspondingly varied palatability across fig species.

Fig consuming weevil larvae have been found in several neotropical fig species (Bronstein 1988; Palmieri and Pereira 2018), including at our study site (Gripenberg et al., 2019). However, other than the single weevil larva from *F. maxima*, we did not find any fig consuming beetle larvae in our samples. It is possible that in central Panama fig consuming weevil larvae are relatively rare compared to moth larvae, but we are reluctant to draw conclusions about relative abundance from our data because both the timing and technique of sampling (collecting fallen figs (as in Gripenberg et al., 2019) versus figs still on the tree (as we did here)) will affect the fauna one encounters. In this study we typically collected figs close to the stage of wasp emergence (late C-phase or early D-phase; Galil and Eisikowitch, 1968), so if figs infested with weevil larvae had already fallen off the tree by then we would have missed them. For a comprehensive survey of all types of fig consuming larvae a different sampling technique would be needed.

When insect herbivory affects reproductive success as severely as found here (up to 80% of fruits destroyed), trees would benefit from having defenses. This study shows that in trees with ants present, the proportion of destroyed figs was significantly lower than when ants were absent. The distribution of data points that is shown in Fig. 2b suggest that azteca ants might protect a tree better (having more cases of zero infested figs) than the category "other ants". This corresponds to the observation that azteca ants are generally more aggressive than other ant genera encountered on fig trees in the area (*pers. obs. LD, KCJ*). Housing predatory ants is a way for plants to outsource their defenses against herbivores (Janzen 1966; Agrawal 1998; Rosumek et al., 2009). In *Ficus*, ants are known to feed on the insects that get attracted to the figs, including pollinating and non-pollinating fig wasps, and earlier studies have shown that the presence of ants reduced the number of non-pollinating wasps, fig consuming larvae, and aborted figs (Bronstein 1988; Compton and Robertson 1988; Schatz et al., 2006; Wang et al., 2014; Jandér 2015). Piatscheck et al. (2018) suggested that ants on *F. petiolaris* in Mexico might not have an effect on the ovipositing *Omiodes stigmosalis* because the ants there were diurnal and the moths nocturnal. We hypothesize that even strictly diurnal ants can eat exposed moth eggs that were oviposited during the night. Although we have not yet observed this at our study site, studies on other species confirm ant predation of lepidopteran eggs (Mansfield et al., 2003; Suenaga 2017; Baldwin et al., 2020). Some ant species may also be active at night (Reid et al., 2011; Narendra et al., 2017; Sheehan et al., 2019), directly preventing moths from ovipositing, in a manner similar to that of ants deterring parasitic wasps from ovipositing into figs (Jandér 2015). Preventing ant access to fig-bearing twigs (Compton and Robertson 1988; Schatz et al., 2006; Wang et al., 2014) would be a good way to experimentally test the effect that we found in this study. A question that remains is whether ants are specifically attracted to *Ficus* trees and if so, by what mechanism? Some other *Ficus* species are known to provide hollow structures in which ants can build nests or to attract ants by secreting nectar from extrafloral nectar glands (Koptur 1992; Maschwitz et al., 1994; Blüthgen 2003; Bain et al., 2014; Harrison 2014). In contrast, the *Ficus* species of this study have no known extrafloral nectar glands or specific plant-provided structures for ants to live within. Possibly the insects that get attracted to the various stages of developing figs are a sufficiently attractive food source for ants to reside in fig trees (e.g. Schatz et al., 2008), or perhaps the surface structure or branch geometry of fig trees are appealing for nest-building. Studying whether ants reside in *Ficus* more often than in other tree genera could be a start. Another possibility is that ants residing in nearby trees temporarily forage in fig trees during their fruiting period.

5. Conclusion

Our results contribute to the wider understanding of fig communities and suggest an important role for regulation of antagonists by a third party. By studying multi-trophic interactions, we can understand the complexity of ecological communities, how they assemble and how they persist. Reduction in reproductive success for the fig tree host was extensive and similar for both feeding guilds of moth, but species behavior and preferences can shift in response to changing abiotic conditions. The fig mutualism is a useful study system because the reproductive success of both mutualistic partners is easily quantified, but our findings have more general implications to more open networks.

Moreover, while biotic protection in this case appears to have low maintenance costs, relying on such a strategy may cause potential vulnerability, should the interaction break down due to shifts in resource availability or climate that may then pose as existential threats. Our approach also highlights the information gain that can be achieved by direct canopy sampling (as opposed to seed traps) and encourages further study in figs and beyond.

Data accessibility

Sequence data are available on BOLD (dx.doi.org/10.5883/DS-KOLF22) and on GenBank and EMBL: accession numbers OQ865378–OQ865403. Ecological data and R code are available on FigShare (https://doi.org/10.6084/m9.figshare.22762226).

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CRediT authorship contribution statement

Lisette van Kolfschoten: Formal analysis, Funding acquisition, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Miriam Adu Asantewaa:** Formal analysis, Investigation, Writing – original draft. **Lovisa Dück:** Investigation, Methodology, Writing – review & editing. **Simon T. Segar:** Formal analysis, Investigation, Methodology, Supervision, Visualization, Writing – original draft, Writing – review & editing. **K. Charlotte Jandér:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data are available in the repositories listed in the 'Data accessibility' section at the end of the manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.actao.2024.104016.

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