Received: 14 November 2023 | Accepted: 2 April 2024
DOI: 10.1111/1365-2435.14575

Functional Ecology BRITISH ECOLOGICAL SOCIETY

RESEARCH ARTICLE

# Fitness effects of symbiotic relationships between arthropod predators: Synergy in a three-way spider symbiosis

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**Funding information**
Holsworth Wildlife Research Endowment; Australian Government Research Training Program; National Geographic Society, Grant/Award Number: WW-058R-17

**Handling Editor:** Mayra Vidal

## Abstract

1. Symbiotic relationships shape ecological communities and often involve more than two species. Yet few experimental studies examine the impact of symbioses involving three species, particularly any mediating role of third parties, and none involving symbioses of predators.
2. We investigated experimentally the synergistic and antagonistic fitness effects of three symbiotic spider predators across a broad latitudinal range and involving different species combinations.
3. The three-dimensional web complex of *Cyrtophora* spiders is a habitat patch to different associates—species of *Argyrodes* and web-building *Leucauge* spider guests. Our field experiments, which manipulated the presence of each guest species and determined the subsequent host weight change, revealed a remarkable consistency in fitness outcomes across the three populations, with the consequences of the interactions between two species depending upon the services provided by a third.
4. *Cyrtophora* hosts intercepted more prey when web-building *Leucauge* guests were present and thus gained more weight. In contrast, *Argyrodes* guests exerted a fitness cost on their *Cyrtophora* host, but only when *Leucauge* guests were absent. A comparison of the prey consumed by *Cyrtophora* hosts and *Argyrodes* guests revealed that their diets (reflected in the size of prey) overlapped less in the presence of *Leucauge* web-building guests.
5. Our novel experimental study highlights the importance of exploring synergistic effects in multi-species symbioses.

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KEYWORDS
arthropod predators, interspecific interactions, multi-species networks, symbioses

# 1 | INTRODUCTION

The nature of symbiotic associations, where different species live together (De Bary, 1879; Saffo, 1992), is traditionally couched in terms of the relative benefits and costs for the species, that is, whether one symbiont has positive, negative or neutral effects on its partner. Symbioses may range from obligate, species-specific associations to those where one species may associate with one of many different species. Accordingly, investigations of symbioses typically adopt a dyadic perspective, generally quantifying the consequence for one species of engaging (or not) with the other species. For example, in protection mutualisms, individuals of one species may assist defending individuals of the other species against natural enemies (sensu Crawley, 1992), such as predators and parasitoids (e.g. Heil & McKey, 2003; Pierce & Dankowicz, 2022). However, symbioses frequently involve more than two species, and additional participants can alter the nature of the relationships between symbionts (Medina et al., 2022). For example, a third party may exploit a mutually supportive interaction between the primary symbionts (Elgar et al., 2016; New, 2017). While we know that the presence of third-party species strongly drives outcomes in positive (mutualistic) interactions among mixed-guild symbioses, less attention has focused on whether another species can mitigate against the effects of a negative (e.g. parasitic) symbiotic relationship (Chamberlain et al., 2014). Identifying the net effects of species interactions, particularly in multi-species networks, has proved challenging because they can vary according to environmental conditions (Daskin & Alford, 2012) and contexts (Hoeksema & Bruna, 2015). Mutualisms are especially prone to context (Hoeksema & Bruna, 2015) because the benefits for both partners also attract costs, which can depend on individual context: if these costs exceed the benefits for one partner, then both will typically experience negative net effects (Bronstein, 2001).

Arthropod natural enemies are typically described as predators, parasites or competitors (following Crawley, 1992), and predatory spiders, who typically react antagonistically to other spiders, including conspecifics, might be regarded as unlikely candidates to form symbiotic relationships (but see Cocroft & Hambler, 1989). Nevertheless, some spiders form multi-species symbioses, typically involving at least one web-building host species (Elgar, 1993), which may provide benefits in the form of food resources, including intercepted prey, proteinaceous web silk and even the host. The host web can be a microhabitat, attracting and supporting many species of spider predators and guests (see Figure 1; Vollrath, 1987), who, unlike other typical multi-species symbioses, can interact simultaneously. Laboratory experiments involving simple manipulations of single species highlight the exploitative nature of these interactions (Koh & Li, 2002, 2003), but field experiments reveal mutualistic interactions (Elgar, 1994; Peng et al., 2013), and highlight the largely unrecognised complexity of symbioses involving arthropod predators.

Tent-web spiders *Cyrtophora* (Araneidae) build substantial three-dimensional webs, comprising a sheet web supported by a tangle of threads that forms an upper barrier web (Figure 1a). The single resident spider typically hangs beneath the sheet web, where it captures prey, and only ventures onto the barrier web to conduct repairs. Small (ca. 10 mm) web-building *Leucauge* (Tetragnathidae) spiders, who use the host tangle threads to support their single resident orb-webs, are typically described as commensals, based on the resource division between these web-building guests (Rypstra, 1979). Finally, the host tent-web supports *Argyrodes* (Theridiidae) spiders, which can also be found on the webs of other, large (>20 mm) web-building spiders, including orb-weavers *Nephila* (Whitehouse, 2011) and sheet web spiders *Psechrus* (Elgar, 1994). The smaller (<10 mm) *Argyrodes* species form symbiotic relationships with their web-spinning hosts (Elgar, 1993) and are commonly reported as kleptoparasitic because they gain food resources from the host's web without apparent cost to the host (Tso & Severinghaus, 2000). The co-location of different species of spiders on the same web offers considerable opportunities to investigate, using field experiments, the nature of interspecific interactions between arthropod predators: the web complexes are easy to find and monitor; the mobility of the host spiders is low, allowing field experiments to reveal fitness effects; and the behaviour of the spiders is readily observed. The combination of partners in this multi-species symbiosis may vary geographically because the geographic range of the *Leucauge* and *Argyrodes* guests often exceeds that of their common hosts across different spatiotemporal scales.

Here, we use field experiments to reveal the foraging consequences for participating spiders of multi-species symbioses involving *Cyrtophora* hosts and their two cohabiting spider guests—orb-weaving *Leucauge* and kleptoparasitic *Argyrodes*. Significantly, we provide unprecedented geographic generality of our empirical inferences by replicating the field experiments at different geographic locations across a broad latitudinal range, with each experiment involving different species' combinations of participating partners (Figure 1). We ask the following questions: (1) do each of the three participants have individual effects on host hunting efficiency; (2) are the feeding regimes (reflecting the range of prey sizes) of *Cyrtophora* hosts and *Argyrodes* guests moderated by the presence of *Leucauge* web-building guests; and (3) what are the potential fitness consequences of the symbioses for the host, estimated by weight change, when alone or co-occurring with different combinations of guest species? By manipulating the species composition of arthropod predators in this system, our study reveals the additive and synergistic effects of different symbionts in a multi-species symbiosis.

# 2 | METHODS

## 2.1 | Species and locations of field sites

This study focuses on species of *Argyrodes* and *Leucauge* found on the webs of *Cyrtophora*, which are mainly distributed in Southeast Asia and Australia (Platnick, 2020). To increase the generality of our results, we selected three field sites that provided variation in habitat, climate zone and symbiont species composition, constrained by

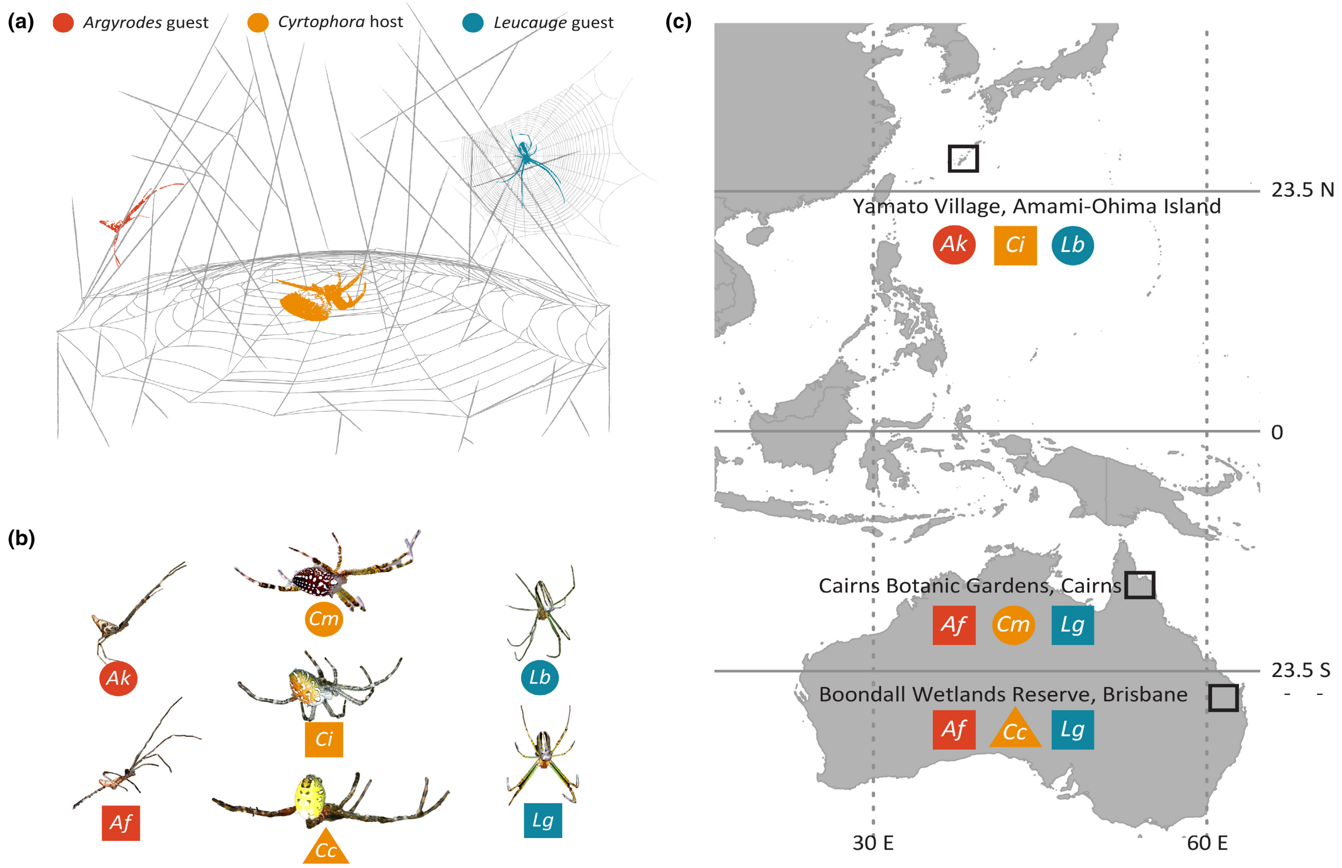

FIGURE 1 (a) schematic illustration of the symbioses between arthropod predators (not drawn to scale); (b) photographs of *Cyrtophora* host, *Argyrodes* and *Leucauge* guests included in this study (Photo credits: Dr Akio Tanikawa). The species names are denoted as the following: *A. fissifrons* as '*Af*'; *A. kumadai* as '*Ak*'; *C. cylindroides* as '*Cc*'; *C. ikomosanensis* as '*Ci*'; *C. moluccensis* as '*Cm*'; *L. granulata* as 'Lg'; *L. blanda* as '*Lb*'; and (c) field sites and host–guest pairs.

logistic and practical considerations. These field sites included two locations in Australia: Boondall Wetlands, Brisbane (BNE), which is dominated by broad-leaved paperbark forests and woodlands within the subtropical zone; and Cairns Botanic Gardens (CNS), which is a partially modified forest within the tropical zone; and one location in Japan: Yamato Village, Amami-Ōshima Island (ASJ), which is dominated by an evergreen broad-leaved forest within the subtropical zone. Among these field sites, we focused on two *Cyrtophora* host species in Australia: *C. cylindroides* (Walckenaer, 1841) in Brisbane and *C. moluccensis* (Doleschall, 1857) in Cairns; and one *Cyrtophora* host species in Japan: *C. ikomosanensis* (Bösenberg & Strand, 1906). These *Cyrtophora* hosts form associations with different pairs of *Argyrodes* and *Leucauge* guest species: *A. fissifrons* (O. Pickard-Cambridge, 1869) in Australia and *A. kumadai* (Chida & Tanikawa, 1999) in Japan; *L.* granulata (Walckenaer, 1842) in Australia and *L. blanda* (L. Koch, 1878) in Japan. The identities of the host and guest species at these three sites are summarised in Figure 1 and Table S1. We note that three very similar species of *Leucauge* occur in Amami-Ōshima: *L. blanda*, *L. subblanda* (Bösenberg & Strand, 1906) and *L. celebesiana* (Walckenaer, 1841). While *L. blanda* appears to be the most common species on *Cyrtophora* webs, distinguishing between these species is very challenging in the field, and juveniles of *L. subblanda* and *L. celebesiana* may have been included as *L. blanda*. We refer to *Cyrtophora* hosts, *Argyrodes* guests and *Leucauge* guests, as collective reference to the different species within each genus.

## 2.2 | Field experiments

For each field site, we located the webs of *Cyrtophora* hosts, accumulating a sample of 183 webs in Boondall Wetlands, 176 webs in Cairns Botanic Garden and 178 webs in Amami-Ōshima. We calculated the surface of the host web as $\pi r\sqrt{r^2+h^2}+\pi r^2$, where $r$ represents the radius of the horizontal orb, and $h$ represents the height of the tangle web. We recorded the average number of web-building *Leucauge* and *Argyrodes* guests per web (see also Table S1).

We estimated the availability of local insect prey for each site by recording the number and size of prey intercepted by the webs of *Cyrtophora* whose hosts had been removed. We used this as a baseline estimate of prey abundance for each field site. This method arguably provides a more accurate sample of the population of insects *available* to the spiders, than other methods, such as sticky traps, malaise traps or coloured bucket traps (see also Eberhard, 2020). However, it is biased against those prey species

**TABLE 1** Scale of inference and sample sizes (specific details included in Table S1).

| Scale of inference | Scale at which the factor of interest is applied | Number of replicates at the appropriate scale |
|---|---|---|
| Triadic symbioses involving predators across geographic regions | Field sites | 3 field sites |
| Prey abundance across geographic regions | Field sites (3) and Treatments (2) | 32–36 webs per treatment |
| Fitness outcomes following manipulation of presence/absence of symbionts | Treatments (5) within field experiment | 24–35 webs per treatment |

that can escape from the web in the absence of the resident spider. We investigated the impact of different combinations of guests on the *Cyrtophora* host web by manipulating the presence of *Argyrodes* and *Leucauge* guest species and by measuring the prey interception rates and weight change of the host. Spiders in the genus *Leucauge* build orb-webs, and so the experimental manipulation included the presence/absence of both the spider and its web.

The field experiment involved trials lasting 14 days. First, we located 38–53 *Cyrtophora* spiders (with intact webs), which were randomly assigned to one of the six treatments: (1) all guest species removed; (2) *Argyrodes* guest present (*Leucauge* spiders and their webs excluded); (3) *Leucauge* spiders and their webs present (*Argyrodes* guest absent); (4) all guest species present; (5) *Argyrodes* and webs of *Leucauge* guests present (*Leucauge* spiders removed); (6) Webs of *Leucauge* guests present (*Leucauge* and *Argyrodes* spiders removed). For treatments 5 and 6, where the webs of *Leucauge* spiders are present but the spiders are excluded, we released the *Leucauge* spider back onto its web if the web had deteriorated, and subsequently removed the spider after she had repaired it, usually within a couple of hours. Following our initial census (Table S1), we added or removed spider guests of either species to ensure that the experimental host webs contained four individuals or their webs, since this most closely reflected the average (Table S1). We removed *Leucauge* guest webs using a USB Soldering Pen (SI-168U, Pro'sKit, Xindian District, New Taipei City, Taiwan), taking care to minimise any damage to the web and ensuring that the remaining host and guest spiders remained on the web. The weight of the *Cyrtophora* host was measured at the start and the end of the trial with a digital scale accurate to 0.001 g (GemPro-250 Precision, MyWeigh, Phoenix, Arizona, USA), and the assigned treatments were maintained continually for each host web over the 14-day trial.

It was not possible to census every *Cyrtophora* web within each of the five treatments, and so we randomly selected 3–5 webs each day, and for those webs we collected the following data in hourly censuses between 08:00 h and 16:00 h: (1) the number and size of prey intercepted by the host webs; and (2) the number and size of prey consumed (either wrapped in silk and suspended on the web or being manipulated in the spider mandibles) by *Cyrtophora* hosts and *Argyrodes* guests in treatments where *Argyrodes* guests were present. We did not collect data on prey taxa, since we could not identify most prey items beyond Order (proportions of unidentifiable prey: 46% in empty webs and 54% in experimental webs): removing the prey would disturb the spiders or damage the web and thus affect subsequent prey interception. Prey size was estimated using digital callipers, accurate to 0.01 mm, that were placed near the prey. We converted the prey size into bins with 0.4 cm intervals to reduce any measuring bias. We followed this daily procedure until we had obtained a single daily census of prey capture for all webs that had been located at the start of the trial. We conducted the field experiment for four 14-day trials at each field site, resulting in sample sizes ranging from 24 to 36 replicates for each treatment (Table S1). There was no spatial segmentation of webs between each of the four 14-day trials. The time between trials within each field site were determined by uncontrolled events, such as a typhoon or heavy rain, which made it impossible to observe the spiders.

## 2.3 | Permits

We thank the Brisbane City Council for permitting us to conduct fieldwork in the Boondall Wetlands and the staff of the Amami Wildlife Conservation Center (奄美野生生物保護センターでは) for permission to conduct fieldwork on their site. We did not require permits to conduct fieldwork at the Cairns Botanical Gardens.

## 2.4 | Data analyses

The scale at which we seek to make inferences are given in Table 1. Broadly, we make inferences from individual experiments, and then combine these inferences to make a broader inference at the scale of triadic symbioses involving different combinations of invertebrate predators. Data analyses were performed using the ‘vegan’ package in R version 4.3.0. Fitting generalised linear mixed-effects models was conducted using the ‘lme4’ package. Additionally, lognormal pseudo-R-squared for generalised mixed-effect models was calculated with the ‘MuMIn’ package, and the ‘multcomp’ package was used for multiple comparisons for generalised linear models.

Generalised linear mixed models (GLMMs) with a Poisson distribution were used to compare the total number of prey items intercepted by host webs among the treatments. For empty webs, the field site was included as a categorical independent variable; for experimental webs, the field site and presence of each of the two guest spiders were used as three categorical independent variables, along with the interactions between them. We used type III Wald Chi-square tests to examine the significance of the main effects. The date of censoring prey interception for each web was included as a random group intercept, and the web surface area and period of monitoring were the log-link offset variables in GLMMs. We did not detect any autocorrelated effects. Since the size of the intercepted prey is likely to be influenced by the web architecture of guest spiders (Ludwig et al., 2018) or guest spiders themselves (Peng et al., 2013), we calculated the effect size (Hedges' *g*) to compare the size of prey items between treatments, which allows us to evaluate the effects of treatments on prey size and determine at which prey size interval the effect was greatest. The number of prey items in each size category consumed by *Cyrtophora* hosts or *Argyrodes* guests were compiled into contingency tables. We analysed these prey consumption data using group-wise Fisher tests. The null hypothesis is that the number of consumed prey items for each size bin is independent of the spider subject (*Cyrtophora* host or *Argyrodes* guest). Accepting the null hypothesis indicates that *Cyrtophora* hosts and *Argyrodes* guests fed on prey of similar size, while rejection indicates that *Cyrtophora* hosts and *Argyrodes* guests fed on different-sized prey. We used this statistical approach to examine if the difference in the feeding regimes (i.e. size of prey) of *Cyrtophora* hosts and *Argyrodes* guests vary with the presence of *Leucauge* web-building guests. Therefore, we conducted these tests at each level of manipulation of *Leucauge* guest species: webs and spiders both absent, only webs present and webs and spiders both present, where *p* values were computed by Monte Carlo simulation with 10,000 replicates and corrected following Benjamin and Hochberg (1995).

The weight gain for each host was estimated by subtracting the initial weight from the weight at the end of the experimental period, and transforming these measures using Ordered Quantile (ORQ) normalisation to fit normality with the formula $g(x) = \Phi^{-1}((\mathrm{rank}(x)0.5)/(\mathrm{length}(x)))$, where $x$ refers to the value of the weight measure, $\mathrm{rank}(x)$ refers to its rank in the weight measures and $\Phi$ refers to the standard normal cumulative distribution function. The normalised weight measures (Shapiro–Wilk Normality Test: $W = 0.998$, $p = 0.752$) were then analysed using a linear mixed-model, in which the field site and manipulation of two guest spiders were included as three fixed factors, along with their interactions. Type III Wald Chi-square tests were used to determine the significance of the main effects. The order of experimental period was treated as a random factor to determine the intercept in the model.

## 3 | RESULTS

### 3.1 | Host hunting efficiency

We estimated local prey availability between geographic locations by noting the number of prey items intercepted by naturally constructed webs from which the *Cyrtophora* host had (vacant) or had not (present) been experimentally removed. Data from vacant webs revealed that the local prey availability differed between climate zones. The total number of prey items intercepted by vacant *Cyrtophora* webs located in Boondall Wetlands, Brisbane, subtropical eastern Australia (BNE) was significantly lower than that at Cairns Botanic Gardens, tropical eastern Australia (CNS) or Amami-Ōshima Island, subtropical Japan (ASJ; Figure 2a; Table S2B), with no significant difference between webs located at Cairns and Amami-Ōshima Island (Tukey HSD tests for GLMMs: $Z = 1.320$, $p = 0.38$). The number of insects intercepted by *Cyrtophora* webs with the host present also differed among field sites (Figure 2b; Table S2B) and was highest on Amami-Ōshima Island (Tukey HSD tests for GLMMs: CNS vs. BNE: $Z = 10.836$, $p < 0.001$; ASJ vs. BNE: $Z = 17.670$, $p < 0.001$; ASJ vs. CNS: $Z = 7.254$, $p < 0.001$).

*Cyrtophora* hosts improve their foraging success by forming a heterospecific web complex with *Leucauge* orb-weaver guests, a pattern that was remarkably consistent across different combinations of species in different geographic locations. *Cyrtophora* webs with *Leucauge* guests intercepted more prey items than host webs from which *Leucauge* guests had been experimentally removed, while prey interception rates were not influenced by the presence or absence of *Argyrodes* guests (Figure 2b; Table S2B). Post-hoc comparisons showed that this effect was due to the presence of the *Leucauge* webs: there was no significant difference between treatments in which the webs of *Leucauge* guests were vacant or not (Tukey HSD tests for GLMMs: $Z = 1.629$, $p = 0.23$). The number and size of prey intercepted did not change significantly following the removal of *Argyrodes* guests (comparisons in number are shown in Figure 2b and Table S2B, while differences in size are presented in Figure S1a and Table S3). Manipulation of the presence of orb-web-building *Leucauge* guests had considerable influence on both the number and size of intercepted prey, with webs of *Cyrtophora* intercepting smaller prey (<0.8 cm) more frequently when webs of *Leucauge* guests were present (Table S4; Figure S1b).

### 3.2 | Third party influence on feeding regimes

The degree of overlap in the feeding regime (reflected in the size of the prey) of *Cyrtophora* hosts and *Argyrodes* guests varied depending upon the presence of *Leucauge* guests and overlapped substantially in the absence of web-building guests and their webs. We recorded 384 prey consumption events across the three field sites, and group-wise Fisher tests revealed that there was a difference in the diet (reflected in the size of the prey) of *Cyrtophora* hosts and *Argyrodes* guests when *Leucauge* orb-webs were present (with or without *Leucauge* spiders), but not when *Leucauge* webs were absent. *Argyrodes* guests fed more frequently on smaller prey (<1 cm; the bottom two rows in Figure 3) when webs of *Leucauge* were present ($p < 0.001$ in all field sites) or both *Leucauge* spiders and their webs were present ($p = 0.002$ in Brisbane and $p < 0.001$ in the other two field sites). However, the size of the prey of *Cyrtophora* hosts and *Argyrodes* guests were similar in the absence of *Leucauge* spiders and their webs: there was no significant difference in the distribution of

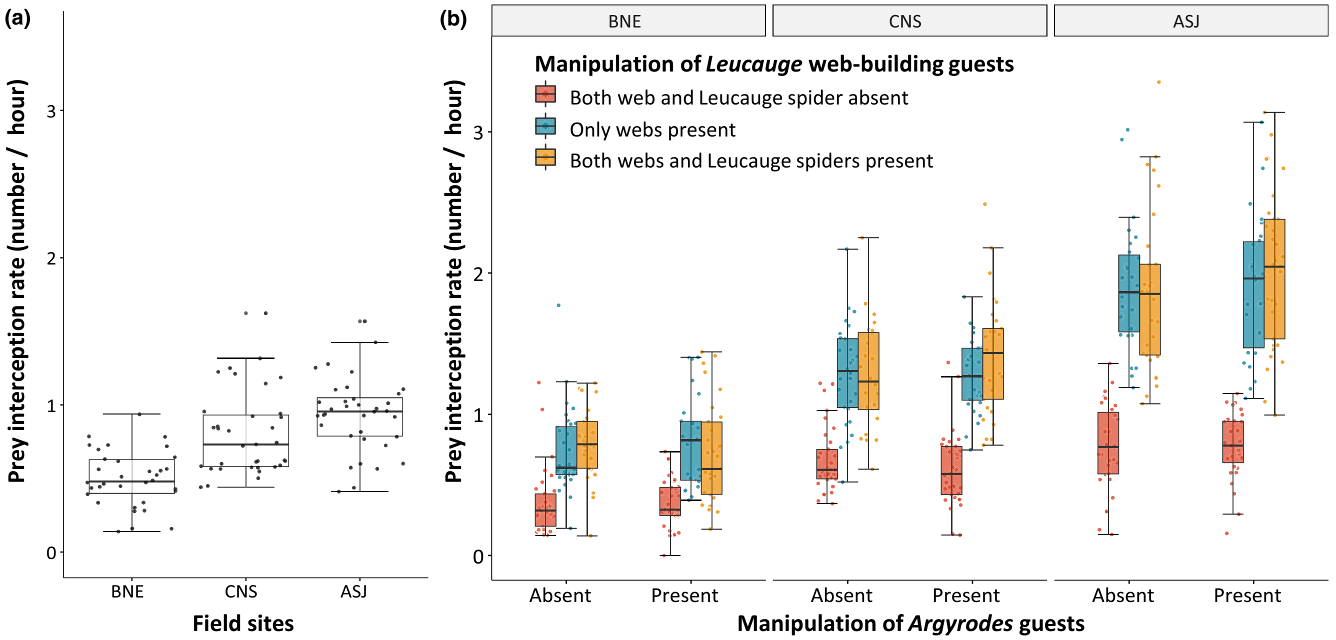

FIGURE 2 Comparisons of prey interception rates for (a) empty webs in different field sites and (b) experimental webs across treatments. Each dot corresponds to the prey interception rate of one host web. The centre lines in boxes represent the medians; box limits indicate the 25th and 75th percentiles; whiskers extend 1.5 times the interquartile range from the 25th and 75th percentiles. Boondall Wetlands in Brisbane (BNE), Cairns Botanic Gardens (CNS) and Amami-Ōshima Island (ASJ).

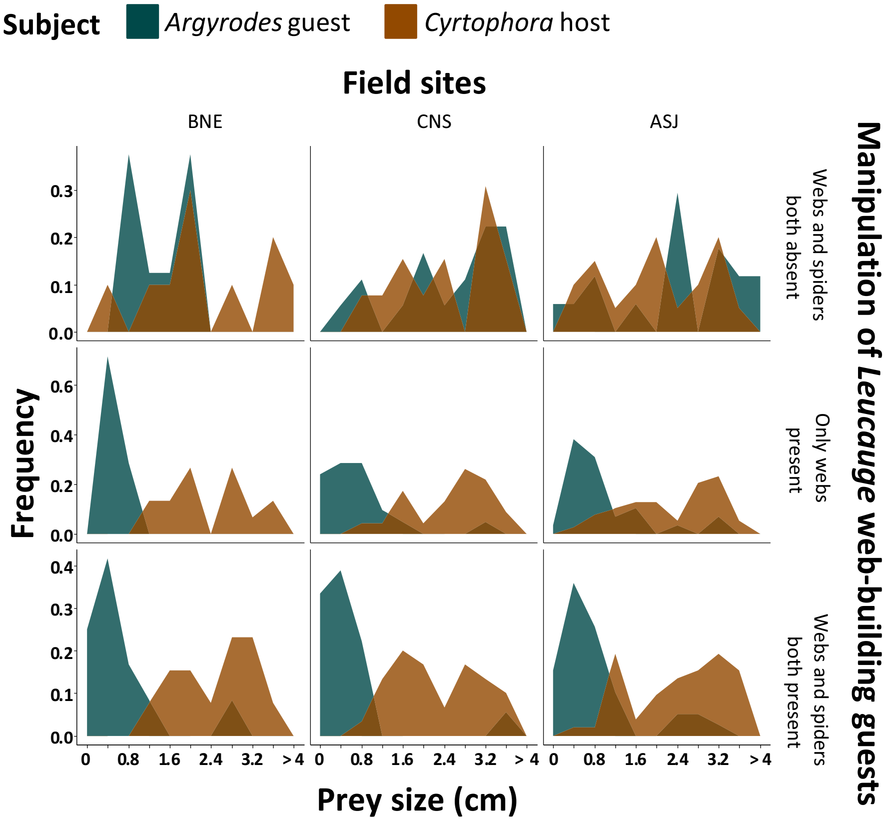

FIGURE 3 Effects of manipulation of *Leucauge* web-building guests on size distributions of prey consumed by *Cyrtophora* hosts and *Argyrodes* guests. Frequency was calculated as the number in each size bin divided by the total for these two subjects, *Argyrodes* guests and *Cyrtophora* host. The ticks on the *x*-axis indicate the lower end of each bin, with increments of 0.4 cm. Boondall Wetlands in Brisbane, Cairns Botanic Gardens and Amami-Ōshima Island are denoted as 'BNE', 'CNS' and 'ASJ', respectively.

the size of prey consumed by *Cyrtophora* hosts and *Argyrodes* guest spiders following the removal of *Leucauge* spiders and their webs ($p = 0.363$ in Brisbane; $p = 0.79$ in Cairns; $p = 0.17$ in Amami-Ōshima Island; the top row in Figure 3).

### 3.3 | Host growth

The presence of *Argyrodes* guests exerted a fitness cost on *Cyrtophora* hosts, through a lower rate of weight gain, but this

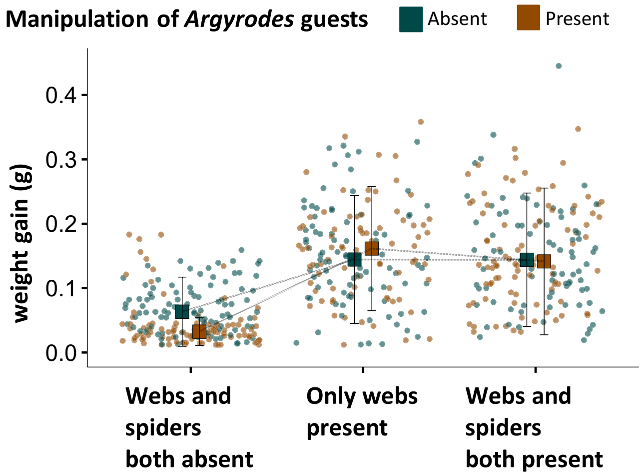

**FIGURE 4** Main and interaction effects of the manipulation of two guest spider species on host weight gain. Each circle indicates the weight gain of one host. Squares and whiskers represent the median and interquartile range of weight gain in each experimental treatment. Dotted lines link the medians between treatments with and without *Argyrodes* guests.

negative impact was mitigated by the presence of web-building *Leucauge* guests. The growth rate of *Cyrtophora* hosts tended to be higher if webs of *Leucauge* guests were present (Figure 4; Table S5). The interaction effect between manipulations of *Argyrodes* guests and *Leucauge* guests was significant (Figure 4; Table S5). Following the removal of *Leucauge* guests, *Cyrtophora* hosts gained less weight when *Argyrodes* guests were present. In contrast, the presence of *Argyrodes* guests did not influence the weight gain of *Cyrtophora* hosts when *Leucauge* guests were present (Table S5; Figure 4).

## 4 | DISCUSSION

Our novel experimental framework, which manipulates the species composition of a multi-species symbiosis, highlights the additive and synergistic effects of different symbionts within arthropod predator symbioses. Specifically, the presence or absence of one symbiont can have profound consequences for the nature of the relationship between the other two symbionts. We discovered that *Cyrtophora* hosts gain more weight when their web complex is host to web-building *Leucauge* guests, consistent with an earlier report of mutualistic associations with two web-building spiders (Elgar, 1994). For adult female spiders, weight gain translates directly into fecundity (Marshall & Gittleman, 1994; Simpson, 1995), especially as the difference is likely to compound over the life of the host spider. However, the impact of *Argyrodes* guests on the foraging success of *Cyrtophora* hosts depended upon the presence of *Leucauge* guests. While *Cyrtophora* webs that were hosts to the webs of *Leucauge* guests were unaffected by the presence of *Argyrodes* guests, the foraging success of *Cyrtophora* hosts without *Leucauge* guests was reduced in the presence of *Argyrodes* guests. This pattern arose because the size of prey consumed by *Argyrodes* guests and *Cyrtophora* hosts changed with the presence of *Leucauge* guests; removing the webs of *Leucauge* guests resulted in fewer prey being arrested and a convergence in the size of prey consumed by *Argyrodes* and *Cyrtophora*. Clearly, the nature of the relationships among these symbionts is more nuanced than traditionally described; in this system, the opportunity to engage with a mutualist partner is a key factor in the context-dependent outcomes of symbioses.

Conventional, context-independent classifications of symbiotic interactions can be misleading if they fail to reveal the complexity of interactions within multi-species symbioses (Trager et al., 2010), and our experiments reveal that the fitness consequences of an interaction with one partner depend upon the presence of another partner. There is extensive evidence of the negative effects of *Argyrodes* guests on host fitness (Grostal & Walter, 1997; Koh & Li, 2002; Larcher & Wise, 1985; Rypstra, 1981; Tanaka, 1984), but subsequent field experiments reveal the positive effects of these guests (Peng et al., 2013). Similarly, *Leucauge* guests were traditionally described as commensals, utilising the barrier web of the *Cyrtophora* host as a supporting structure for their own orb-webs (Rypstra, 1979), but field experiments reveal the positive impact of these orb-weaver guests on host foraging success (Elgar, 1994). These different interpretations of the nature of the symbiotic relationship reflect the traditionally dyadic-species perspective, which does not take account of the synergistic effects of other guest spiders that may or may not inhabit the host web. Thus, while interspecific foraging groups of predatory spiders have been documented for over 150 years (e.g. Belt, 1874; Elgar, 1993; Rypstra, 1979), our understanding of the ecological patterns or processes associated with these web complexes remains surprisingly limited.

Orb-weaver spiders are traditionally described as classic 'sit-and-wait' predators, but this perspective obscures the diverse tactics they employ to enhance foraging success, including visual attractants such as body coloration (Peng et al., 2020); silken decorations (Tan et al., 2010; Tan & Li, 2009; Walter & Elgar, 2012); olfactory cues (Bjorkman-Chiswell et al., 2004; Henneken et al., 2017); and modifying web characteristics according to the heterogeneity in prey availability (Scharf et al., 2011). These strategies may increase prey capture rates but may also increase the risk of predation (Bruce et al., 2001; Fan et al., 2009; Li, 2005) or the nutrient costs of silk production (Opell, 2008). Unlike other guests (Peng et al., 2013), the body coloration of *Leucauge* spiders apparently did not appear to function as a visual lure, but there are other, non-mutually exclusive mechanisms by which the presence of *Leucauge* guest webs may enhance the capture success of *Cyrtophora* hosts. First, the silk of *Leucauge* webs may, like that of other web-building spiders, reflect light within the UV spectra, thereby exploiting the tendency of dipteran insects to fly towards UV light and thus increase the likelihood of being arrested by the web (Théry & Casas, 2009; Walter & Elgar, 2012). Second, the combination of the silk threads of *Cyrtophora* hosts and the webs of *Leucauge* guests may produce a ricochet effect (Uetz, 1989), in which flying insects bounce between webs and thus provide a foraging benefit for those two web associates (Elgar, 1994). Third, the silk of *Leucauge* webs may contain chemical prey attractants, such as putrescine, that can attract prey to within the vicinity of the web and thus increase the prey capture rate, as documented for *Argiope* orb-weavers (Henneken et al., 2017). Although the gain in host weight in the presence of *Leucauge* webs was consistent across the different study sites, the prey interception rate in experimental webs of *C. ikomosanensis* in Japan was the highest across the three locations, perhaps reflecting a visual luring function of the comparatively brighter body coloration of this host species (Blamires et al., 2014; Peng et al., 2020).

The remarkable consistency in the nature of the symbioses across the three populations, which differ in both species' composition and types of prey, highlights the propensity for outcome change to be related to biotic (i.e. the presence of third-party symbionts) rather than abiotic (spatial gradient) factors. In diverse defensive symbioses, insect hosts can be protected against pathogens and parasites when they are also associated with microbial symbionts (Hajek et al., 2019). For example, the same insect species can engage with different facultative bacterial symbionts that provide protection against the same fungal entomopathogen, or different host species can associate with the same fungal symbionts against different parasitic nematode species (Hajek et al., 2019). Clearly, the process of biotic factors driving the patterns of outcome variation exists in both positive and negative species interactions. Nevertheless, theoretical and empirical studies indicate that interspecific interactions may shift to competition or collapse under extreme conditions (Michalet et al., 2014). The nuanced relationships revealed in the present study may similarly change under stress caused by extreme weather disturbance events, especially if they target a particular type of prey.

## AUTHOR CONTRIBUTIONS

P.P., D.S.-F. and M.A.E. conceived the ideas and designed methodology; P.P., Y.-C.S. and Y.G.B. collected the data; P.P. and Y.-C.S. analysed the data; and P.P. and M.A.E. led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication. Our study brings together authors from several different countries, including scientists based in the countries where the study was carried out.

## ACKNOWLEDGEMENTS

We are grateful for the support provided by the staff and mongoose busters of the Amami Wildlife Conservation Center(奄美野生生物保護センターでは), Japan. The research was supported by the Australian Government Research Training Program (PhD scholarship to P.P.), the Holsworth Wildlife Research Endowment (to P.P.), and the National Geographic Society (WW-058R-17 to M.A.E.). The funders had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript. Open access publishing facilitated by The University of Melbourne, as part of the Wiley - The University of Melbourne agreement via the Council of Australian University Librarians.

## CONFLICT OF INTEREST STATEMENT

All authors declare no conflict of interest.

## DATA AVAILABILITY STATEMENT

The datasets and statistical analyses supporting this article are deposited in Dryad: https://doi.org/10.5061/dryad.vq83bk40h.

## ETHICS

Our research, which involves invertebrate species only, did not require ethical approval from a human subject or animal welfare committee.

## DECLARATION OF AI USE

We have not used AI-assisted technologies in creating this article.

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

**Table S1.** *Cyrtophora* host, *Argyrodes*, and web-building *Leucauge* guest spp., where they are found, and sample size for empty and experimental webs.

**Table S2.** Effects of (a) field site on the prey interception rate of empty *Cyrtophora* webs and (b) field site, manipulation of guest species (*Argyrodes* and web-building *Leucauge*) on the prey interception rate of experimental *Cyrtophora* webs.

**Table S3.** Effect size (Hedges' *g*) of comparisons in size of prey intercepted in experimental webs with manipulation of *Argyrodes* guests.

**Table S4.** Effect size (Hedges' *g*) of comparisons in size of prey intercepted in experimental webs with manipulation of web-building *Leucauge* guests.

**Table S5.** Frequencies of different prey sizes consumed by Cyrtophora host and Argyrodes guest in three field sites: (A)

Boondall Wetlands in Brisbane; (B) Cairns Botanic Gardens; (C) Amami-Oshima Island.

**Table S6.** Effects of manipulation of two guest spider species on the weight gain of *Cyrtophora* hosts.

**Figure S1.** Number of prey items intercepted by experimental webs with manipulation of (A) *Argyrodes* and (B) *Leucauge* web-building guests. Solid square and whiskers show the average and standard error of the mean.

**How to cite this article:** Peng, P., Stuart-Fox, D., Su, Y.-C., Baba, Y. G., & Elgar, M. A. (2024). Fitness effects of symbiotic relationships between arthropod predators: Synergy in a three-way spider symbiosis. *Functional Ecology*, *00*, 1–10. https://doi.org/10.1111/1365-2435.14575