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Symbiosis between arthropod predators

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Dedicated to my parents, Ming-Fui Pang and Lin-Chun Wu.

Abstract

In natural communities, species may evolve characteristics in response to a variety of complex interactions involving heterospecific or conspecific members. Symbioses between arthropod predators, traditionally considered to be predatory and thus antagonistic, provide intriguing opportunities to explore this topic. Recent studies of interspecific interactions among certain spider symbiotic systems reveal outcomes ranging from araneophagy to mutualism. The latter is apparently unusual among any predators, and may reflect the unappreciated role of visual and chemical signalling as prey-luring traits in symbioses. In this thesis, I used a tractable symbiosis allowing long-term trials, which comprises web-building host spiders whose mobility is low and their associates whose behaviour is easily observed, to develop an understanding of the evolutionary significance of symbioses among arthropod predators.

First, as orb-weavers are generally the focal species in these symbiotic systems, I investigated the deceptive signal and trait evolution of these web-building spiders. By performing field experiments conducted under contrasting light conditions with paper model spiders, I examined the visual signal design for prey luring. The results show that both the colour (yellow) and pattern (yellow and black mosaic) are essential for luring prey in a high ambient light environment. Subsequently, using a phylogenetic comparative approach, I investigated the association between prey viewing environment and the ventral signal of orb-weavers among 63 species and found that yellow mosaic patterns are more prevalent for web-building spiders living in high ambient light intensity. Combined, my data indicate that high contrast yellow mosaic colour patterns are highly effective in luring prey for orb-weavers occurring under high ambient light conditions, suggesting that prey colour preferences may be important in the evolution of visually mediated prey-luring systems.

Second, to highlight the direct and indirect ecological effects in multispecies arthropod symbioses, I conducted field experiments that manipulated the presence of web-building and non-web-building guests to test the synergistic and antagonistic effects of these inquilines on their hosts across a broad latitudinal range. I discovered that *Cyrtophora* hosts may promote their foraging success by forming heterospecific aggregations with web-building *Leucauge* guests. Specifically, a *Cyrtophora* host web complex intercepted more prey

when *Leucauge* guest webs were attached, and thus these hosts experienced higher weight gain. However, non-web building *Argyrodes* guests imposed fitness cost on their hosts, but only when *Leucauge* guests were excluded. This pattern was consistent among different host-guest pairs and over an extensive spatial distribution, in which the negative interaction between *Cyrtophora* host and *Argyrodes* guest was mitigated by the mutualism between web-building host and its guest – *Leucauge* orb-weaver. This suggests that the outcome of interspecific interactions between arthropod predators may be more associated with biotic factors – the presence of a third partner species, rather than an abiotic factor – environmental gradients.

Lastly, I investigated the underlying mechanism facilitating the establishment of symbioses between arthropod predators. Using a series of field experiments performed on guest species that form distinct interspecific interactions with their host, I evaluated if the settlement pattern of *Argyrodes* guest spiders is affected by the nature of their relationship with, and the cues from the host and conspecifics. The field surveys confirmed that more guests settled onto the webs with a host and fewer conspecifics, and that the effect of conspecifics was stronger for kleptoparasites than mutualists. This suggests that intraspecific competition may be stronger in kleptoparasitism. Using behavioural assays with y-maze olfactometers, I revealed the role of volatile odours in intra- and interspecific communication. Both *A. kumadai* from Japan and *A. fissifrons* from Taiwan preferred host related odours, but klepoparasitic *A. kumadai* appeared to be repelled by the odours of conspecifics, and the mutualistic *A. fissfrons* was indifferent to the odours. The differences in cue discrimination ability correspond with the density of sensilla on the front legs of these guest species. These results highlight how the nature of species interactions can act as a selection pressure on not only the strength of cues for partnership recognition but on odorant perception traits.

Declaration

This is to certify that:

- the thesis comprises only my original work towards the PhD except where indicated in the preface,
- due acknowledgement has been made in text to all other material used,
- the thesis is fewer than 100,000 words in length, including tables, maps, bibliographies and appendices.

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Preface

I conceived the research project and made primary contribution to all the work contained in this thesis, with support from Devi Stuart-Fox and Mark Elgar. I am also responsible for the statistical analyses, seeking advice from others as appropriate. The contributions made by others to the conception, design, and editing of individual chapters is appropriately acknowledged.

The thesis is presented as three main chapters (Chapter 2-4) written as in-principle publishable manuscripts, each of which comprises multiple components and/or experiments. Rather than including both an introductory and concluding chapter, I have included a single introductory chapter (Chapter 1) that provides the theoretical and biological context for the overall thesis; highlights important knowledge gaps; and synthesises the key original findings and the significance of my key findings presented in the thesis.

A modified version of Chapter 2 was submitted for publication in *Functional Ecology* and was accepted on 10th December 2019. It is included in this thesis, in recognition of my primary contribution, with the permission of all co-authors:

Po Peng, Devi Stuart-Fox, Szu-Wei Chen, Eunice J. Tan, Guan-Lin Kuo, Sean J. Blamires, I-Min Tso, and Mark A. Elgar. (2020). High contrast yellow mosaic patterns are prey attractants for orb-weaving spiders. *Functional Ecology* 34: 853–864. https://doi.org/10.1111/1365-2435.13532.

I conducted field experiments with the assistance of Szu-Wei Chen and Guan-Lin Kuo; Eunice Tan and I-Min Tso helped develop the research framework and interpretation of the results; Sean Blamires contributed to drafting and revising the manuscript.

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My PhD journey begins and ends with gratitude.

A several years ago, while I read the ground-breaking scientific achievements by my supervisors – Mark Elgar, who fundamentally changed the way how arachnologists examined evolutionary questions using arthropod predator systems, and Devi Stuart-Fox, whose research outputs have been exploring the frontiers of visual ecology research, I felt divinely enlightened and aspired to pursuit a postgraduate degree in ecology and evolutionary biology. As the surest way to make your dreams come true is to live them, I came to Melbourne and had both of them as my supervisors. During my PhD, I often felt that I was living in a fantasy world, since everything feels not quite normal when you are supervised by the people who you have idolised and worshiped for years. I am truly thankful for their unconditional support and insightful feedback. My PhD would not have been possible without them, as they are not only research supervisors but also life mentors.

My advisory committee members Michael Keough (Chair) and Luke Holman have provided valuable comments which have improved the quality of my research substantially.

If you feel the above text is sycophantic, then you shouldn't read the following.

Here we go.

I thank members of all the labs I have stayed, for they have been an amazing source of encouragement. In particular, I am indebted to the following people for their patient help, without whom this thesis would not have been achievable. Caroline Dong, my favourite lab member who was also my officemate during the last stage of PhD. I was so enlightened by her writing skills and whimsically inventive mind. Thanks for making my everyday delightful. Katrina Rankin and Leslie Ng, for their fabulous company. Iliana Medina Guzman, for discussions on comparative analysis. Yong-Chao Su and Ren-Chung Chen, as well as their lab groups, for their help in processing molecular data.

Thanks to my best friends James Maino and Oliver Thomas, who introduced me to other projects which broadened my horizons. I am grateful for their camaraderie. I thank Shawn

Goodwin and Sean Deville for being my housemates. They provided me a lot of support and fun (Moreland Devils Motorcycle Club never dies; it just fades away). My doctoral degree would not have been possible without my family. All the members of my family are truly, deeply and sincerely committed to offering their love regardless of time and distance.

I was supported by a scholarship from Australian Government Research Training Program. The empirical work was supported by research grants provided by The Holsworth Wildlife Research Endowment – Equity Trustees Charitable Foundation & the Ecological Society of Australia, and National Geographic Society (WW-058R-17 to Mark Elgar and Po Peng).

Thanks are also due to Brisbane City Council for permitting the experiment in Boondall Wetlands. Fieldwork in Japan was supported 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). Many thanks for their hospitality and assistance during my research visit in Amami Oshima. I thank Dimitar Dimitrov for providing the BEAST tree and the aligned DNA matrix files for comparative analyses in Chapter 2.

Unmentioned friends, if you are reading this, I would be happy to buy you a beer to alleviate your resentment (only if you can catch me in person, which might be far away and I will not cover your travel costs).

Thank you all for sharing this enjoyable journey with me.

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Chapter 1
General introduction

Understanding the complexity of species interactions in diverse natural communities, characterised as Darwin's tangled bank, is an enduring challenge in ecology and evolution. While many studies, in diverse ecological systems, have investigated the selective forces driving evolution in complex natural environments (Endler, 1986), the focus is on how focal species respond to selection pressure from another species, as direct ecological effects can be easily qualified with the net effect on organismic fitness (Fox, Roff & Fairbairn, 2001). For example, investigations of feedback processes in eco-evolutionary dynamics typically involve interactions between two species (van Velzen & Gaedke, 2017), since the negative effects of one species, e.g. predator, on the other species, e.g. prey, is analytically tractable in population dynamics. Such an approach has also revealed numerous behavioural adaptations that derive from two-species coevolutionary arms races (Brodie Jr., Ridenhour & Brodie III, 2002; Benkman, Parchman, Favis & Siepielski, 2003). However, members of any species may sequentially or simultaneously engage with various competitors, predators, parasites, or mutualists during the course of their life. These interspecific interactions can act as selective agents (terHorst, Miller & Powell, 2010), and elucidating evolutionary processes requires a more integrative view of these direct and indirect interactions between species (terHorst *et al.*, 2018).

1.1 Focal species in symbioses

Symbioses generally arise when the focal species has functional traits to provide "goods" in exchange of "services" from other ancillary species (Douglas, 2010). The symbiotic relationship among arthropod predators – spiders, commonly involves at least one web-building species as the host (Elgar, 1993). Due to the rich resources provided by webs, many species of spider guests settle in these habitat patches (Whitehouse *et al.*, 2002). The focal species, web-building spiders, were regarded as sit-and-wait predators, yet several lines of evidence demonstrate that these arthropod predators adopt numerous strategies to improve their foraging success. This includes web relocation according to prey availability (Rypstra, 1985; Herberstein & Fleisch, 2003), optimisation of web architecture to retain prey (Sensenig, Kelly, Lorentz, Lesher & Blackledge, 2013), and exploiting the sensory bias of prey across different sensory modalities by adding olfactory attractants in silk thread (Henneken, Goodger, Jones & Elgar, 2017) and web decorations as visual lures (Walter & Elgar, 2012). Additionally, the colourful body parts of orb-weavers from several genera may function as a visual lure by increasing the prey interception rate (review in White and Kemp (2015)).

While experimental studies largely focus on the function of orb-web spider colouration, it is not known which visual elements are vital for prey attraction. Furthermore, as viewing conditions (e.g. ambient light intensity) influence the perceptual basis and thus determine signal efficacy (Endler & Thery, 1996; Seehausen, Alphen & Witte, 1997), a link between body colouration and prey viewing environment seems likely. In Chapter 2, I conducted field experiments under distinct light conditions with dummies resembling real spider bodies, in which the area, arrangement, and spectral characteristics of the conspicuous body parts were manipulated. Comparisons of the prey attraction rates for different dummies confirmed that yellowness is effective for luring both diurnal and nocturnal flying insects, and that a high contrast (bright versus dark) mosaic pattern is important in prey attraction under high ambient light intensity for the focal species *Nephila pilipes*. Combined, these findings suggest that both the colour (yellow) and pattern (yellow and black mosaic) are key to luring prey in a high ambient light environment. If high contrast yellow mosaic patterns are the most effective colour patterns for prey luring in high ambient light environments, I would expect an evolutionary association between this colour pattern and ambient light conditions across orb weavers. Accordingly, to evaluate the generality of the findings from the field experiment, I compiled a dataset describing the body colour patterns and natural history characteristics across a taxonomically broad range of orb-weaving spiders. Using a phylogenetic comparative approach, I investigated the evolutionary association between spider ventral colour patterns and ambient light. The results indicated that the colour pattern which optimises prey luring efficiency (high contrast yellow mosaic pattern) is associated with foraging in bright light conditions, suggesting that prey sensory biases and signal efficacy are key to spider foraging ecology.

1.2 Multi-species interactions

Living in a group also promotes individual foraging advantage, as commonly seen in social spiders (Whitehouse & Lubin, 2005), where aggregations may increase prey capture rate by cooperative foraging (Uetz, 1988) or allow individuals to capture bigger prey using larger communal webs (Yip, Powers & Aviles, 2008). Nevertheless, an evident cost of group living is increased intraspecific competition between group members, with resources becoming limited as conspecific density increases (Sharpe & Avilés, 2016). In interspecific symbioses involve arthropod predators, spiders in the genus *Argyrodes* are the most reported guest species, and previous studies revealed that they exploit their host for a

variety of food resources, including small prey intercepted by the host web (Vollrath, 1979), large prey captured by the host (Whitehouse, 1997a), and silk materials containing proteins and other physiologically essential compounds (Tillinghast & Townley, 1987). Traditionally, *Argyrodes* spider guests were considered as kleptoparasites that exert a fitness cost on their host (Koh & Li, 2002; Koh & Li, 2003).

Most research investigating interspecific relationships has focused on the effect of one species on the other in pairwise interactions. While this approach is necessary to identify the nature of the relationship between species, it also ignores the possibility that the outcome of the interaction may vary with the presence of a third-party. Early investigations of the ecological and evolutionary significance of *Argyrodes*-host systems adopted this one-on-one perspective, and a consistent pattern emerged: hosts experience fitness costs when *Argyrodes* guests are present on their web (Larcher & Wise, 1985; Grostal & Walter, 1997; McCrate, Andrea, Uetz & George, 2010). Recent experimental evidence, however, revealed that colourful *Argyrodes* guests may be mutualists, whose body colouration can attract prey onto the host web and thus elevate the prey capture success of the host spider (Peng, Blamires, Agnarsson, Lin & Tso, 2013). The geographic range of particular *Argyrodes* species often exceeds that of their common hosts (Platnick, 2018), which means that colour-mediated mutualisms are not necessarily obligate, and thus may not constrain the biogeographic distributions of *Argyrodes*-orb web spider networks. This highly speciose clade, comprising around 100 species is characterised by remarkable variation in the number of hosts per species, in which the host may engage with other guest spiders in addition to *Argyrodes* species (Elgar, 1993; Agnarsson, 2004). This suggests that the relationships between *Argyrodes* guests and their hosts may vary, depending upon the combination of partners.

Host spiders of the genus *Cyrtophora* build a dome-shaped web complex, which comprises a horizontal dome web supported from above by dense vertical tangle threads. This substantial, three-dimensional web not only represents a microhabitat for *Argyrodes* guests, but its upper tangle barriers are also used by small orb-weavers in the genus *Leucauge* as structural support for their orb-webs. These web-building guests appear to be commensals, as their niche is arguably separate from their host (Rypstra, 1979). While multispecies symbioses between arthropod predators were documented decades ago, very few attempts have been made to experimentally examine the synergistic and antagonistic effects of these

guest spiders on their hosts. The broad literature on multi-species networks provides compelling evidence that the magnitude of the direct interactions between two species could be ameliorated by the indirect effects arising from the presence of a third species (Strauss, 1991; Menge, 1995). Examples are often found in cascading trophic interactions, where the presence of a predator species may reduce the competition between two prey species, as it alters the population density of either one party (Werner & Peacor, 2003). This raises intriguing questions about mixed-species symbioses among arthropod predators, such as whether the antagonistic interaction between host and *Argyrodes* guests can be mediated when a third-party, web-building *Leucauge* guests are present. In Chapter 3, I conducted field experiments using three *Cyrtophora* host spiders that occur in an extensive spatial range, and form associations with different pairs of *Leucauge* and *Argyrodes* guest species. By manipulating the presence of each guest spider, I investigated the effects of co-habiting with these participants on host hunting efficiency. The field data show that the prey interception rate of *Cyrtophora* webs was higher when web-building *Leucauge* guests were present and thus *Cyrtophora* hosts gained more weight.

Game theory suggests that the contrasting capabilities of partner species allows mutualisms to evolve (see Leigh Jr (2010) for a review). The three-dimensional web of *Cyrtophora* hosts likely functions as a predator defence architecture for the host (Blackledge, Coddington & Gillespie, 2003). My study reveals that these hosts can also enhance their foraging success by forming alliances with orb-weaving *Leucauge* spiders, suggesting that the webs of these guests could function as an additive, but resource-cost free, prey capture device. This contrasts with the communal webs constructed by colonial and social spiders, which provide groups of conspecifics with foraging, reproduction, and protection benefits (Whitehouse & Lubin, 2005), but also attract costs in the form of competition, risk of predation and silk production (Krause & Ruxton, 2002). Although not specifically investigated here, the cost of group living might be mitigated in heterospecific symbioses, since *Leucauge* guests exclusively feed on smaller prey items captured by their own webs and thus do not compete for food with their *Cyrtophora* hosts (Hénaut, Delme, Legal & Williams, 2005).

By contrast, *Cyrtophora* hosts experience negative effects from *Argyrodes* guests, but only following the removal of *Leucauge* web-building guests (Chapter 3). This is because excluding the webs of *Leucauge* orb-weavers resulted in fewer, prey being intercepted. This effect was strongest for smaller prey items, which are typically captured by *Argyrodes* guests,

resulting in considerable overlap in the size of prey consumed by *Argyrodes* and *Cyrtophora*. Remarkably, this pattern was consistent across all three field sites regardless of the distinct species assemblage and types of prey, where the host-*Argyrodes* interaction becomes less antagonistic when there is an alliance between the web-building *Cyrtophora* host and orb-weaving *Leucauge* guests. Accordingly, I conclude that biotic (i.e., the presence of third-party symbionts) rather than abiotic (i.e., spatial gradients) factors are more important in determining the interaction outcome in symbioses among arthropod predators.

1.3 How symbioses form in ecological time

The webs spun by *Cyrtophora* hosts, and which function as the habitat patch for spider guests, do not persist indefinitely. Therefore, guest spiders need to locate their host webs in order to form the symbiotic relationship with their hosts. Compared with web-building *Leucauge* guests, selection may act more strongly on non-web-building *Argyrodes* inquilines, as they do not build foraging webs and rely solely on their hosts to secure prey items. The host characteristics that may influence the settlement patterns of *Argyrodes* guest spider and their consequences on host fitness have been broadly documented. Across diverse species pairs of host-kleptoparasites, there is a strong correlation between host web size and the number of guests inhabiting it, where larger webs accommodate more spider guests (Cangialosi, 1990; Rypstra & Binford, 1995). Additionally, the kleptoparasite load is associated with the degree of host web aggregation, suggesting that the costs of kleptoparasitism may be greater for hosts that live in larger group or aggregation sizes (Elgar, 1989; Agnarsson, 2002; McCrate, Andrea, Uetz & George, 2010; Agnarsson, 2011; Fernandez-Fournier & Avilés, 2018). While the impact of spider guests on host foraging success has been extensively documented, the underlying mechanisms used by these inquilines to find their hosts are poorly understood. Interspecific communication is essential to the establishment and maintenance of symbioses in ecological time (Caro & Allen, 2017). It is likely that communication between different spider species, including that between *Argyrodes* guests and their hosts, would utilise olfactory and vibratory channels, as these are comprehensively used by spiders for intraspecific communication (Schulz, 2013; Trabalon, 2013; Witt & Rovner, 2014).

More importantly, the signals or cues used in interspecific communication will depend upon the nature of the symbiotic relationship (Nieh, Barreto, Contrera & Imperatriz–Fonseca, 2004; Morales, Barone & Henry, 2008). In mutualistic networks, signallers will

actively attract receivers to ensure engagement with their benefiting partners (Caves, Green & Johnsen, 2018). By contrast, in symbioses that involve antagonistic interactions, selection may favour mechanisms that conceal the presence of the disadvantaged symbiont (Kilner & Langmore, 2011), which in turn may favour more sensitive sensory organs in the exploiting species. Although several studies have compared the antennal morphology of insects with different life-history strategies, such as generalist or specialist parasitoids, there are no consistent patterns (Elgar *et al.*, 2018).

The symbioses between spider predators provide exciting opportunities to explore whether the nature of the relationship between symbionts, ranging from negative to positive outcomes (as discussed in Chapter 3 and Peng, Blamires, Agnarsson, Lin and Tso (2013)), acts as a selection pressure on the morphology of the receptor organs. In spiders, the olfactory sensilla are located on the legs of spiders and their numbers can be easily quantified. Using field experiments that manipulated the presence of host and other spider guests, I was able to compare the settlement rate of guests onto host webs and found several general attributes that affect the settlement decision of *Argyrodes* species, including the presence of the host on the web, and the density of conspecifics. While this was a consistent pattern among *Argyrodes* species, the influence of conspecific density on settlement was greater for kleptoparasites than mutualists. Behavioural assays with y-maze olfactometers confirmed the role of chemical cues in communication between arthropod predators. Both kleptoparasitic and mutualistic *Argyrodes* species preferred host relevant odours, and while kleptoparasites expressed a stronger response against conspecifics, this cue did not appear to influence the behaviour of mutualists. These differences correspond with the density of sensilla on the legs of *Argyrodes* guests, which was greater in kleptoparasites than mutualists. This is consistent with the view that selection has favoured investment in receptor organ that optimises host-derived cue detection according to the nature of the symbiotic relationship.

By using a tractable symbiosis, which comprises web-building host spiders and their associated web-building and non-web-building guests, I explored both the evolutionary significance of these relationships, and how the relationships emerge in ecological time. My approach included phylogenetic comparative analyses, field experiments and surveys conducted across biogeographic regions, and laboratory-based behavioural assays. The results of my research highlight the value of: (1) combining experimental and comparative analyses in examining pairwise species interactions (Chapter 2); (2) considering the

importance of a third-party in evaluating the nature of species interactions (Chapter 3); (3) conducting similar experiments with symbioses involving different species across broad biogeographic ranges (Chapter 3 & 4); and (4) novel experimental designs that manipulate treatments beyond the bounds of naturally existing variation (Chapter 2-4). Combined, my project demonstrates the value of spider symbioses as excellent model systems to test the evolutionary significance of behavioural traits and eco-evolutionary feedbacks in interspecific symbioses.

Chapter 2

High contrast yellow mosaic patterns are prey attractants for orb-weaving spiders

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2.1 Summary

Many animals improve their foraging success by producing signals that exploit the sensory biases of potential prey, but the specific properties that make these sensory traps effective remain unclear. I combine field experiments with phylogenetic comparative analyses to investigate the visual luring properties of different signal designs in web-building spiders. My field experiments used cardboard spider models to examine the effects of size, colour and pattern on the foraging success of the colourful giant wood spider, *Nephila pilipes*. These experiments revealed that both the colour (yellow) and pattern (yellow and black mosaic) are essential for prey luring in a high ambient light environment. I subsequently used phylogenetic comparative analyses to demonstrate an evolutionary association between foraging in bright ambient light and yellow mosaic body colour patterns among 63 species of orb-weaving spiders from 53 genera. Together, my data show that: (i) the colour of the bright body parts of orb-weavers is essential for diurnal and nocturnal prey attraction, while the pattern and area alone are important for diurnal foraging; and (ii) the evolution of these visual lures is associated with the viewing environment, specifically ambient light intensity. I conclude that the effectiveness of colour-luring might be a major driver of the convergent evolution of yellow mosaic patterns in phylogenetically divergent orb weavers.

2.2 Introduction

The evolution of colour signals is shaped by the visual capabilities and sensory biases of receivers. In this regard, most research has focused on how conspecific receivers shape sexual signals (Taylor, Clark & McGraw, 2011), or how predators shape the protective coloration of prey (warning signals and camouflage) (Stuart-Fox, Moussalli, Marshall & Owens, 2003). Comparatively few studies have examined how the sensory biases of prey shape the evolution of predator colour patterns. In particular, colour-based deception is frequently applied in a prey-luring context, where predators exploit the sensory biases of prey (White & Kemp, 2015) to lure or attract the prey to within striking distance. However, the underlying attributes influencing the efficacy of colour-lures remain unclear.

Web-building spiders are an excellent system to study the properties of effective prey lures. They are sit-and-wait foragers that rely on a range of strategies to acquire prey. These strategies include modifying the architecture of the web in response to fluctuating prey availability (Sandoval, 1994; Schneider & Vollrath, 1998; Heiling & Herberstein, 2000; Blamires, 2010; Blamires & Tso, 2013; Blamires, Martens & Kasumovic, 2018), and exploiting different sensory channels to attract prey to the vicinity of the web, for instance by adding odours (Henneken, Goodger, Jones & Elgar, 2017), colours (Craig, Weber & Bernard, 1996; Hsiung, Justyn, Blackledge & Shawkey, 2017), silken decorations (Tan *et al.*, 2010; Walter & Elgar, 2012; Yeh, Blamires, Liao & Tso, 2015) or prey remains (Bjorkman-Chiswell *et al.*, 2004; Tan & Li, 2009) to their webs, as well as conspicuous colour patterns on the body (Tso, Lin & Yang, 2004; Peng, Blamires, Agnarsson, Lin & Tso, 2013). There is remarkable variation in the colour patterns of web-building spiders, and the commonly seen yellow or orange mosaic pattern on the ventral surface of orb-weaver spiders can serve as visual lures to enhance foraging success (Tso, Tai, Ku, Kuo & Yang, 2002; Chuang, Yang & Tso, 2007; Tso, Liao & Huang, 2007; Blamires *et al.*, 2011; Liao, Liao, Blamires & Tso, 2019) (see also White and Kemp (2015) for a review). However, conspicuous colour patterns may also have a protective function (Tan, Reid & Elgar, 2016) – aposematic or disruptive – and it is unclear which colour pattern elements are essential for effective prey luring.

Web building spiders live in a broad range of ambient light conditions, both in terms of when they are actively foraging and where they locate their webs. As ambient light conditions strongly influence the efficacy of visual signals (Endler & Thery, 1996;

Seehausen, Alphen & Witte, 1997), the viewing environment could influence the evolution of visual lures (see also Elgar, Allan and Evans (1996)). The diversity in both morphology and spectral environment of orb-weavers offers an opportunity to investigate this potential association experimentally.

Here, I tested the visual luring properties of different signal designs in web-building spiders using a combination of field experiments and phylogenetic comparative analyses. First, using artificial models, I manipulated the colour, adjacency, and area of individual colour patches to reveal which of these attributes of signal design (see Endler (1992); Endler and Thery (1996); Osorio and Vorobyev (2005); Endler (2012) for a review) are the essential elements of orb-weaver spider bodies that exploit prey sensory biases and thus act as lures. Second, I compiled an inter-specific dataset describing spider body colouration and natural history characters across a taxonomically broad range of orb-weaving spiders. I analysed this dataset using a phylogenetically controlled comparative approach to determine if the evolution of spider body colouration is associated with the viewing environment of the targeted prey.

2.3 Materials and methods

2.3.1 Signal design and prey attraction

2.3.1.1 Field experiment

I conducted a field experiment using paper models to manipulate the area and shape of the conspicuous body colouration of *Nephila pilipes* (Figure 2.1a and b). Golden orb-weaver spiders are active both diurnally and nocturnally. This means their body colouration might be shaped by the combination of different light conditions (day/night) and visual systems of predator/prey species, and hence they represent an excellent species to study deceptive signal efficacy and evolution.

The field experiment was conducted in Huoyan Mountain (24°06'42.2"N, 121°11'51.1"E), Sanyi Township, Miaoli County, Taiwan, in July and August of 2008 and 2009. The study site was located on a trail through a secondary forest where *N. pilipes* are abundant (about 1–2 females every 10 m along the trail). I haphazardly selected webs of *N. pilipes* at the study site. From preliminary surveys, I found that most individuals of *N. pilipes* (84.48%,

116 individuals censused) built webs on understory shrubs with their dorsal side facing the dense foliage and the ventral side facing the open space. This indicates that the body colouration on the ventral side of spiders might have greater opportunities for prey luring as they are more exposed to the insect viewers. Nevertheless, the perception by predators of the ventral surfaces has been largely neglected (e.g., White and Kemp (2016); White and Kemp (2017)). A drab looking spider on the dorsal surface may have a conspicuous yellow mosaic ventral surface (see Figure 2.1c and d). There could be an evolutionary benefit to having bright/yellow mosaic patterns on the ventral rather than dorsal surfaces, because the web exists as a partial barrier to the spider, presumably to protect it from being easily accessible to predators. Accordingly, my model spiders followed the colouration patterns of the ventral side of *N. pilipes* (Tso, Lin & Yang, 2004).

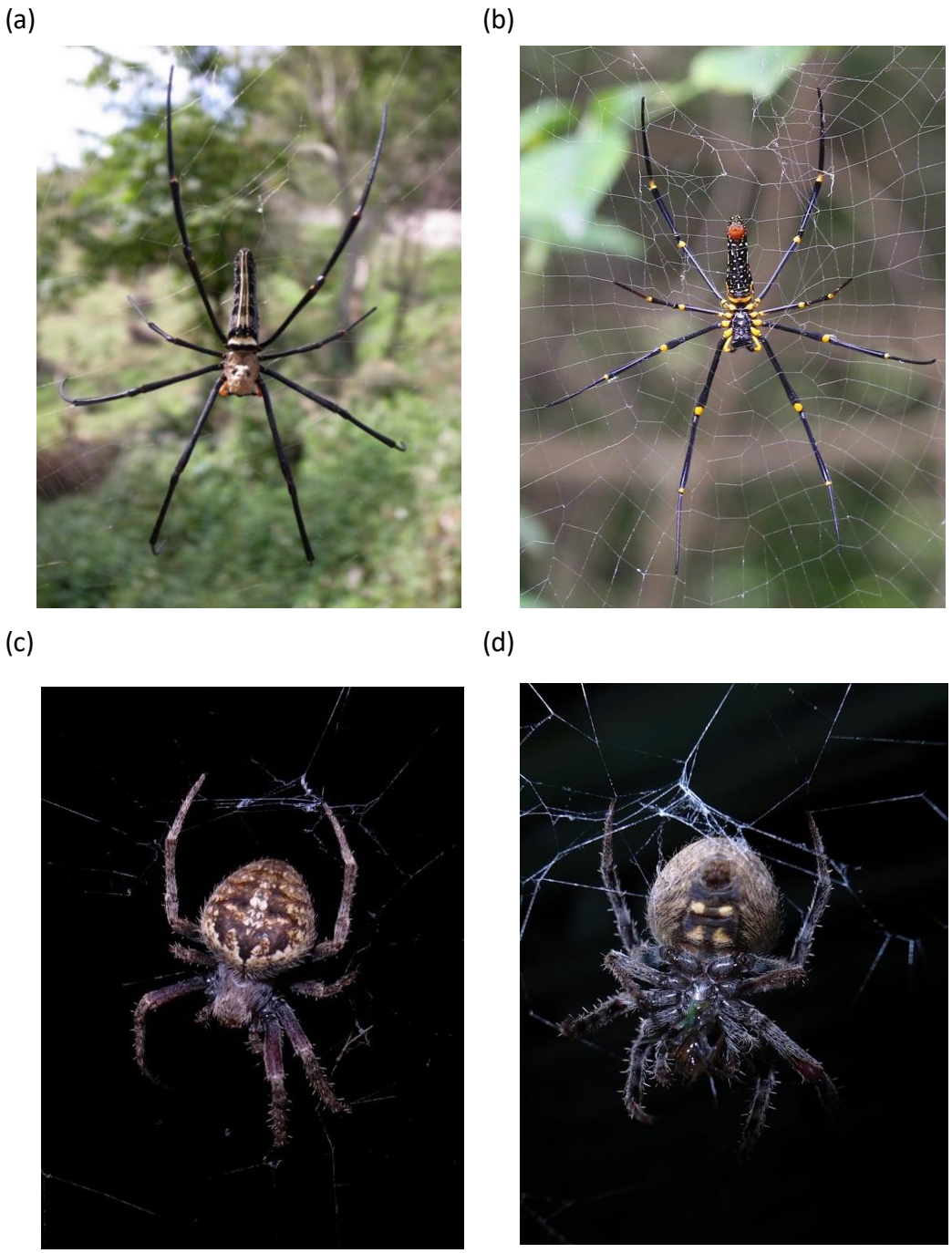

Figure 2.1. The dorsal (a) and ventral (b) views of an adult female giant wood spider *Nephila pilipes*, and the dorsal (c) and ventral (d) views of an adult female *Neoscona* cf. *punctigera*. Photo credits: (a), (c), and (d) Chen-Pan Liao; (b) Ying-Yuan Lo.

2.3.1.2 Experimental treatments

Five types of spider models were constructed for the field experiment (Figure 2.2): First, I created standard models to mimic the natural ventral colouration of *N. pilipes* (standard treatment). Second, to test the significance of the chromatic property of the signal, I

created models with blue spots rather than yellow spots on the abdomen (blue treatment). The blue and yellow had similar luminance to control for achromatic properties. Thirdly, to evaluate whether the pattern arrangement is an essential element for prey attractiveness, I made models in which the mosaic yellow pattern fused into one single yellow patch (aggregated treatment). The area of the yellow patch in this model was the same as the total area of yellow spots in the standard model. A previous study conducted during the day found that increasing the area of bright yellow not only increased prey attraction rate, but also predator attack rate, suggesting that the observed pattern reflects a trade-off between the opposing selection pressures of feeding performance and predation risk in a diurnal foraging environment (Fan, Yang & Tso, 2009). However, *N. pilipes* forages during both the day and night, and thus experiences very different degrees of exposure to light, raising the question of whether there is a similar trade-off during nocturnal foraging. I tested this possibility by creating entirely yellow and dark models (yellow and dark treatments). Spider webs without models (web treatment) were also monitored, and data from the web treatment were used as a control to test for the effectiveness of the standard treatment in attracting insects.

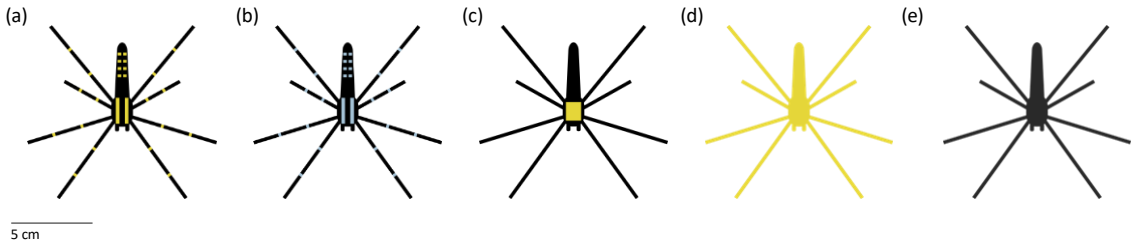

Figure 2.2. Colouration patterns of cardboard models used in the field manipulation. The shape and colour patches mimic that of the ventral side of the giant wood spider *Nephila pilipes*. (a) standard, (b) blue, (c) aggregated, (d) yellow, and (e) dark.

I created spider models from cardboard of different texture and cut them into a spider-shape, with all eight legs extended. Yellow or blue paper spots were attached to the cardboard models with odourless transparent glue to represent the colouration of un-manipulated and manipulated ventral spots, respectively. I followed the protocol of Tso, Lin and Yang (2004), to ensure the model spiders resembled real spider body colouration. Briefly, I randomly selected 5 points for each cardboard model, and measured the reflectance spectra using a spectrometer (S4000, Ocean Optics Inc., Dunedin, FL, USA) connected to a 450 W Xenon arc lamp, and compared these to spectra obtained from the corresponding body surface of *N. pilipes* (Tso, Lin & Yang, 2004).

2.3.1.2 Matching dummy and spider colours based on insect colour spaces

Preliminary surveys conducted prior to the field experiment revealed a variety of hymenopteran and dipteran insects that constitute the primary diurnal prey types of *N. pilipes* (52.34% and 24.30%, respectively), while a number of different species of moths comprised the majority (54.32%) of nocturnal prey. Therefore, four psychophysical colour spaces were applied to determine how the different models and ventral body colouration of female *N. pilipes* were likely to be perceived by insects under different light conditions. First, I computed (1) the bee colour hexagon model (Chittka, 1992). This model was originally derived from the visual physiology of the honeybee, but it is applicable to other hymenopterans given the similar spectral sensitivities across this clade (Briscoe & Chittka, 2001). Due to the uncertainty of colour perception in fly vision (Hannah, Dyer, Garcia, Dorin & Burd, 2019), I modelled two fruit fly colour spaces: (2) fruit fly categorical model (Troje, 1993), and (3) fruit fly tetrahedral model (Endler & Mielke, 2005). Lastly, as moths represent the primary nocturnal prey type of *N. pilipes*, I used the (4) hawkmoth Maxwell triangle model (Johnsen *et al.*, 2006), a neuroethological model developed for nocturnal hawkmoth vision, as representative of nocturnal moths, because hawkmoths are active in a similar ambient light environment. By calculating colour space coordinates using spectra of cardboard and real spider body parts with different models of colour space (see **A.1 Calculating colour contrast in psychophysical colour spaces** for details), I assessed the resemblance between model spiders and real spiders in the visual spaces of relevant receivers. I chose yellow and black paper samples with coordinates in colour spaces that were closest to the yellow and black patches of *N. pilipes* (thereby resembling real spider bodies), and chose blue paper with the lowest achromatic contrast to the yellow patch (i.e., a similar brightness but with a different chromatic property).

2.3.1.3 Extracting prey attraction data

I used video cameras to record the responses of prey and insects to the cardboard spider models, with approximately 8 h of recordings during each period of light exposure (diurnal: 0800h to 1600h; nocturnal: 2000h to 0400h). Before recording, I removed the live spider from her web, randomly chose one type of model spider, and placed it at the central hub of the web. I measured the hub radius, web radius, and number of spirals and radii of the four cardinal directions of the webs. These variables were used to estimate the capture area

of each spider web (Herberstein & Tso, 2000), thus ensuring that each model was placed on a similar sized web. Video cameras (Sony DCR-TRV series and Sony DCR-SR series) were placed 1–2 m away from the spider webs, depending on the nearby vegetation. Data used in the subsequent analyses were separately obtained from 656 hours of diurnal and 1122 hours of nocturnal footage. Of the diurnal monitoring hours, 161 were from aggregated treatments (n= 25), 163 from blue treatments (n = 24), 177 from standard treatments (n= 26), and 155 from web treatments (n = 24). Of the nocturnal recordings, 194 were from aggregated treatments (n= 29), 198 from blue treatments (n = 29), 195 from standard treatments (n = 29), 177 from yellow treatments (n = 25), 184 from dark treatments (n = 27), and 174 from web treatments (n = 26). From the footage, I recorded attraction events, which were defined as events where either the prey flew toward or physically contacted the model spiders, or the prey were intercepted by the webs.

For each individual spider model, I calculated the prey attraction rate (number of prey items attracted per hour) by dividing the total number of attraction events by the number of monitoring hours. I include only sequences with more than four hours of recording time.

2.3.1.4 Data analysis – field experiment

All of the field data failed Kologorov–Smirnov tests for normality ($P < 0.05$): prey attraction data were skewed leftward or over-dispersed owing to a high proportion of zero values. Therefore, I applied negative binomial regression models for data analyses, where prey interception count was the dependent variable, experimental manipulations (treatments) were used as a fixed independent variable, in which the standard treatment group (model spider that resembles the ventral coloration pattern of *N. pilipes*) was deliberately designated as the reference level in the models to facilitate comparisons of probabilities of different treatments, and the web capture area and the period of monitoring (in hours) were set as offset variables ($\log_e$-link). I also ran power analyses for these models and extracted the effect size (standardised mean difference, SMD). The model fitting was examined using deviance in the goodness of fit test. The G-test likelihood –ratio test was used to compare the compositions of attracted prey taxa of the spider model treatments. Student's t-tests were used to compare the chromatic contrast values of the

cardboard model spiders against corresponding spider body parts with applicable colour discrimination thresholds in the bee colour hexagon model.

2.3.2 Evolutionary association between spider ventral signal and prey viewing environment

2.3.2.1 Inter-specific comparative approach

I sourced natural history information of web-building spiders to score (1) the degree of exposure of these orb-weavers and thus their opportunity for prey luring, and (2) the ambient light condition of orb-weavers that rest on the webs. Specifically, I used phylogenetic comparative analyses to test if there is an association between: (i) the presence of either yellow or white mosaic pattern and the opportunity for prey luring; and (ii) the presence of either yellow or white mosaic pattern and the ambient light intensity.

2.3.2.2 Species selection

A robust genus-level phylogeny of the Araneidae (derived from Dimitrov *et al.* (2017)) with 363 species was used as an initial framework for species selection. The specific species within this genus-level phylogeny were not always identified, so I designated them as the most well-documented species within that genus, based on records in the World Spider Catalog (Platnick, 2018) and Zoological Record database in Web of Science (Reuters, 2012). For example, the genus "*Callobius*" in the original phylogeny is labelled "*Callobius* sp.", which I replaced with "*Callobius claustrarius*" as this is the most extensively documented species of this genus, according to the World Spider Catalog (Platnick, 2018) and Zoological Record database on Web of Science (Reuters, 2012), and thus most likely to have relevant natural history descriptions.

2.3.2.3 Scoring spider colour patterns

I obtained information on ventral body colouration patterns of species represented in the above phylogeny from photographs obtained from field guides (Lissner, 2014; Huber, 2015; Atkinson, 2017; Huang, 2017; Lissner & Scharff, 2017; Nentwig, Blick, Gloor,

Hänggi & Kropf, 2017; Oger, 2017; Tanikawa, 2017; Víquez & Longhorn, 2017; Whyte & Anderson, 2017) and online galleries that are maintained exclusively by arachnologists, arachnological societies, or funded by museums, and thereby minimising any mislabelling of photographs. I focused my search to online resources that aligned with the geographic distribution of the taxa, since this is likely to improve the reliability of species identification. I included photographs of adult females only, because adult males rarely build foraging webs once they become sexually mature (Savory, 1928) (but see Hirscheimer and Suter (1985)). Finally, I only included photographs of living spiders, as the colours of preserved spiders typically fade.

I first categorised each species as either with or without either yellow or white body parts. Next, I distinguished between a yellow or white mosaic pattern from a single continuous yellow or white patch if any of the following criteria were met: (i) yellow or white banded legs; (ii) yellow or white stripes (including bars and streaks) on the ventral side of the body, or (iii) mottled (flecked, stippled, or spotted) patterns on the ventral side of the body. For species with known polymorphisms, I obtained photographs of all known morphs, and only scored a yellow or white mosaic pattern if all of the morphs met the above criteria. Photos of representative species that were categorised as "presence of yellow mosaic pattern" and "presence of white mosaic pattern" can be found in Figure 2.4c.

2.3.2.4 Natural history

For those species for which I had scored colour pattern data, I sourced information about their natural history characters using references listed on the World Spider Catalog (Platnick, 2018) and Zoological Record database on Web of Science (Reuters, 2012). The intention was to quantify the opportunity (or potential) for prey luring. For example, a species that hides within its retreat has no opportunity for prey luring because its body, no matter how colourful, is obscured by the retreat. In contrast, the body colour pattern of species that rest at the hub of the foraging web, which is located in an open habitat, is clearly exposed and thus has an opportunity to lure prey. In my first analysis, I scored for each species the opportunity for prey luring according to three criteria (macrohabitat, microhabitat and refuge, Table 2.1a), and deemed the species as having little or no opportunity if it scored low opportunity for any of those three criteria (see Table A1). In my second analysis, I excluded species that remained in a refuge, and scored the viewing

environment for these species according to two criteria (macrohabitat and microhabitat, Table 2.1b), and deemed the species as in a dim environment if it scored dim in either of those criteria (see Table A2). These two comparative analyses did not use temporal foraging pattern as a binary measure for the opportunity for prey luring or ambient light environment because there is seasonal variation in the temporal activity patterns of orb-weavers, which is seldom reported and lacks consistency across the literature.

Table 2.1. Natural history characters used in the comparative analyses to score (a) the opportunity for prey luring, and (b) the ambient light condition.

Natural history characters	Description	Binary value
a) ***Opportunity for prey luring***		
Macrohabitat	Semi-closed places, e.g. cave or buildings	Small
	Habitat is in open space, e.g. scrub, woodland	Large
Microhabitat	Concealed locations, such as crevices in rock or outer covering of tree	Small
	Proximity to accessible locations, e.g. building aerial webs distant from obstacles	Large
Refuge	Present, e.g. when the spider hides in a refuge made of silk or dried leaves	Small
	Absent, i.e. the spider rests on the web	Large
b) ***Ambient light condition***		
Macrohabitat	Overall dark space	Dim
	Habitat is bright, e.g. forest edges, forest gaps	Bright
Microhabitat	Dark, e.g. when the web is built in narrow crack in stones and bark, or beneath litter or logs	Dim
	The web is built away from barriers to exposure to light	Bright

2.3.2.5 Phylogenetic comparative analysis

To investigate the association between the presence of yellow/white mosaic pattern and the opportunity for prey luring (see Appendix A: Table A1), I pruned taxa with missing

colour or illumination values from the phylogeny by Dimitrov et al (2017), resulting in a sample of 79 genera with 90 species. Next, to test the association between the spider ventral signal and ambient light condition (see Appendix A: Table S2), I used a subset of 53 genera with 63 species. My comparative analysis followed Pagel (1994), which employs transition probabilities between character states along the phylogenetic tree as either independent of or dependent on each other, and then compares the likelihoods of these two models to detect any correlation between two discrete traits. I used the "fitPagel" function within the phytools (Revell, 2012) package in R (R Core Team, 2018), which depends on R packages "ape" (Paradis, Claude & Strimmer, 2004; Popescu, Huber & Paradis, 2012) and "geiger" (Harmon, Weir, Brock, Glor & Challenger, 2007). I used the function "make.simmap" to first fit the continuous-time reversible Markov process for the evolution of the binary traits for each of the four different models (yellow and white mosaic colour patterns with the opportunity for prey luring and ambient light condition), and then simulated stochastic trait evolution using maximum likelihood estimation (Pagel, 1994) and the tip states on the phylogeny. The "densityMap" function was applied to plot the phylogeny with the posterior density for the mapped characters from stochastic character mapping on the phylogeny.

2.3 Results

2.3.1 Signal design and prey attraction

The yellow paper used for the model spider has a similar chromatic property as the yellow body parts of *N. pilipes* (see Appendix A: A.2 Chromatic properties of dummies; Table A3 and A4). Thus, the colour signals of standard models resembled, in the eyes of flying insects, those of real spiders . Blue cardboard spider models had similar or slightly higher luminance but a significantly different chromatic property, while aggregated cardboard spider models resembled colour signals of real spiders except for the arrangement of the pattern. The capture area (cm^2) of webs did not differ across treatments in either ambient light environment (diurnal: Kruskal-Wallis test, $\chi^2 = 0.8924$, df = 3; $P = 0.8273$; nocturnal: Kruskal-Wallis test, $\chi^2 = 6.2059$, df = 5; $P = 0.2867$). The Pearson goodness-of-fit statistics showed that the generalised linear models fitted the prey interception data (daytime: Pearson $\chi^2 = 103.949$, df = 95, $P = 0.249$; night-time: Pearson $\chi^2 = 170.543$, df = 159, $P = 0.252$).

The field experiment demonstrated that both the colour (yellow) and pattern (yellow and black mosaic pattern) are essential for prey luring in a high ambient light environment. Under diurnal conditions, the standard treatment resembling natural ventral colouration of *N. pilipes* attracted significantly more prey than either the blue or aggregated yellow treatments (Table 2.2a, Figure 2.3a), suggesting that chromatic properties and pattern, but not luminance, are important in luring diurnal insect prey. Under nocturnal foraging conditions, increasing the area of the yellow colour signal (yellow treatment) did not increase prey attraction (Table 2.2b, Figure 2.3b). However, yellow appears to be essential for prey attraction at night as the standard, aggregated and yellow treatments all had higher prey attraction than the blue or dark treatments. The higher prey attraction of the standard models than blue models (both day and night), indicates that the efficacy of yellow for prey attraction is not simply due to high luminance. Under both diurnal and nocturnal conditions, the model spiders resembling the ventral colour pattern of *N. pilipes* (standard treatment) attracted proportionally more Hymenoptera and Lepidoptera, compared with blue models (Figure A2; log likelihood ratio test: Daytime: $G = 6.501$, $df = 2$, $P = 0.039$; Night-time: $G = 10.645$, $df = 3$, $P = 0.014$). Standard models also attracted more prey insects than webs without models (web treatment; Table 2.2, Figure 2.3). This indicated that the standard models were attractive to prey insects and the colour signal alone was sufficient to lure prey. The standardised mean difference of the model using data collected during daytime was more negative (Table 2.2a, SMD < -0.008 for all treatments) than the model with nocturnal data (Table 2.2b, SMD > -0.009 for all treatments), suggesting that the effect of manipulation of visual properties on prey attraction is stronger during bright ambient light conditions.

Table 2.2. Results of the negative binomial regression, showing the effect of no visual signal (web without a spider), changing chromatic property (blue), arrangement pattern (aggregated), and colour area (yellow and dark) of the standard model, which resembles the ventral coloration pattern of *N. pilipes* on the rate of prey attraction in the field experiments during (A) daytime and (B) night-time.

Treatment	Estimate of *ß*	SE	*Z*	*P*	Standardised mean difference
a) Daytime					
Intercept	-8.550	0.241	-35.459	<0.0001	-
blue	-1.205	0.415	-2.908	0.004	-0.010
aggregated	-0.921	0.399	-2.308	0.021	-0.008
web	-3.137	0.781	-4.017	<0.0001	-0.013
b) Night-time					
Intercept	-9.137	0.257	-35.512	<0.0001	-
blue	-1.088	0.414	-2.627	0.009	-0.007
aggregated	0.197	0.365	0.541	0.589	0.002
yellow	0.352	0.369	0.955	0.340	0.004
dark	-1.064	0.442	-2.409	0.016	-0.007
web	-2.468	0.653	-3.776	0.0002	-0.009

*Standard treatment group was deliberately designated as the reference level to facilitate comparisons of probabilities of different events. The ratio between probabilities of two certain events is e ß

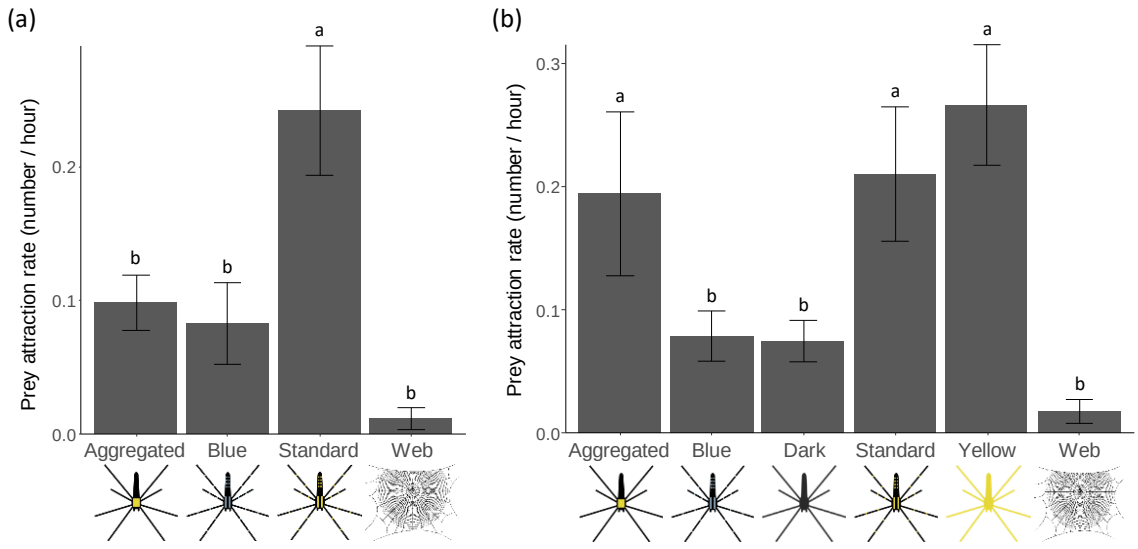

Figure 2.3. Mean (±SE) prey attraction rate (number of prey attracted/hour/cardboard model spider) recorded in (a) diurnal and (b) nocturnal field experiments. Letters represent comparisons of prey attraction with the standard treatment, where different letters indicate statistically significant differences.

2.3.2 Evolutionary association between spider ventral signal and prey viewing environment

Among orb-weaving spiders, there is an evolutionary association between yellow mosaic colour pattern and both the opportunity for prey luring and the ambient light intensity. A yellow mosaic colour pattern was more likely to evolve in species that have an opportunity for prey luring, and, among these species, the colour pattern was more likely to evolve in those species that forage in high ambient light conditions (Table 2.3, left panel of Figure 2.4a and b). There was no significant association between white mosaic colour pattern and the opportunity for prey luring or foraging ambient light intensity (Table 2.3, right panel of Figure 2.4a and b).

Table 2.3. Test for association between the presence of a yellow/white mosaic colour pattern and the opportunity for prey luring/occurrence in high ambient light intensity in web-building spiders. *L(I)* is the log-likelihood of the model of independent changes between characters; *L(D)* is the log-likelihood of the model of correlated changes.

Models of evolution	**L(I)**	**L(D)**	**Likelihood-ratio**	**p-value**
Substitution rate of the opportunity for prey luring depends on the presence of yellow mosaic colour pattern and vice versa	-87.414	-81.148	12.535	0.014
Substitution rate of the opportunity for prey luring depends on the presence of white mosaic colour pattern and vice versa	-104.540	-100.978	7.121	0.130
Substitution rate of the ambient light intensity depends on the presence of yellow mosaic colour pattern and vice versa	-66.028	-61.151	9.758	0.045
Substitution rate of ambient light intensity depends on the presence of white mosaic colour pattern and vice versa	-73.615	-70.350	6.530	0.163

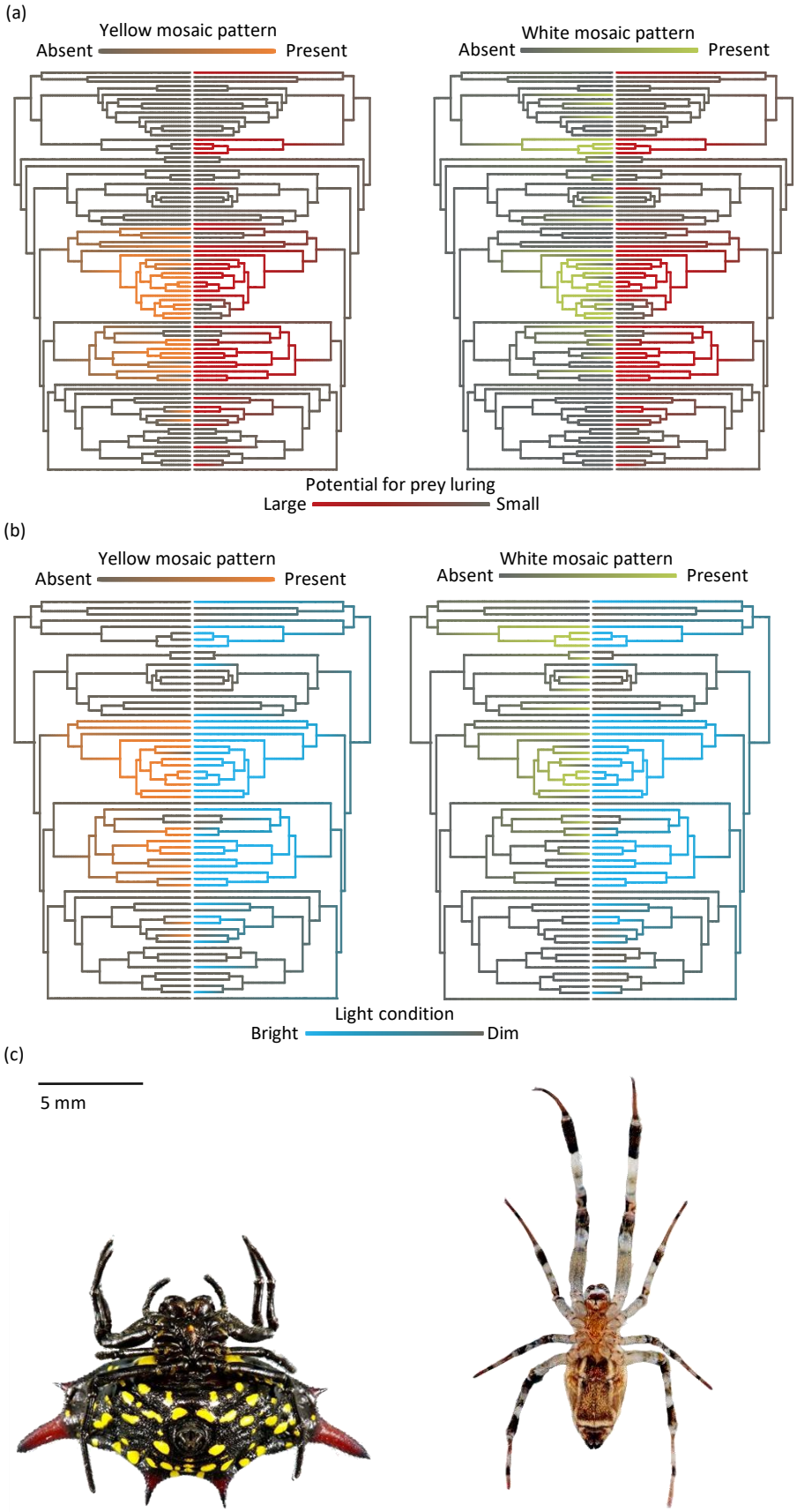
(a)
Yellow mosaic pattern
Absent
Present
White mosaic pattern
Absent
Present
Potential for prey luring
Large
Small
(b)
Yellow mosaic pattern
Absent
Present
White mosaic pattern
Absent
Present
Light condition
Bright
Dim
(c)
5 mm

Figure 2.4. The araneid genus-level phylogeny (derived from Dimitrov *et al.* (2017)) with colour gradient bars indicating the posterior density for each mapped character, depicting the evolution of yellow and white mosaic colour patterns with (a) the opportunity for prey luring, and (b) the occurrence of the species in high light exposure. Orange denotes the presence of yellow mosaic colour pattern (left panel), lime green denotes the presence of white mosaic colour pattern (right panel), red denotes large opportunity for prey luring, and blue denotes bright light condition. (c) Photos of representative species which were categorised as "presence of yellow mosaic pattern" (left; Gasteracantha sauteri), and "presence of white mosaic pattern" (right; Zosis geniculata). Photo credits: Wen-Chun Huang.

2.4 Discussion

My field experiments demonstrated that the visual luring properties of orb-weavers are shaped by the chromatic properties of the colour patches on their body, which are broadly consistent with a previous study (Tso, Lin & Yang, 2004). Yellow colouration is effective during both diurnal and nocturnal foraging and a mosaic of yellow and dark patterning is especially effective under bright light conditions. Surprisingly, aggregating the area of yellow in a single patch reduced prey attraction during the day, and aggregating or increasing the area of yellow had no effect at night. Furthermore, my results show that the efficacy of coloration in prey luring, even under nocturnal conditions, is strongly associated with spectral properties. My phylogenetic comparative analysis provides evidence that yellow, but not white, mosaic patterns are associated with the foraging ambient light intensity— that is, the viewing context. Combined, my study shows that orb-weaving spiders that forage in high ambient light conditions typically evolve yellow mosaic colour patterns that are highly effective in luring prey.

My study highlights the heuristic value of combining experimental and comparative analyses. The experimental results indicate that the yellow colour pattern of the body of *Nephila* spiders provides a foraging advantage under both bright (diurnal) and dim (nocturnal) ambient light. This result is consistent with the general association I found between yellow mosaic patterns and the opportunity for prey luring. However, in contrast to the experimental results, the comparative analysis also revealed an association between yellow mosaic patterns and ambient light condition. Nevertheless, the experimental and comparative results may not necessarily be contradictory – the experimental effect size was greater under diurnal than nocturnal conditions (Table 2.2), so selection may have favoured yellow colour patterns in the majority of *Nephila* because of the foraging benefits under diurnal conditions alone. Clearly, it would be useful to examine the effects of these colour patterns on the webs of spiders that are strictly nocturnal. For example, nocturnal *Neoscona* spiders attract prey with the yellow abdominal spots under dim light conditions, suggesting

that the prey luring function of ventral yellow mosaic patterns might not be exclusively applicable in high ambient light environments (Chuang, Yang & Tso, 2008).

My results suggest that chromatic properties, specifically yellow colouration, are effective for prey luring, even under nocturnal foraging conditions. The traditional view is that colour preference only applies to the diurnal viewing environment. Notwithstanding the limitations of colour vision in dim light, multiple lines of evidence show that nocturnal animals can discriminate colours under a range of illumination intensities between 1 cd m^2 (early twilight) and 0.0001 cd m^2 (starlight intensity) (Kelber & Lind, 2010). Under diurnal conditions, chromatic properties of various floral parts play important roles in the signalling efficacy of flowers to pollinators (Dyer *et al.*, 2012; Burd, Stayton, Shrestha & Dyer, 2014), where yellow centres or yellow pollen are common attributes of flowers (Lunau, 1995; Lunau, 2000). Because nectar could represent food resources for insects, the inner parts of many flowers resemble the colour of pollen, with pollinators like Hymenoptera and Diptera innately associating this visual cue with a food reward (Heuschen, Gumbert & Lunau, 2005; Ushimaru, Watanabe & Nakata, 2007). Additionally, recent empirical and permutation studies reveal that some nocturnal Lepidoptera also have the ability to discriminate colour under dim light conditions, and innately prefer yellow (White, Stevenson, Bennett, Cutler & Haber, 1994; Cutler, Bennett, Stevenson & White, 1995; Kelber, 1997). For example, yellow sticky papers or pan traps can function as better visual lures to dipteran and hymenopteran insects (Alyokhin, Messing & Duan, 2000; Wu, Chen & Yang, 2007). I consistently found in my field experiments that naturalistic yellow mosaic (standard) cardboard spider models lured proportionally more pollinators – Hymenoptera and Lepidoptera in daytime, and Lepidoptera in night-time, compared with blue models that had slightly higher luminance but differed substantially chromatically (Table 2 and 3; Fig. S2). Combined, these results suggest that the chromatic property of yellow, rather than its high luminance, is an essential visual element for luring pollinator insects (but see (White & Kemp, 2016; White & Kemp, 2017)).

Previous studies (Edmunds, 1990; Fan, Yang & Tso, 2009), suggest that the area of yellow is constrained by the risk of predation by diurnally active, visually hunting predators. In the colour luring system of orb weavers, a larger area of yellow attracts more prey and predators during the day (Fan, Yang & Tso, 2009). Diurnal pollinators, such as honeybees and bumblebees, adopt achromatic vision when stimuli subtend a small visual angle and

chromatic vision when a large one is subtended (Giurfa, Vorobyev, Brandt, Posner & Menzel, 1997; Papiorek, Rohde & Lunau, 2013). It is possible that entire yellow models subtended a larger angle with a large-sized yellow area and hence could be perceived chromatically from a longer distance and became more attractive to diurnal flying insects (both prey and predators). On the other hand, nocturnal pollinators, such as hawkmoths, prefer inspecting flowers with heterogeneously coloured marks compared with homogeneously coloured ones (Goyret, 2010). This is consistent with my results showing that an increased area of yellow does not increase nocturnal prey attraction. Therefore, unlike diurnal spiders, the yellow mosaic pattern on nocturnal spiders does not represent a compromise between prey attraction and predator avoidance. The effect of both colour and pattern on risk from predators or parasitoids warrants further investigation.

Both colour and pattern are important in luring prey: I found that compared with standard cardboard spider models, the diurnal prey attraction rate was significantly lower for the aggregated treatment, in which the yellow component was a single patch with the same total area. This supports the view that the pattern preference of diurnal prey might be one major driving force for the body colouration of orb-weavers. Studies of diurnal pollinators suggest that a contrasting pattern may be more important in mediating insect attraction than the spectral properties; for example, the elimination of pattern led to a decrease in insect attraction to flowers (Koski & Ashman, 2014). Furthermore, bright and dark colours as well as their combined patterns convey different messages for different species. The conventional view is that high contrast yellow and dark patterns are more likely to be aposematic to advertise the prey's unprofitability to predators (Stevens & Ruxton, 2012), or prevent recognition by disrupting the outline of the body or salient features (Stevens, Winney, Cantor & Graham, 2009). However, as the resolution of every vision system is limited, a conspicuous colour pattern can be aposematic or disruptive at a short distance, whereas it can merge to appear uniform and cryptic at a long distance, especially for predators (Endler, 1978; Marshall, 2000; Tullberg, Merilaita & Wiklund, 2005; Bohlin, Tullberg & Merilaita, 2008). This distance-dependent effect may not only exist in the context of defensive coloration but also in the colour-luring system of orb-weavers. In almost 1800 hours of video recording, I only noted three events where wasps (potential predators of orb-weavers) hovered around my cardboard model spiders. These events were all recorded within one treatment group –aggregated models, where the yellow component was merged into one patch. Natural selection is expected to optimise signal design to elicit

the maximum possible response from "intended" receivers, like prey, but avoid detection by "non-intended" receivers like predators (Endler, 1992). One possibility is that the yellow mosaic coloration pattern of diurnally foraging orb weaving spiders can simultaneously attract prey and reduce predator detection as a result of distance dependence.

Many web-building spiders in numerous genera have various yellow markings (Platnick, 2018), and this body colouration functions as a visual lure in at least two families (Araneidae, Tetragnathidae; see (White & Kemp, 2015). My study provides evidence that colour luring traits are shaped by selection to maximise diurnal and nocturnal prey attractiveness and suggests a general evolutionary association between this trait and ambient light conditions. I conclude that the effectiveness of colour-luring might be one major driver of the convergent possession of yellow body colouration in phylogenetically divergent orb weavers. More broadly, my study highlights that prey colour preferences and signal efficacy play a significant role in the evolution of visually mediated prey-luring systems.

Chapter 3
Fitness effects of symbiotic relationships among arthropod predators

3.1 Summary

Research that investigates the evolutionary significance of inter-specific interactions typically focus on the outcome from either a single species or pairwise perspective. However, natural ecological communities typically involve multispecies networks, and the importance of interactions with third parties is often overlooked. The three-dimensional web-complex of *Cyrtophora* spiders serves as a habitat patch to different pairs of associates – kleptoparasitic *Arygyrodes* and web-building *Leucauge* guests. I investigated experimentally the synergistic and antagonistic effects of these guest spiders on their hosts across a broad latitudinal range by manipulating the presence of each guest and determining the consequences for host weight gain. *Cyrtophora* hosts intercepted more prey when web-building *Leucauge* guests were present and thus gained more weight. Conversely, *Argyrodes* guests do not promote the foraging success of their *Cyrtophora* host, and instead exert a fitness cost when *Leucauge* guests were absent. A comparison of the prey consumed by *Cyrtophora* hosts and their *Argyrodes* guests revealed that their diets overlapped less in the presence of *Leucauge* web-building guests: *Argyrodes* guests fed on smaller prey items because these were more frequently intercepted by the host web when *Leucauge* spiders were present. These data not only reveal the mutualism between two web-building spiders, but also highlights how the severity of a negative symbiotic association can be mitigated by the service provided by a third-party.

3.2 Introduction

The nature of the association between symbiotic species is traditionally couched in terms of the balance between the benefits and costs for the focal species: that is, whether one symbiont has positive, negative, or neutral effects on its partner. Accordingly, the majority of investigations on heterospecific partnerships take a one-on-one-species perspective, generally quantifying the influence of engaging with only one given partner species. However, many symbioses involve more than two species and additional participants can alter the nature of the relationships between the other symbionts (Krause & Ruxton, 2002). In protection mutualisms, a defensive partner enables a host to defend against a natural enemy (Heil & McKey, 2003). By contrast, the impacts of additional species in a mutualism can be negative, as a third party may invade and exploit the mutually supportive interaction between the primary symbionts (Elgar, Nash & Pierce, 2016; New, 2017). While I know that the presence of third-party species strongly drives outcomes in positive (mutualistic) interactions among mixed-species symbioses, less attention has focussed on whether the negative effects of an interspecific relationship between two symbionts (e.g., parasitism) could be mitigated by another species (Chamberlain, Bronstein & Rudgers, 2014).

Identifying the net effects of species interactions, particularly in multi-species networks, has proved challenging because they can vary under different contexts. For example, a host-pathogen interaction can be altered due to changes in environmental temperature (Daskin & Alford, 2012). More generally, species interactions that result in positive effects (mutualism) are expected to be prone to context dependency (Hoeksema & Bruna, 2015). That is because the benefits derived from an exchange of "goods" and "services" for both partners are also accompanied with a cost, which will vary depending on individual context. For example, while host plants provide nectar as a "good" in exchange for pollinating as a "service", the investment that secures this mutualistic relationship carries costs for both parties: resources for host plants to attract pollinators, and time and energy costs for pollinators to find host plants. Costs to pollinators will depend on time and energy searching host plants, while costs to the host plant will depend on resources investing on attracting pollinators. Broadly, once the costs are greater than the benefit for one partner, then both will typically experience negative net effects (Bronstein, 2001). By contrast, negative outcomes of antagonistic interactions (predation, parasitism, and competition), are expected to be relatively fixed. Despite these general expectations, I lack data on

whether and how the outcomes of beneficial symbioses vary depending on biotic or abiotic context.

Interactions between arthropod predators are usually described in terms of competition, predation, or parasitism (Crawley, 2009). Generalist predators, like spiders whose diet frequently includes other spiders, are typically regarded as unlikely candidates to form symbiotic relationships. Nevertheless, some spiders form multi-species symbioses, typically involving at least one web-building species (Elgar, 1993). The web-building host species can provide a variety of food resources, including the host itself, recently intercepted prey, stored prey, and even the proteinaceous silk. Furthermore, the larger web can serve as habitat to guest spiders that do not build foraging webs, and other orb-weaving guests can attach their smaller webs to the host web (see Figure 3.1A). Thus, spider webs can represent an unusual microhabitat that attracts many species of spider predators and guests (Vollrath, 1987). Unlike other typical multi-species symbioses, the hosts can simultaneously interact with multiple partners. Laboratory experiments involving simple manipulations of single species highlight exploitative interspecific interactions (Koh & Li, 2002; Koh & Li, 2003). However, field experiments have revealed mutualistic interactions involving different species of spiders (Elgar, 1994; Peng, Blamires, Agnarsson, Lin & Tso, 2013), highlighting the previously unrecognised complexity of symbioses between arthropod predators.

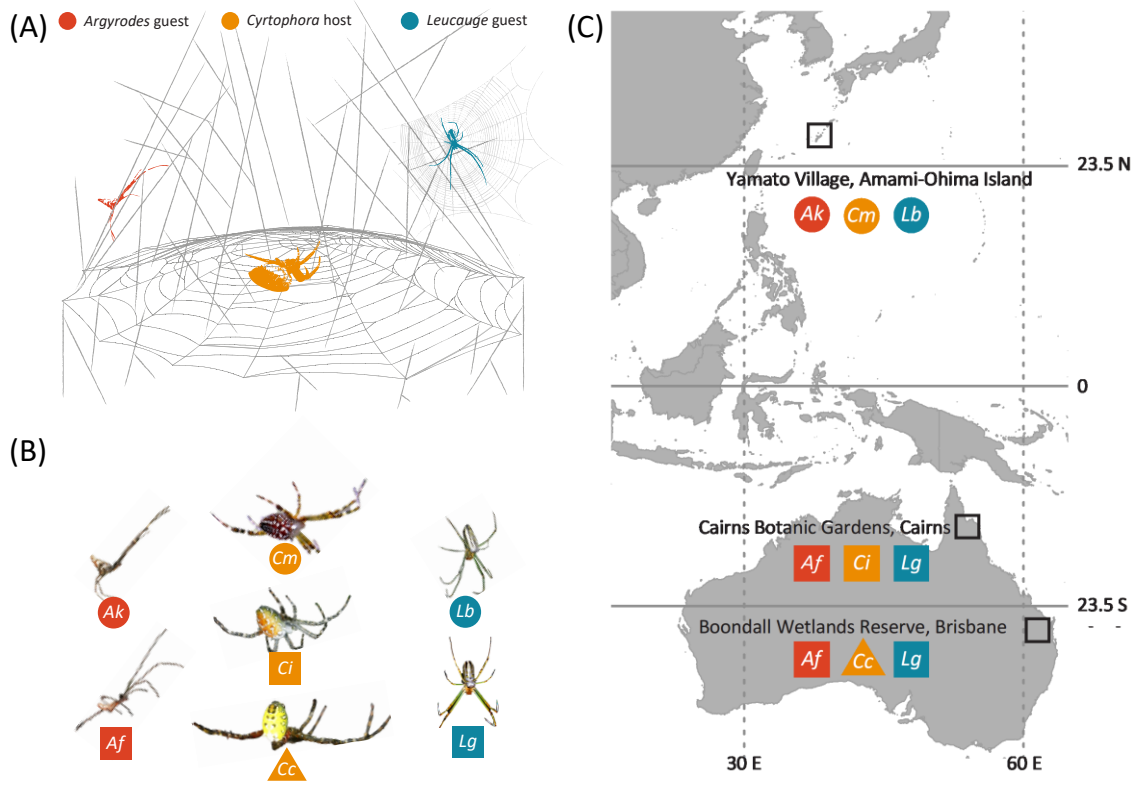

Figure 3.1. (A) schematic illustration of the symbioses between arthropod predators (not dawn to scale); (B) photographs of *Cyrtophora* host, *Argyrodes* and *Leucauge* guests included in this study (Photo credits: Dr Akio Tanikawa). The species names are denoted as the following: *A. fissifrons* as "*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.

Tent-web spiders of the genus *Cyrtophora* build a substantial three-dimensional web, comprising a sheet-web supported by a tangle of threads that forms an upper barrier-web (Figure 3.1A). The *Cyrtophora* host spider mostly remains in the centre of the web or hangs beneath the sheet-web where it captures prey, and only patrols the barrier-web in order to conduct repairs. Small (ca. 10 mm) web building spiders in the genus *Leucauge* are often found in the barrier-webs of *Cyrtophora*, utilising the tangle threads as support structures for their orb-webs. Species of *Leucauge* have been described as commensals, based on the resource division between these web-building guests (Rypstra, 1979). Spiders in the genus *Argyrodes* also utilise the web spun by their host, which are typically large (>20 mm) orb-web spiders belonging to the genus *Nephila* and *Cyrtophora*, but may also include other genera (Whitehouse, 2011). The smaller (<10 mm) *Argyrodes* spiders 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 hetero-specific 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 trackable; the mobility of the host spiders is low, allowing long-term trials; and the behaviour of subjects is easily observed. The number of guest spiders per host web varies between species, and the geographic range of these spider guests often exceeds that of their common hosts across different spatiotemporal scales. This means that the relationships between guest spiders and their hosts may vary geographically, depending upon the combination of partners and local selection pressures.

Here, I examine the foraging consequences of spider guests on their hosts in three-species symbioses between *Cyrtophora* hosts and *Leucauge* and *Argyrodes* guests. To examine the consistency of these outcomes, I use three *Cyrtophora* host spiders that occur across a broad latitudinal range, and form associations with different pairs of *Leucauge* and *Argyrodes* guest species. First, I manipulated the presence of guest spiders to reveal whether each of these participants have individual effects on host hunting efficiency. Second, I compared the feeding regimes of *Cyrtophora* host and *Argyrodes* guest spiders to assess whether these effects are moderated by the presence of a third party – *Leucauge* web-building guests. Lastly, to evaluate the potential fitness consequences of the symbioses, I measured the weight change of the host when alone or co-occurring with different combinations of guest species.

3.3 Materials and methods

3.3.1 Field experiments

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, 2018). I selected three field sites across climate zones, including two locations in Australia: Boondall Wetlands, which is dominated by broad-leaved paperbark forests and woodlands within the subtropical zone; and Cairns Botanic Gardens, which is a partially modified forest within the tropical zone; and one location in Japan: Yamato Village, Amami-Ohima Island, which is dominated by an evergreen broad-leaved forest within the tropical zone. Among these field sites, I focused on two *Cyrtophora* host species in Australia: *C. cylindroides* in Brisbane and *C. ikomosanensis* in Cairns; and one *Cyrtophora* host species in Japan: *C. moluccensis*. These *Cyrtophora* hosts form associations with different pairs of *Argyrodes* and

Leucauge guest species across considerable latitude: *A. rainbow* in Australia and *A. kumadai* in Japan; *L. granulata* in Australia and *L. blanda* in Japan. The identity of the host and guest species at these three sites are summarised in Table 3.1 and Figure 3.1B & C.

Table 3.1. *Cyrtophora* host, *Argyrodes*, and web-building *Leucauge* guest spp., where they are found, and sample size for empty and experimental webs. Values in parentheses from column "*Cyrtophora* host" to "*Leucauge* web-building guest" are mean±SEM for web surface (in cm), average number of *Aryrodes* guest species, and average number of web-building *Leucauge* guests respectively for each field site.

Cyrtophora host	*Argyrodes* guest	*Leucauge* web-building guest	Location	Climate zone	Experimental period	Representative latitude and longitude coordinates	Sample size						
							Empty webs	All guest species removed	*Argyrodes* guest present	*Leucauge* spiders and their webs present	All guest species present	*Argyrodes* guest and webs of *Leucauge* guest present	Webs of *Leucauge* guests present
C. cf. cylindroides (6481.57±150.61)	*A. cf. fissifrons* (4.33±0.17)	*L. cf. granulate* (3.66±0.14)	Boondall Wetlands Reserve, Brisbane, Queensland, Australia	Subtropics	December and January of 2016 and 2017	-27.545879, 153.048577	34	33	35	29	29	29	32
C. cf. ikomosanensis (6178.43±133.69)	*A. cf. fissifrons* (3.89±0.17)	*L. cf. granulate* (3.43±0.14)	Cairns Botanic Gardens, Cairns, Queensland, Australia	Tropics	January and February 2018	-16.897129, 145.748163	35	33	33	24	29	29	28
C. moluccensis (6099.22±132.29)	*A. cf. kumadai* (3.73±0.17)	*L. cf. blanda* (3.54±0.14)	Yamato Village, Amami-Ohima Island, Kagoshima Prefecture, Japan	Tropics	July and August 2017	28.333555, 129.355667	36	32	31	29	33	33	27

For each field site, I haphazardly selected webs of *Cyrtophora* hosts, accumulating a sample of 183 webs in Boondall Wetlands, 176 webs in Cairns Botanic Garden, and 178 webs in Amami-Oshima. I 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) and recorded the average number of web-building *Leucauge* and *Aryrodes* guests per web. These descriptive statistics are also given in Table 3.1.

I 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. I used this as a baseline measure of prey abundance for each field site. I 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 both the presence of the spider and of its web

The field experiment involved trials lasting 14 days. First, I located 38 to 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, I released the *Leucauge* spider back onto its web if the web had deteriorated, and subsequently removed the spider after she had repaired it. Following my initial census (Table 3.1), I added or removed spider guests of either species to ensure that the experimental host webs contained four individuals or attached webs, in which *Leucauge* guest webs were removed 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 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 continually maintained for each host web over the 14-day trial.

On each day, I randomly selected 3-5 experimental webs and collected the following data in hourly censuses between 0800 hr to 1600 hr: (1) the prey intercepted by host webs; (2)

the prey consumed by *Cyrtophora* hosts and *Argyrodes* guests in treatments where *Argyrodes* guests were present. I could not identify most prey items beyond order (proportions of unidentifiable prey: 45.95% in empty webs and 53.98% in experimental webs) because 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, and placed near the prey. I converted the prey size into bins with 0.4 cm intervals to reduce any measuring bias. These measuring procedures were repeated until all of the webs located at the start of the trial were censused. I conducted the field experiment for four 14-day trials at each field site, resulting in sample sizes ranging from 24 – 36 replicates for each treatment (Table 3.1).

3.3.2 Data analyses

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, 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. I 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. Since the size of the intercepted prey is likely to be influenced by the web architecture of guest spiders (Ludwig, Barbour, Guevara, Avilés & González, 2018) or guest spiders themselves (Peng, Blamires, Agnarsson, Lin & Tso, 2013), I 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. I analysed this 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. I used this statistical approach to examine if the difference in the feeding regimes of *Cyrtophora* hosts and *Argyrodes* guests vary with the presence of *Leucauge* web-building guests. Therefore, I

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 10000 replicates and corrected following Benjamini and Hochberg (1995).

The weight gain for each host was estimated by comparing the weight at the end of the experimental period with the initial weight, and transforming these measures using Ordered Quantile (ORQ) normalisation to fit normality with the formula $\mathrm{g}(x) = \Phi^{-1}((rank(x) - 0.5)/(length(x)))$, where x refers to the value of the weight measure, $rank(x)$ refers to its rank in the weight measures, and Φ 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.

3.4 Results

3.4.1 Prey interception

Field experiments using empty webs revealed that the local prey availability differed between climate zones. The total number of prey items intercepted by empty *Cyrtophora* webs in Boondall Wetlands was significantly lower than that at the other two field sites (Table 3.2B; Figure 3.2A), with no significant difference between Cairns Botanic Gardens and Amami-Ohima Island (Tukey HSD tests for GLMMs: $Z = 1.320$, $P = 0.383$). Additionally, data collected for experimental webs where the *Cyrtophora* host was present showed that the number of insects intercepted by experimental webs differed among field sites (Table 3.2B; Figure 3.2B), and was highest on Amami-Oshima Island (Tukey HSD tests for GLMMs: Cairns Botanic Gardens vs. Boondall Wetlands Reserve in Brisbane: $Z = 10.836$, $P < 0.001$; Amami-Oshima Island vs. Boondall Wetlands Reserve in Brisbane: $Z = 17.670$, $P < 0.001$; Amami-Oshima Island vs. Cairns Botanic Gardens: $Z = 7.254$, $P < 0.001$).

Table 3.2. Effects of (A) field site on the prey interception rate of empty *Cyrtophora* webs and (B) field site, manipulation of guest species on the prey interception rate of experimental *Cyrtophora* webs. Cairns Botanic Gardens and Amami-Oshima Island are denoted as "CNS" and "ASJ" respectively in fixed effects, while date of censoring the individual web is denoted as "date_census" in random effects. The significant terms in fixed effects are in bold.

(A)

Fixed Effects	χ^2	**df**	***P***
Intercept	7410.156	1	**<0.001**
Site	26.967	2	**<0.001**
Random Effects			
σ_{date_census}	0.208		
N_{date_census}	55		
Marginal R^2	0.278		
Observations	105		

(B)

Fixed Effects	χ^2	**df**	***P***
Intercept	172080	1	**<0.001**
Site	312.38	2	**<0.001**
Presence of *Argyrodes* guests	0.078	1	0.781
Manipulation of *Leucauge* web-building guests	305.45	2	**<0.001**
Site X Presence of *Argyrodes* guests	0.790	2	0.674
Site X Manipulation of *Leucauge* web-building guests	2.096	4	0.718
Presence of *Argyrodes* guests X Manipulation of *Leucauge* web-building guests	0.677	2	0.713
Random Effects			
σ_{date_census}	0.124		
N_{date_census}	180		
Marginal R^2	0.656		
Observations	537		

* To facilitate comparisons of incidence rate ratio, the reference level in the categorical independent variables in fixed effects were deliberately designated as the following: "Boondall Wetlands in Brisbane" in "Site"; "absent" in "Presence of Argyrodes guests "; "Spiders and their webs both absent" in "Manipulation of Leucauge web-building guests".

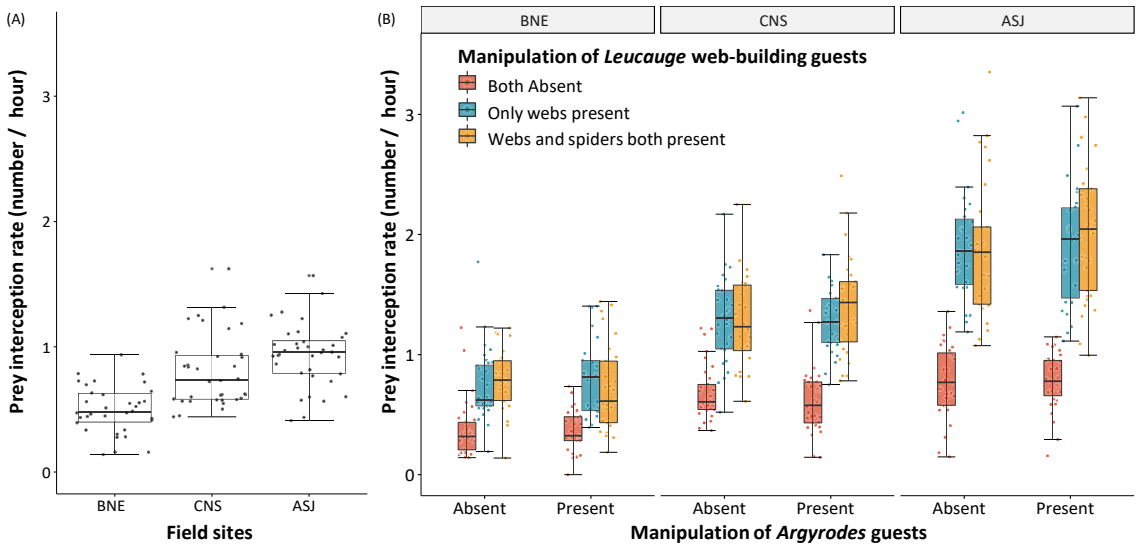

Figure 3.2. Comparisons of prey interception rates for (A) empty webs in different field sites, and (B) experimental webs across treatments. Each dot corresponds to the prey interception of one host web; the centre lines in boxes represent the medians; box limits indicate the 25th and 75th percentiles; whiskers extend 1.5 times the interquartile range from the 25th and 75th percentiles; "Absent" and "present" refer to whether the guest species (and/or their webs) were or were not experimentally removed from the host web; Boondall Wetlands in Brisbane, Cairns Botanic Gardens, and Amami-Oshima Island are denoted as "BNE", "CNS", and "ASJ" respectively.

By manipulating the presence of guest spiders, I discovered that *Cyrtophora* hosts may enhance their foraging success by forming a heterospecific web complex with *Leucauge* guests. Experimental *Cyrtophora* webs with orb-webs of *Leucauge* guests intercepted more prey items than those without, while prey interception rates were not influenced by the presence or absence of *Argyrodes* guests (Table 3.2B; Figure 3.2B). Post-hoc comparisons showed that the presence of *Leucage* spiders on their webs did not influence prey interception rates: there was no significant difference between treatments in which *Leucauge* guests were or were not present on their orb-webs (Tukey HSD tests for GLMMs: Z = 1.629, P = 0.232). The number and size of prey intercepted did not change significantly following the removal of *Argyrodes* guests (comparisons in number are showed in Table 3.2B and Figure 3.2B, while differences in size are presented in Table 3.3 and Figure 3.3A). Manipulation of the presence of orb-web-building *Leucauge* guests had considerable influence on both the number and the size of prey intercepted, with webs of *Cyrtophora* intercepting smaller prey (< 0.8 cm) more frequently when webs of *Leucauge* guests were present (Table 3.4; Figure 3.3B).

Table 3.3. Effect size (Hedges' g) of comparisons in size of prey intercepted in experimental webs with manipulation of *Argyrodes* guests. Text in bracket corresponds to the assessed magnitude defined by Cohen (1992). Boondall Wetlands in Brisbane, Cairns Botanic Gardens, and Amami-Oshima Island are denoted as "BNE", "CNS", and "ASJ" respectively

Field site	Comparison	Prey size (cm)										
		0-0.4	0.4-0.8	0.8-1.2	1.2-1.6	1.6-2	2-2.4	2.4-2.8	2.8-3.2	3.2-3.6	3.6-4	> 4
BNB	*Argyrodes* guests absent vs. *Argyrodes* guests present	0.128 (negligible)	0.173 (negligible)	-0.213 (small)	0.191 (negligible)	0.026 (negligible)	-0.141 (negligible)	0.305 (small)	-0.02 (negligible)	0.05 (negligible)	-0.26 (small)	-0.092 (negligible)
CNS	*Argyrodes* guests absent vs. *Argyrodes* guests present	-0.167 (negligible)	0.051 (negligible)	-0.163 (negligible)	-0.24 (small)	-0.062 (negligible)	-0.078 (negligible)	-0.162 (negligible)	0.106 (negligible)	0.248 (small)	0.096 (negligible)	0.162 (negligible)
ASJ	*Argyrodes* guests absent vs. *Argyrodes* guests present	0.184 (negligible)	-0.068 (negligible)	-0.147 (negligible)	-0.185 (negligible)	0.326 (small)	-0.122 (negligible)	0.233 (small)	-0.26 (small)	-0.018 (negligible)	-0.121 (negligible)	0.071 (negligible)

Table 3.4. Effect size (Hedges' g) of comparisons in size of prey intercepted in experimental webs with manipulation of web-building *Leucauge* guests. Text in bracket corresponds to the assessed magnitude defined by Cohen (1992), where large level is in bold. Boondall Wetlands in Brisbane, Cairns Botanic Gardens, and Amami-Oshima Island are denoted as "BNE", "CNS", and "ASJ" respectively.

Field site	Comparison	Prey size (cm)										
		0-0.4	0.4-0.8	0.8-1.2	1.2-1.6	1.6-2	2-2.4	2.4-2.8	2.8-3.2	3.2-3.6	3.6-4	> 4
BNB	*Leucauge* guests and their webs both absent vs. Only webs of *Leucauge* guests present	**-0.829**	**-1.066**	-0.755	-0.306	-0.36	0.067	0.035	-0.211	-0.021	-0.547	0.077
		(large)	**(large)**	(medium)	(small)	(small)	(negligible)	(negligible)	(small)	(negligible)	(medium)	(negligible)
	Leucauge guests and their webs both absent vs. *Leucauge* guests and their webs both present	**-0.888**	**-0.808**	-0.417	-0.424	-0.327	-0.119	-0.376	-0.377	-0.306	-0.148	0.234
		(large)	**(large)**	(small)	(small)	(small)	(negligible)	(small)	(small)	(small)	(negligible)	(small)
	Only webs of *Leucauge* guests present vs. *Leucauge* guests and their webs both present	-0.048	0.256	0.351	-0.104	0.011	-0.198	-0.408	-0.199	-0.308	0.399	0.187
		(negligible)	(small)	(small)	(negligible)	(negligible)	(negligible)	(small)	(negligible)	(small)	(small)	(negligible)
CNS	*Leucauge* guests and their webs both absent vs. Only webs of *Leucauge* guests present	**-1.226**	**-1.061**	-0.681	-0.703	-0.493	-0.115	-0.183	-0.526	-0.232	-0.027	0.342
		(large)	**(large)**	(medium)	(medium)	(small)	(negligible)	(negligible)	(medium)	(small)	(negligible)	(small)
	Leucauge guests and their webs both absent vs. *Leucauge* guests and their webs both present	**-1.14**	**-1.173**	**-0.81**	-0.477	-0.317	-0.263	-0.169	-0.686	-0.444	-0.114	0.336

Field site	Comparison	Prey size (cm)										
		0-0.4	0.4-0.8	0.8-1.2	1.2-1.6	1.6-2	2-2.4	2.4-2.8	2.8-3.2	3.2-3.6	3.6-4	> 4
		(large)	**(large)**	**(large)**	(small)	(small)	(small)	(negligible)	(medium)	(small)	(negligible)	(small)
	Only webs of *Leucauge* guests present vs. *Leucauge* guests and their webs both present	0.098	-0.223	-0.147	0.239	0.151	-0.153	0.011	-0.221	-0.182	-0.071	0
		(negligible)	(small)	(negligible)	(small)	(negligible)	(negligible)	(negligible)	(small)	(negligible)	(negligible)	(negligible)
ASJ	*Leucauge* guests and their webs both absent vs. Only webs of *Leucauge* guests present	**-1.439**	**-1.339**	**-0.951**	-0.668	-0.754	-0.139	-0.452	-0.723	-0.89	-0.134	0.348
		(large)	**(large)**	**(large)**	(medium)	(medium)	(negligible)	(small)	(medium)	(large)	(negligible)	(small)
	Leucauge guests and their webs both absent vs. *Leucauge* guests and their webs both present	**-1.411**	**-1.21**	-0.782	-0.607	-0.479	-0.466	-0.6	-0.782	-0.519	-0.532	0.24
		(large)	**(large)**	(medium)	(medium)	(small)	(small)	(medium)	(medium)	(medium)	(medium)	(small)
	Only webs of *Leucauge* guests present vs. *Leucauge* guests and their webs both present	-0.237	-0.034	0.055	0.012	0.29	-0.315	-0.121	-0.048	0.392	-0.386	-0.172
		(small)	(negligible)	(negligible)	(negligible)	(small)	(small)	(negligible)	(negligible)	(small)	(small)	(negligible)

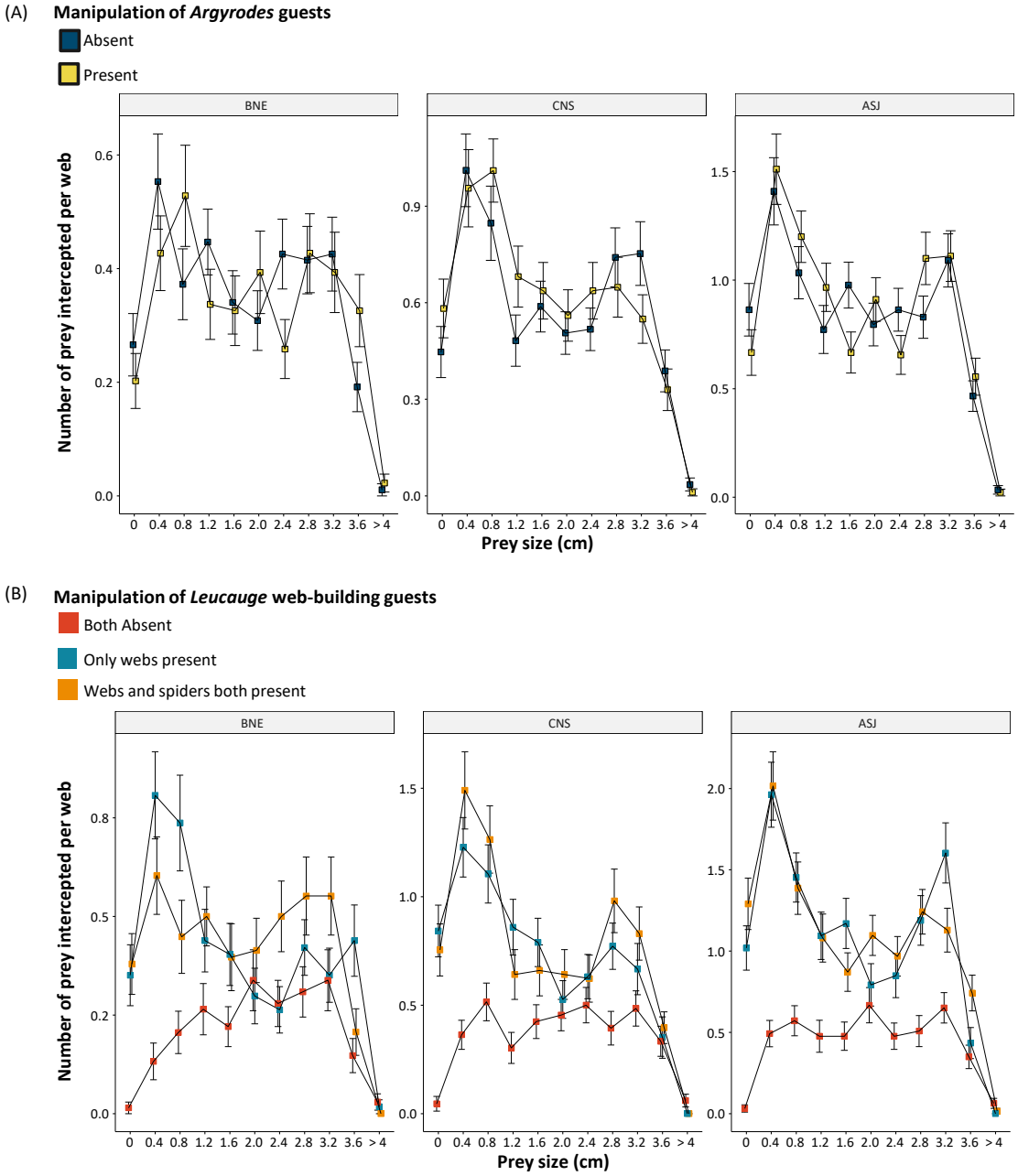

Figure 3.3. Size of prey intercepted by experimental webs with manipulation of (A) *Argyrodes* and (B) *Leucauge* web-building guests. Solid square and whiskers show the average and standard error of the mean. The ticks on x-axis indicate the lower end of each bin. Boondall Wetlands in Brisbane, Cairns Botanic Gardens, and Amami-Oshima Island are denoted as "BNE", "CNS", and "ASJ" respectively

3.4.2 Prey consumption

Field observations showed that the degree of overlap in the feeding regimes of *Cyrtophora* hosts and *Argyrodes* guests varied when *Leucauge* guests were present. I recorded 384 prey consumption events across three field sites, and the results of group-wise Fisher tests revealed that there was a difference in the diet of *Cyrtophora* hosts and *Argyrodes* guests when

Leucauge orb-webs were present (with or without *Leucauge* spiders), but not when *Leucauge* spiders and their webs were absent. *Argyrodes* guests fed more frequently on smaller prey (< 1 cm; the bottom two rows in Figure 3.4) 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 Boondall Wetlands in Brisbane, and P <0.001 in the other two field sites). However, the prey sizes of *Cyrtophora* hosts and *Argyrodes* guest spiders were similar when *Leucauge* spiders and their webs were absent: there was no significant difference in the distribution of 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 Boondall Wetlands in Brisbane; P = 0.787 in Cairns Botanic Gardens; P = 0.166 in Amami-Oshima Island; the top row in Figure 3.4). These data indicate that the diets of guest and host spiders overlapped substantially when the webs of web-building guests were absent.

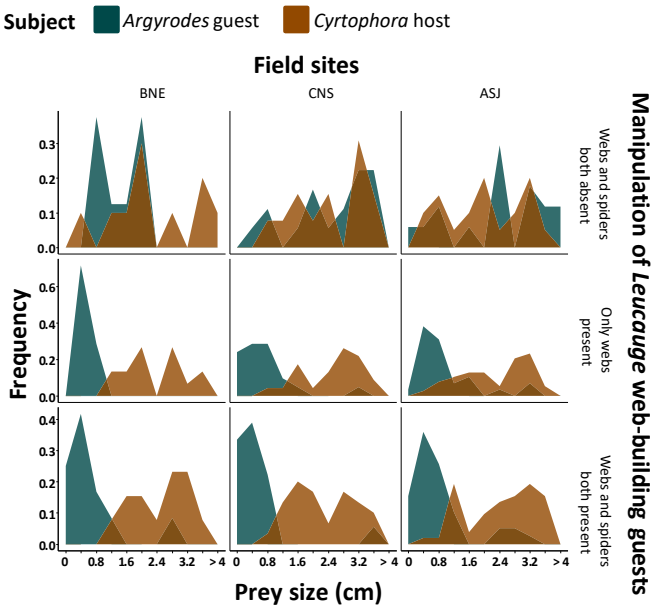

Figure 3.4. Effects of manipulation of *Leucauge* wb-building guests on size distributions of prey consumed by *Cyrtophora* hosts and *Argyrodes* guests. Frequency was calculated as the number in each size bin divided by the total for these two subjects, *Argyrodes* guests *and Cyrtophora* host. The ticks on x-axis indicate the lower end of each bin with increment given by 0.4 cm. Boondall Wetlands in Brisbane, Cairns Botanic Gardens, and Amami-Oshima Island are denoted as "BNE", "CNS", and "ASJ" respectively.

3.4.3 Host growth

The data of host weight gain indicated that *Argyrodes* guests exerted a fitness cost on *Cyrtophora* hosts, but this negative impact was mitigated by the presence of *Leucauge* guests. The growth rate of *Cyrtophora* hosts tended to be higher if webs of *Leucauge* guests were present (Table 3.5; Figure 3.5). The interaction effect between manipulations of *Argyrodes* guests and *Leucauge* web-building guests was significant (Table 3.5; Figure 3.5). Following 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 3.5; Figure 3.5).

Table 3.5. Effects of manipulation of two guest spider species on the weight gain of *Cyrtophora* hosts. The experimental period is denoted as "period" in random effects.

Predictor	Sum of Squares (type III)	*df*	Mean Square	*F*	*P*
Site	1.953	2	0.977	1.568	0.209
Manipulation of *Argyrodes* guest	1.588	1	1.588	2.55	0.111
Manipulation of web-building *Leucauge* guests	185.442	2	92.721	148.872	< 0.001
Site X Manipulation of *Argyrodes* guest	0.537	2	0.269	0.431	0.65
Manipulation of *Argyrodes* guest X Manipulation of web-building *Leucauge* guests	11.097	2	5.548	8.908	< 0.001
Site X Manipulation of web-building *Leucauge* guests	2.761	4	0.69	1.108	0.352
Random Effects					
σ_{period}	0.1509				
N_{period}	12				
Marginal R^2	0.375				
Observations	537				

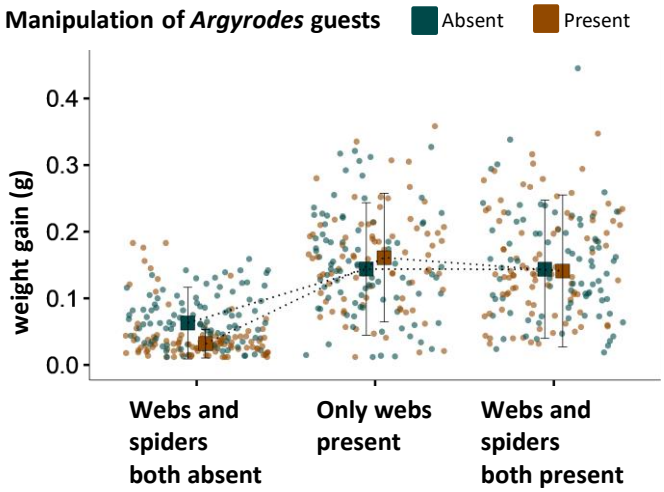

Figure 3.5. Main and interaction effects of manipulation of two guest spider species on host weight gain. Each circle indicates the weight gain of one host. Squares and whiskers represent the median and interquartile range of weight gain in each experimental treatment. Dotted lines link the medians between treatments with and without *Argyrodes* guests.

3.5 Discussion

For multi-species symbioses involving arthropod predators, the presence and absence of one symbiont can have profound implications for the nature of the relationship between the other two symbionts. Consistent with an earlier report of mutualistic associations with two orb-weaving spiders (Elgar, 1994), my experimental manipulations revealed that web-building *Leucauge* guests have a positive impact on the prey interception rate of their *Cyrtophora* hosts: host *Cyrtophora* spiders whose web complex includes the webs of *Leucauge* guests gain more weight, which for adult spiders will translate directly into fecundity (Marshall & Gittleman, 1994; Simpson, 1995), especially as the difference is likely to compound over the life of the host spider. In contrast with previous experiments showing that *Argyrodes* guests with conspicuous body parts may improve the prey capture of their host (Peng, Blamires, Agnarsson, Lin & Tso, 2013), *Argyrodes* species included in this study do not enhance the foraging success of the *Cyrtophora* host, but rather exert a fitness cost, but only when *Leucauge* guests are absent. This arises because of changes in the size of prey size consumed by *Argyrodes* guests and *Cyrtophora* hosts according to the presence of *Leucauge* guests: removing the webs of *Leucauge* orb-weavers resulted in fewer prey being arrested and a convergence in the size of prey consumed by *Argyrodes* and *Cyrtophora*.

Orb-weaver spiders are traditionally considered sit-and-wait predators, but this belies the diverse tactics they employ to enhance prey encounter rates and thus foraging success,

including visual attractants such as body colouration (see Chapter 2); silken decorations (Tan & Li, 2009; Tan *et al.*, 2010); olfactory cues (Bjorkman-Chiswell *et al.*, 2004); and modifying web characteristics according to the heterogeneity in prey availability (Scharf, Lubin & Ovadia, 2011). These strategies may increase prey capture rates, but may also increase the risk of predation (Li, 2005; Fan, Yang & Tso, 2009) or the nutrient costs of silk production (Opell, 2008). The body colouration of *Leucauge* spiders may not function as a visual lure (Chapter 2) because the presence of the spiders did not influence the foraging success of *Cyrtophora* hosts. There are at least three other mechanisms by which the presence of *Leucauge* guest webs may enhance the capture success of *Cyrtophora* hosts. First, the silk of *Leucage* webs may, like that of other web-building spiders reflect light within the UV spectra, thereby exploiting the tendency of dipteran insects to fly toward UV light and thus increase the likelihood of being arrested by the web (Théry & Casas, 2009; Walter & Elgar, 2012). Second, the combination of 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 (see also Elgar (1994)). Thirdly, the silk of *Leucage* webs may contain chemical prey attractants, such as putrecene, that can attract prey to within the vicinity of the web and thus increase prey capture rate, as documented for *Argiope* orb-weavers (Henneken, Goodger, Jones & Elgar, 2017). These mechanisms may combine additively to enhance foraging success for *Cyrtophora*. Although the gain in host weight in the presence of *Leucage* webs was consistent across the different study sites, the prey interception rate in experimental webs of *C. moluccensis* in Japan was highest across the three locations, perhaps reflecting the visual luring function of the body colouration of this species (Blamires, Hou, Chen, Liao & Tso, 2014).

My field experiments illustrate that the antagonistic interaction outcomes of symbioses may vary with the presence of other symbionts. This possibility is typically ignored, as investigations of symbioses typically adopt a one-on-one perspective, and is rarely documented in empirical studies of invertebrate systems. The presence of web-building *Leucauge* guests not only promotes the fitness of the habitat-forming *Cyrtophora* host, but also changes the spectrum of available food and thus the nature of its relationship with *Argyrodes* guest species. *Cyrtophora* host webs allied with the webs of *Leucauge* orb-weavers intercepted more and smaller insect prey, which allowed greater separation in the size of prey captured by hosts and their *Argryrodes* guests. In contrast, when *Leucauge* webs were

experimentally excluded, the hosts experienced significantly less weight gain, presumably because the *Argyrodes* guests impinged on the ability of *Cyrtophora* hosts to capture prey, perhaps through competitive interference. 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.

My study included three sites across distinct climate zones with differences in resources and species composition of symbionts. While my study was not designed to examine how interactions between guests and their hosts respond to local selection pressures, my results suggest that the outcome of multispecies interactions can be similar despite biogeographic differences, including species composition and types of prey. Theoretical and empirical studies indicate that interspecific interactions may shift to competition or collapse under extreme conditions (reviewed in Michalet, Le Bagousse-Pinguet, Maalouf and Lortie (2014)). In group-living spiders, colony aggressiveness can be altered by extreme disturbance events, like tropical cyclones (Little, Fisher, Schoener & Pruitt, 2019). The relationships between the arthropod predators in this study may similarly change under stress caused by extreme weather disturbance events, especially if they targeted particular prey types.

The remarkable consistency in the nature and underlying mechanisms of symbioses of arthropod predators reported here for three distinct populations 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. For example, the same insect species can engage with different facultative bacteria 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 (reviewed in Hajek, Morris and Hendry (2019)). Clearly, the process of biotic factors driving the patterns of outcome variation exists in both positive and negative species interactions.

Empirical studies that examine the complexity of interactions within symbioses (Trager *et al.*, 2010) reveal that conventional classifications of symbiotic interactions can be incorrect. Consequently, it may be misleading to identify the nature of the relationship between

symbionts without considering the symbioses more generally across multi-species networks. There is extensive evidence of the negative effects of *Argyrodes* guests on host fitness (Rypstra, 1981; Tanaka, 1984; Larcher & Wise, 1985; Grostal & Walter, 1997; Koh & Li, 2002), but more recent 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 *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 differences may reflect the traditionally dyadic-species perspective, that makes little reference to other guest spiders that may or may not inhabit the host web. While these studies document the pattern of relationships between host and guest spiders, the synergistic effects arising from other guests are largely ignored. Interspecific foraging groups of spiders have been known for decades, but my understanding of the ecological patterns or processes associated with these web complexes is surprisingly limited (but see Elgar (1994)). My 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.

Chapter 4

Kleptoparasites use odour cues from both hosts and conspecifics to determine settlement patterns

4.1 Summary

Research that considers the evolutionary significance of symbioses typically focusses on elucidating the costs and benefits to each partner, with far fewer attempts to identify the underlying mechanisms that contribute to their persistence in ecological time. Symbioses require individuals of the associating species to come together, and the strength of the cues and signals associated with alerting individual identity and location will depend on the nature of the relationship. I conducted field and laboratory experiments with different pairs of *Cyrtophora* host and *Argyrodes* guests, which form distinct interspecific relationships, to explore the role of odours in determining guest settlement patterns. I discovered that the presence of hosts and conspecifics influences the settlement decisions of *Argyrodes* species: *Argyrodes* guests preferred webs with the resident spider present, and preferred webs with fewer conspecifics. I also found that the effect of conspecifics was stronger for kleptoparasitic than mutualist *Argyrodes*, suggesting that the parasitic nature of the relationship intensifies intra-specific competition. Behavioural assays with y-maze olfactometers further highlighted how chemical communication in symbioses varies with the nature of the relationship. Both *A. kumadai* from Japan and *A. fissifrons* from Taiwan were attracted to host-derived odours, however the klepoparasitic *A. kumadai* was repelled by the odours of conspecifics, while the mutualistic *A. fissfrons* was indifferent to this cue. The differences in the cue discrimination of *Argyrodes* species is matched by the abundance of sensilla on their front legs, highlighting how the nature of symbiotic relationships may shape both signals and morphological traits that optimise signal detection.

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4.2 Introduction

Understanding the evolutionary significance of symbioses requires identification of the costs and benefits to each partner, and the mechanisms that allow the symbiosis to form and persist. Although many studies have investigated the fitness costs and benefits to the individuals involved by the addition or removal of different partner species, few have identified the mechanisms that facilitate these symbioses. The establishment and maintenance of many symbioses requires communication between symbionts, which can involve various stimuli in different sensory modalities. For example, in a marine cleaning mutualism, partner species advertise the cleaning service they provide and avoid predation by their clients using conspicuous colour patterning (Cheney, Grutter, Blomberg & Marshall, 2009; Cheney, 2013). Similarly, some caterpillars attract their tending ant partners with chemical cues (Pierce *et al.*, 2002).

As communication is a vital element of symbioses, the strength of the cues and signals associated with establishing symbiotic associations would arguably depend on the nature of the relationship. Specifically, hosts may actively signal their presence to inquilines in a mutualism (Edwards, Hassall, Sutherland & Yu, 2006). Conversely, as hosts have evolved several strategies to avoid parasites, including reducing the likelihood of detection (Sarabian, Curtis & McMullan, 2018). Accordingly, it is likely that parasites make a greater investment in their sensory morphology in order to locate their host (see also Elgar *et al.* (2018)). However, there is no compelling empirical evidence to support this prediction to date.

Spiders are an ideal group in which to examine the cues and mechanisms underlying the formation and persistence of different types of symbioses. The webs of many large orb-weaving spiders, such as *Cyrtophora* and *Nephila*, can be habitat patches that support one or more guest species. Particularly well known are those belonging to the genus *Argyrodes*, which are obligate web associates that do not build webs but instead settle in the web of an orb-weaver host spider, where they forage for prey items arrested by the web. Field experiments involving *Argyrodes* on the webs of *Cyrtophora* host spiders indicate that the nature of their relationship might not be strictly parasitic or mutualistic (see Chapter 3; Elgar (1994); Peng, Blamires, Agnarsson, Lin and Tso (2013)). Specifically, *Argyrodes* in Australia and Japan are more likely kleptoparasites that exert a fitness cost on their host

when prey availability is low (Chapter 3), while *Argyrodes* in Taiwan form a mutualistic association with their host, in which their body colouration functions as a visual lure to enhance the foraging success of their *Cyrtophora* hosts (Peng, Blamires, Agnarsson, Lin & Tso, 2013). The diverse outcomes of symbioses between these arthropod predators provide an excellent framework to examine the settlement patterns of spider guests, and to reveal associated sensory adaptations.

Here, I investigate the role of odour and other cues in the settlement patterns of guests on the three-dimensional webs constructed by *Cyrtophora* host spiders. Using isolated spider webs as habitat patches, I first conducted a field experiment to reveal whether guest spiders made settlement decisions based on the presence of the host or conspecifics. Second, I used a behavioural assay to determine whether guest species were attracted to chemical cues of hosts, their webs, or of conspecifics. Lastly, I compared the sensilla density on the leg segments of guest spider species, which differ in the nature of their relationship to their *Cyrtophora* host (kleptoparasitc or mutualistc). I focussed on the sensilla of leg segments because of their recognised chemo-receptor function (Witt & Rovner, 2014). A long- held, but untested premise is that information exchange between spider species is likely through olfactory and vibratory channels, as these are commonly used by spiders in the context of mating behaviour (Schulz, 2013; Trabalon, 2013).

4.3 Materials and methods

4.3.1 Field experiments

I conducted field experiments using natural populations of *Cyrtophora* host and *Argyrodes* guest species in Boondall Wetlands Reserve, Brisbane, Queensland, Australia (27°32'45.1644"S, 153°2'54.8772"E); Cairns Botanic Gardens, Cairns, Queensland, Australia (16°53'49.6644"S, 145°44'53.3868"E); Yamato Village, Amami-Ohima Island, Kagoshima Prefecture, Japan (28°20'0.798"N, 129°21'20.4012"E); and Chung-Ai Bridge, Orchid Island, Taitung County, Taiwan (22°0'26.7804"N, 121°34'20.6004"E), in January 2017, February 2018, September 2018, and August 2018 respectively.

I focussed on solitary *Cyrtophora* webs (distance between webs > 1 m) for my field experiments. The surface of each host web was estimated 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. Any web-building spiders that formed an association with *Cyrtophora* webs, such as *Leucauge* web-building symbionts, were removed during the experimental period because aggregations of these webs could be a confounding factor when investigating settlement patterns of *Argyrodes* guests. Interconnected webs tend to be located and invaded by these kleptoparasites more frequently than solitary webs (Elgar, 1989; Agnarsson, 2011). Additionally, because my intention was to investigate exclusively the inter- and intra-specific interactions of *A. fissifrons* or *A. kumadai*, and thus other guest species, such as *A. bonadea* were removed to eliminate potential interspecific competition.

I conducted two separate field experiments. The first investigated whether *Argyrodes* guests locate host webs using cues derived directly from the host. I located recently built (< 1 day) *Cyrtophora* webs, thereby minimising the abundance of conspecific cues of Argyrodes guests, and assigned them randomly as either control or experimental treatments. For experimental webs, I removed both the host and guest spiders from the web, while in the control webs I retained the host but removed any *Argyrodes* guests. The number of *Argyrodes* that settled in these control and experimental webs were recorded each day for the next 5 days.

The second experiment investigated whether cues from *Argyrodes* influence the settlement patterns of conspecifics. I located webs of *Cyrtophora* hosts and assigned recently-built (<

1 day) webs to the control group, and *Cyrtophora* hosts that had been observed with *Argyrodes* guests present for more than 5 days to the treatment group. I removed *Argyrodes* guests from control webs and added individual *Argyrodes* to the webs of the treatment group. Following Peng, Blamires, Agnarsson, Lin and Tso (2013) and preliminary surveys (see Chapter 3), I further added or removed spider guests to ensure that the experimental webs contained 8 guests (twice more than the average number of guests found on the *Cyrtophora* webs) for 3 days before the trial commenced. This pre-experimental period was to ensure that the *Argyrodes* inquilines had sufficiently traversed the experimental webs. The hosts were present on both control and experimental webs, allowing guests to locate host webs using host-derived cues, but whether the settlement patterns would be influenced by having conspecific individuals in the same web could be examined explicitly.

The webs of both experimental and control webs were then censored each day for the following 5 days. I examined whether larger individuals have a competitive advantage in settling on host webs by measuring the body length of all *Argyrodes* with digital callipers, accurate to 0.01 mm, at the end of the experimental period. I conducted both experiments at each field site, yielding sample sizes ranging from 19 – 30 replicates for each treatment for each site (Table 4.1).

Table 4.1. Study sites, host-guest species pairs, and sample sizes for field experiments. The number of guests found by the end of experimental period are shown in parentheses.

Location	*Cyrtophora* host	*Argyrodes* guest	Field experiments 1		Field experiments 2	
			Host present	Host absent	Conspecifics present	Conspecifics absent
Yamato Village, Amami-Ohima Island, Japan	*C. moluccensis*	*A. cf. kumadai*	25	22	27 (103)	27 (98)
Boondall Wetlands Reserve, Australia	*C. cf. cylindroides*	*A. cf. fissifrons*	27	21	25 (110)	27 (109)
Cairns Botanic Gardens, Australia	*C. cf. ikomosanensis*	*A. cf. fissifrons*	22	20	30 (129)	27 (108)
Lanyu, Taiwan	*C. unicolor*	*A. cf. fissifrons*	19	19	20 (150)	24 (99)

4.3.2 Behavioural assays

I used behavioural assays with a Y-maze apparatus to determine the role of chemical cues from heterospecific hosts and conspecific guests in the settlement processes of *Argyrodes* guests. I conducted choice bioassays on *A. kumadai* in Japan and *A. fissifrons* in Taiwan. Although they are described as two different species (Chida & Tanikawa, 1999), and are so described here, molecular evidence reveals no significant differentiation between these two groups of spiders (see Appendix B for details; Figure B1). Nevertheless, they are found on the webs of different *Cyrtophora* hosts (*C. moluccensis* in Japan and *C. unicolor* in Taiwan) and the nature of their interspecific interactions with their hosts appear to be different: *A. fissifrons* are recognised as mutualistic partners in Taiwan, but *A. kumadai* are considered as kleptoparasites in Japan (see Chapter 2 and Peng, Blamires, Agnarsson, Lin and Tso (2013)).

The y-maze apparatus (Figure 4.1) consisted of three arms (80 × 35 × 35 mm each; length, width, height). A chamber (60 × 60 × 60 mm each) was attached to each of the two arms and a stimulus was placed in one only of these chambers. An air pump (Hiblow-C5F, Techno Takatsuki Co Ltd, Takatsuki, Osaka, Japan) created an airflow that passed an activated carbon air purifier (Gas Filter Canister R510-31-6, RWD Life Science, Shenzhen, Guangdong Province, China), split to each of the arms, where it flowed over the stimulus towards the Y-junction. The airflow was controlled and equalised with polycarbonate flowmeters (RMA-11, Dwyer Instruments, Inc., Michigan, Indiana, United States). The floor of the maze was covered with 15 mm wide masking tape, which facilitated movement by the subject. The maze was systematically cleaned with purified boiled water and dried with hot air generated by a hair dryer, and the masking tape was replaced after each trial.

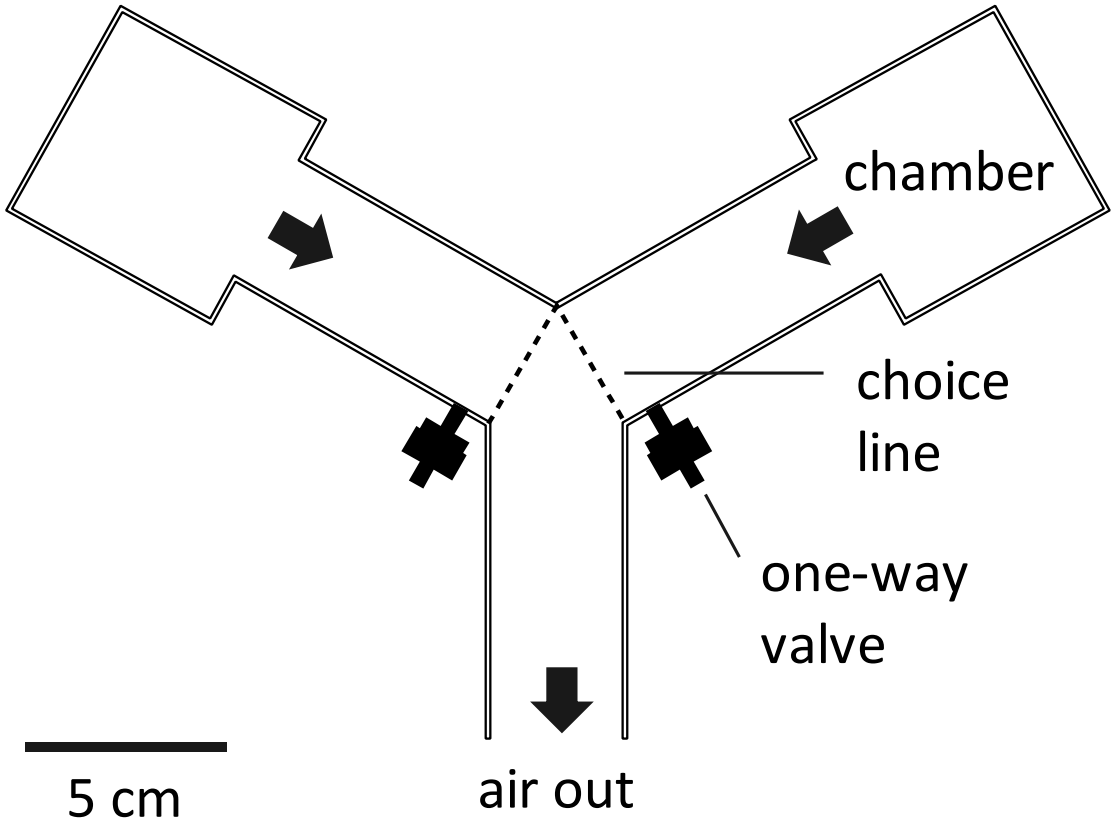

Figure 4.1. Schematic illustration of the Y maze olfactometer for behavioural assays. Arrows show the direction of airflow. The purified and standard air flow was created using an air pump with activated carbon air purifier, with odours flowing toward the Y-junction. The stimulus was randomly assigned to the chamber attached to either arm for each trial. Individual *Argyrodes* subjects were introduced at the bottom of the central arm, and the decision was noted when it passed the choice line (dashed). The airflow was controlled and equalised with the flowmeter and one-way valves on each arm.

Four types of stimuli, collected from the field, were used in the behavioural assays: (1) *Cyrtophora* host (n = 35 in Japan and 23 in Taiwan) and (2) the silk from new (< 1 day) webs of *Cyrtophora* host, thereby minimising the influence of odour from conspecific guests (n = 32 in Japan and 25 in Taiwan); (3) *Argyrodes* guests (n = 44 in Japan and 42 in Taiwan) and (4) the silk from older webs that likely contains the odour from conspecific *Argyrodes* (n = 25 in Japan and 20 in Taiwan). These stimuli were collected from the experimental webs in field experiment 2 (above). The web samples were collected by wrapping silk threads around a glass rod and then scraping off the silk using clean forceps, attaching the silk to a capillary and placing it into a sealed jar until behavioural assays.

I conducted behavioural assays in a temperature-controlled environment (26 ± 1 ℃) at each field site in Taiwan and Japan, in which the only light source was provided by a LED bulb (model: 35004631,5 w/600 lm, TOSHIBA) located approximately 2 meters above the

olfactometer. For each trial, the stimulus was randomly assigned to either arm of the Y-maze apparatus, and the *Argyrodes* subject was introduced at the base of the central arm, from where it was able to walk to the intersection and then choose between the two arms of the maze. The choice line was set as the entrance to the maze arm, and a decision was registered when the prosoma of the subject crossed the choice line. Each *Argyrodes* subject was tested 5 times for each stimulus, and I excluded data from individuals that did not move to the choice line within 10 minutes after the trial began (ca 15% of *A. kumadai* and 12% of *A. fissifrons*). Individual subjects were preserved in 95% ethanol after behavioural assays.

4.3.3 Quantifying sensilla density

I examined the first pair of legs of each specimen of *Argyrodes* subjects used in behavioural assays (Figure 4.5A), since they play a significant role in the foraging behaviour of spiders (Barrantes & Eberhard, 2007; Baba, Osada & Miyashita, 2012). The legs were examined using a Leica microscope (S Apo Stereozoom 1.0x-8.0x) with a stage micrometer accurate to 0.1 mm. The microscopy image was captured by a Nikon D5600 camera motorised and controlled with Helicon Remote software (version 3.9.7. W). I focused on the tarsus (Figure 4.5A), as sensilla are more densely distributed on this part of the leg, compared with other segments (Foelix, 1996). All micrograph files were processed in Helicon Focus (version: 7.0.2 pro), in which images with different depths of the same field were compiled into a single sharp image. These procedures were repeated on both medial and lateral sides of the legs. While I quantified the sensilla density for each micrograph file with ImageJ (Abràmoff, Magalhães & Ram, 2004), the original file name was removed and assigned with a random key number to conceal the species information. I counted the number of sensillum and divided that by the length of the tarsus (estimated with the micrometer), to compute a standardised sensilla density for each specimen.

4.3.4 Data analyses

For field data, I used generalised linear mixed models (GLMMs) with a Poisson distribution to examine the settlement patterns of *Argyrodes* species. The number of *Argyrodes* guests that settled in *Cyrtophora* host webs on each census day was the response variable. Predictor variables were field site (i.e. the host-guest species pair), treatment (i.e. the presence/absence of host and conspecific individuals) and their interactions. Web surface area was included as a log-link offset variable, as abundance of web inhabitants is

strongly associated with the size of the web. For field experiment 1, I included census day and web ID as random variables. For field experiment 2, the number of individuals present on the experimental webs was influenced by the pre-experimental period, therefore the random effects were census day within individual host webs. I employed type III Wald Chi-square tests to determine whether the main effects were significant. For field experiment 2, I compared the body lengths of *Argyrodes* individuals found on control and experimental webs at the end of experimental period using Type III ANOVA. Body length was square-root-transformed to normalize distributions (Shapiro-Wilk normality test, $W = 0.997$, $P = 0.1065$). Predictors were the same as that for the settlement rate models, namely study site, presence of conspecifics and their interaction.

For behavioural assay data, using typical generalised linear models result in singular fits, in which overfitting biased the variance-covariance decomposition parameters towards zero, thereby increasing the probability of false conclusions (Harrison *et al.*, 2018). To avoid singularity, I took Bayesian mixed effects logistic regression with weak prior on the variance (Mai & Zhang, 2018) to analyse the response of *A. kumadai* and *A. fissifrons* toward each type of stimulus. The dependent variable was the subject's choice (1 = chose stimulus; 0 = chose empty arm). The predictor was species (*A. kumadai* or *A. fissifrons*), and the individual subject was included as a random variable. Type III Wald Chi-square tests were used to examine if the impact of guest species on responsiveness to different stimuli was significant.

The sensilla density of medial and lateral sides were averaged for each leg, and the mean sensilla density for each individual was estimated by computing an average of both legs. Mean sensilla density was log-transformed (base = 10) for normalisation (Shapiro-Wilk normality test, $W = 0.958$, $P = 0.066$), and I used a two-sample t-test to compare the sensory hair density of *A. kumadai* with *A. fissifrons*.

4.4 Results

4.4.1 Field experiments

Field experiment 1 revealed that removal of *Cyrtophora* hosts reduced the settlement rate of *Argyrodes* guests (Table 4.2A; Figure 4.2A). When the host spider was present, the number of *Argyrodes* guests increased through successive census days whereas settlement

rate did not change significantly over time following removal of the host. Field experiment 2 showed an effect of conspecific presence on settlement patterns among study sites (Table 4.2B; Figure 4.2B). In Amami-Oshima Island, Boondall Wetlands in Brisbane, and Cairns Botanic Gardens, the number of guests settled in host webs decreased rapidly when conspecifics were present. This pattern was most significant in Japan. By contrast, the settlement rate of guests diminished gradually at Lanyu (Table 4.2B; Figure 4.2B). When conspecifics were absent, the number of *Argyrodes* guests that settled in host webs followed a similar pattern to field experiment 1, in which the number of individuals found on host webs increased through successive census days (Table 4.2B; Figure 4.2B). By the end of the experimental period in field experiment 2, a total of 906 *Argyrodes* were found on *Cyrtophora* host webs (sample sizes are listed in Table 4.1).

Table 4.2. Effects of (A) presence of the host and (B) presence of conspecifics on the settlement rate of *Argyrodes* guests. Significant fixed effects are in bold.

(A)

Fixed Effects	χ^2	**df**	***P***
Intercept	3197.791	1	**< 0.001**
Presence of host	104.076	1	**< 0.001**
Site	3.561	3	0.313
Presence of host X Site	4.651	3	0.173
Random Effects			
$\sigma_{id\_host\_web}$	0.062		
$N_{id\_host\_web}$	175		
σ_{day}	0.312		
N_{day}	5		
Marginal R^2	0.210		
Observations	875		

(B)

Fixed Effects	χ^2	**df**	***P***
Intercept	115310	1	**< 0.001**
Presence of conspecifics	119.08	1	**< 0.001**
Site	9.913	3	**0.019**
Presence of conspecifics X Site	74.338	3	**< 0.001**
Random Effects			
$\sigma_{Day\|web\_id}$	0.049		
$N_{Day\|web\_id}$	207		
Marginal R^2	0.436		
Observations	1035		

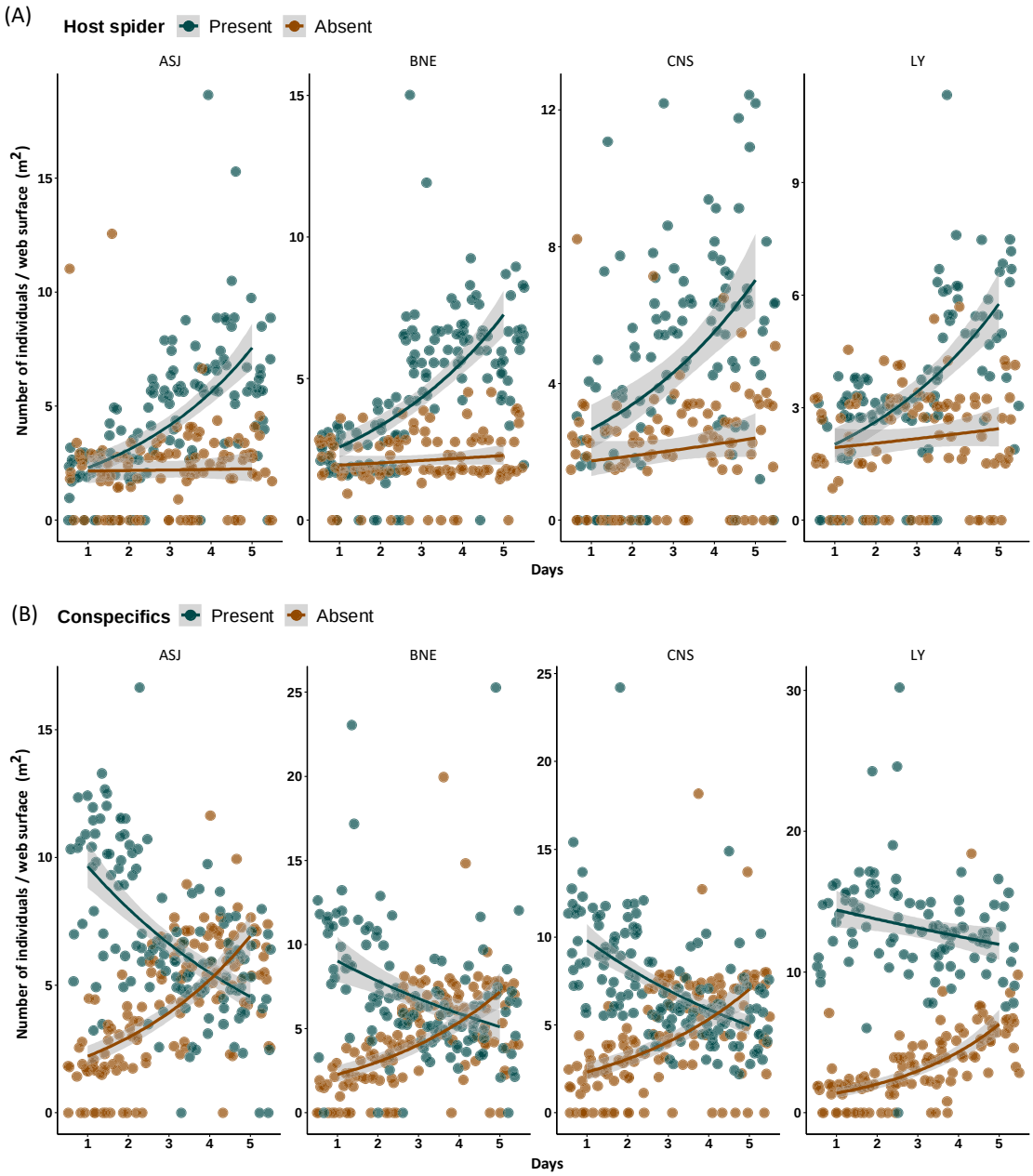

Figure 4.2. The settlement rate of *Argyrodes* individuals in the field experiment: (A) Field experiment 1, in which the presence of the *Cyrtophora* host was manipulated; (B) Field experiment 2, in which the presence of conspecific *Argyrodes* guests was manipulated. Regression lines and 95% confidence intervals (grey areas) were estimated from quasi-Poisson generalised linear models. Each circle represents one *Cyrtophora* host web censored on a given census day. Amami-Oshima Island , Boondall Wetlands in Brisbane, Cairns Botanic Gardens, and Lanyu are denoted as "ASJ", "BNE", "CNS", and "LY" respectively.

The body length of *Argyrodes* found on webs where conspecifics were present was significantly larger than that of individuals that settled on the control webs where conspecifics were absent (Table 4.3, Figure 4.3). Additionally, post-hoc comparisons showed that the guests in Lanyu were the largest among sites (Figure 3; Tukey HSD test:

Lanyu versus Amami-Oshima, $t = 3.014$, $P = 0.014$; Lanyu versus Boondall Wetlands, $t = 3.045$, $P = 0.013$; Lanyu versus Cairns Botanic Gardens, $t = 2.767$, $P = 0.030$).

Table 4.3. Type III ANOVA results comparing body length of guests between treatments among study sites.

Predictor	Sum of square	df	Mean square	*F*	*P*
Intercept	6443.8	1	6443.8	6443.8	**< 0.001**
Presence of conspecifics	3.7	1	3.7	50.126	**< 0.001**
Site	1.0	3	0.333	4.464	**0.004**
Presence of conspecifics X Site	0.4	3	0.133	1.764	0.152
Error	66.6	898	0.074		

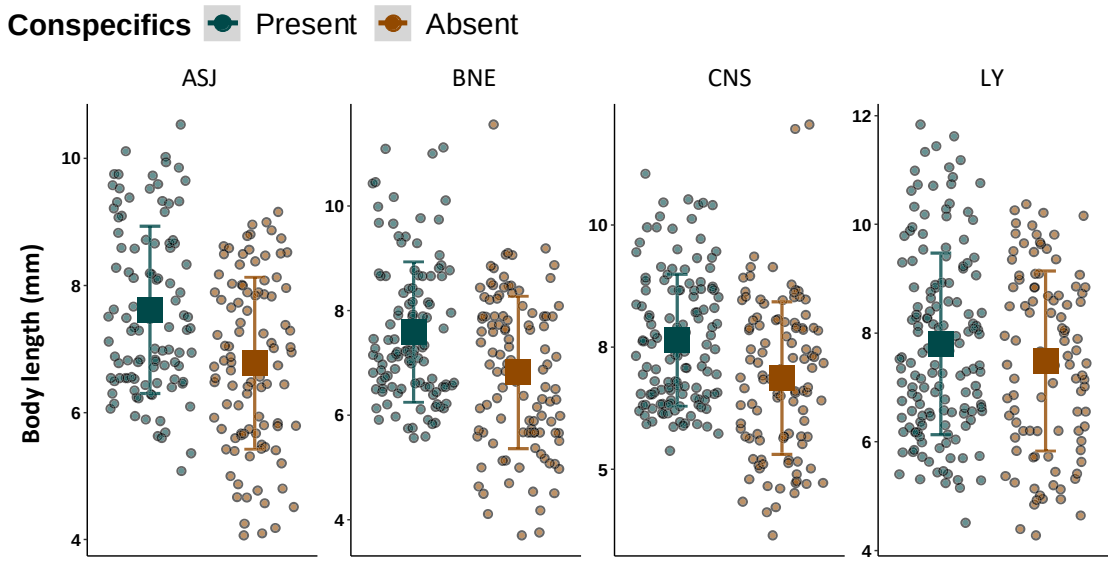

Figure 4.3. Comparisons of body length for guests amongst study sites in Field experiment 2. Solid squares and whiskers indicate the average and standard deviation respectively. Each circle indicates a measure of body length of one guest.

4.4.2 Behavioural assays

52 subjects (25 *A. kumadai* and 27 *A. fissifrons* individuals) were tested in the behavioural assays. When using host spiders as the stimulus, individuals of both *A. kumadai* and *A. fissifrons* preferred the arm with the host odour. Similarly, most subjects of both guest spider species tended to select the arm containing host web silk (Table 4.4; Figure 4.4).

Table 4.4. Results of behavioural assays, indicating the difference in the responsiveness of *A. kumadai* and *A. fissifrons* guests for each type of stimulus using mixed effects logistic regression. Individual subject is denoted as "subject" in random effects. Significant fixed effects are in bold.

Fixed effects				**Random effects**
Stimulus	χ^2	**df**	***P***	$\sigma_{subject}$
Host spider	0.044	1	0.834	0.5173
Host web silk	2.1877	1	0.139	0.2945
Conspecifics	14.209	1	**< 0.001**	0.339
Host web silk with conspecifics cues	32.260	1	**< 0.001**	0.312

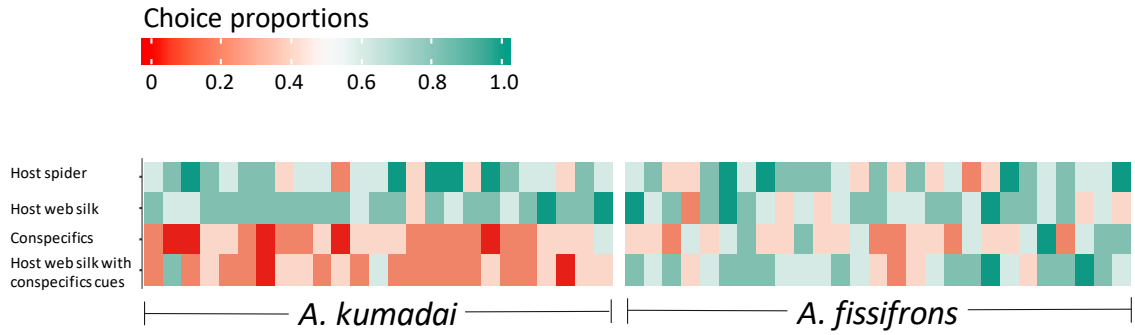

Figure 4.4. Results of choice assay, showing the differences in response to each type of stimulus. Choice proportions were calculated as the proportion of trials that each subject chose the arm containing a given stimulus, where colours in cyan and red reflect preference (value close to 1) and repellence (value close to 0) respectively. From top to bottom, each row corresponds to a given type of stimulus, and each square represents the choice proportion of one subject.

There were differences in the response of individuals of *A. kumadai* and *A. fissifrons*, when conspecifics and host web silk with conspecific cues were used as stimuli (Table 4.4; Figure 4.4). Although *A. fissifrons* guests did not show a significant preference for conspecifics only, they generally preferred the arm containing host silk with conspecific cues. By contrast, *A. kumadai* not only frequently chose the arm without conspecifics but also mostly entered the arm without host silk when it had conspecific cues (Table 4.4; Figure 4.4).

4.4.3 Quantifying sensilla density

Sensilla density of the tarsal segment (estimated by the absolute number of sensilla) of *A. kumadai* guests was significantly higher than that of *A. fissifrons* (Figure 4.5B; two sample t test with equal variance, $t = -9.267$, df = 50, $P < 0.001$).

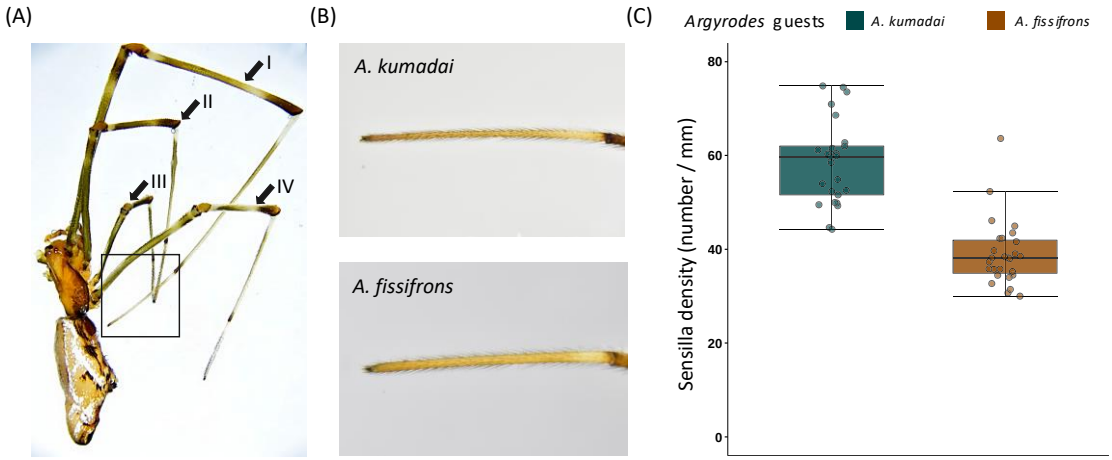

Figure 4.5. Comparison of sensilla density between *A. kumadai* and *A. fissifrons*. (A) image of *Argyrodes fissifrons* – the Roman numerals and arrows indicate the order of leg pairs. The black square at the distal end of leg I indicates the approximate area over which the sensilla density on the first pair of legs was quantified. Photo credit: Ting-Siou Chen. (B) Representative microscopy photos. (C) Sensilla density of tarsus.

4.5 Discussion

My field experiments revealed that the settlement decisions of *Argyrodes* guests are influenced by the presence of both hosts and conspecifics, a pattern that was consistent across different species pairs: the settlement rate of *Argyrodes* onto host webs was slower following experimental removal of the host, and the number of settling guests on the host web dropped when more conspecifics were experimentally added to the host web. Olfactometer assays confirmed that odour perception is important in the settlement process: both *Argyrodes* guest species preferred host cues but differed in their discrimination of *Argyrodes* species. The kleptoparasitic *A. kumadai*, was repelled by the odours of conspecifics, while the mutualist *A. fissfrons* was indifferent to conspecific odours. These differences are reflected in the density of sensilla on the legs of the two species of *Argyrodes*: higher densities were found in *A. kumadai*, which responds to both host and conspecific cues, than on *A. fissifrons*, which responds to host cues only. Combined, my study provides evidence that the nature of symbiotic relationship may shape sensory systems.

My experiments confirmed host dependency in a host-ectoparasite system composed of arthropod predators, a feature that has been widely noted in vertically inherited, nutritional

symbioses (Fisher, Henry, Cornwallis, Kiers & West, 2017). A new web without a host might seem more appealing to kleptoparasites, since the capture spiral can still intercept prey and an absent host removes any possibility of competition or predation. However, the results of my field experiment revealed the opposite pattern, with more guests preferred to settle in the control webs where the host was present, a pattern consistent with other symbioses involving *Argyrodes* (Elgar, 1994). These patterns arise because *Argyrodes* can detect and respond to cues produced by their host, although it is not known whether these chemical cues are host specific. The magnitude of the negative consequence for *Argyrodes* settling on a web without a host is unclear – there may be foraging opportunity costs if webs with hosts intercept more prey than webs without hosts, which would certainly be the case as the webs without hosts would deteriorate and be less effective.

My results highlight how intraspecific competition among kleptoparasite spider guests can determine their settlement patterns. Antagonistic interactions are common among animals living in ephemeral and patchy resources (Strong, Lawton & Southwood, 1984; Holdridge, Cuellar-Gempeler & terHorst, 2016; Roeleke, Johannsen & Voigt, 2018), and *Argyrodes* spiders that reside on host webs are similarly expected to experience strong competition with conspecifics. Several studies of spider kleptoparasitism have similarly found that removing conspecifics results in an influx of other individuals (Elgar, 1989; Miyashita, 2001). Furthermore, Miyashita (2001) discovered that immigrating guest individuals were significantly smaller. While these experimental studies revealed the pattern of intraspecific competition among spider guests, the results might simply reflect age differences in dispersal, such that smaller individuals are more likely to be juveniles with a higher tendency to disperse. My field experiment, which manipulated competition by adding conspecifics, showed that larger individuals prevail when competition increases: the average size of individuals collected from the higher density treatment were larger than those collected from control webs. The consistent pattern across field sites most likely reflects interference competition, where individuals directly prohibit the ability of others to access a common resource, rather than exploitation, where individuals interact indirectly as they compete for common resources (Mishra, Behera & Senapati, 1995). Interactions between *Argyrodes* guests are resolved by body size (Whitehouse, 1997b), where larger individuals have a competitive advantage in obtaining prey items over smaller individuals (Tso & Severinghaus, 2000). Although not specifically examined in this study, it is possible

that larger *Argyrodes* individuals may be more likely to successfully settle in the webs with the host present than smaller ones.

The parasitic nature of the interspecific association between host and *Argyrodes* guest might intensify intraspecific competition, since the risk of being detected and attacked by the host would increase with increasing numbers of *Argyrodes* kleptoparasites on the same web. In contrast, the host may be more tolerant of mutualistic guests, and so the risk of attack may not increase with increasing numbers of *Argyrodes* mutualists. The settlement rate of mutualistic guests in Lanyu diminished gradually, perhaps also indicating that competition was less intense. My choice assays provide additional support: kleptoparasitic *A. kumadai* were repelled by the odours of conspecifics, including host web silk containing conspecific cues, while mutualistic *A. fissifrons* in Lanyu were neither repelled nor attracted to these cues.

Symbioses require effective sensory organs that allow associates to detect each other, and the significance of these cues may depend upon the nature of the symbiosis. I detected a greater density of sensilla on the tarsal segment of kleptoparasitic *A. kumadai* than that of the mutualistic *A. fissifrons*. There are at least two possible selective pressures shaping the development of chemoreceptors on these spider guests. First, hosts that benefit from their mutualistic *Argyrodes* guests may release a more conspicuous chemical cue, thereby more readily attracting *Argyrodes* mutualists. In contrast, selection may favour less conspicuous odours from hosts that suffer from a kleptoparasitic interaction, in which case kleptoparasitic *Argyrodes* guests would require greater investment in sensilla number to detect the more cryptic cues. The settlement rate of *Argyrodes* species did not change significantly across different field sites, which is not unexpected when there is ongoing reciprocal selection pressures arising from interactions between mutualistic/kleptoparasitic spider guests and their host. Second, kleptoparasitic *A. kumadai* guests both avoided settling on a host web with a higher density of conspecifics and were repelled by the odours of conspecifics. This behaviour requires the ability to detect the odours of both hosts and conspecifics, perhaps favouring a higher density of sensilla to support the larger number of receptors.

My study highlights the link between diverse foraging strategies and chemosensory structures, which remain poorly understood. For example, sensilla associated with

chemoreception (basiconica and placoidea) are more abundant on the antennae of specialist than generalist species of braconid parasitoid wasps (Ngumbi, Chen & Fadamiro, 2010), while the opposite pattern was revealed in a phylogenetic comparative analysis of chalcid wasps (Symonds & Elgar, 2013). *Argyrodes* species, with their worldwide distribution, remarkable variation in host specificity (Whitehouse, 2011), and a relatively well sourced phylogenetic history (Su & Smith, 2014), provide great opportunities for comparative analyses of the association between host specialization and micro-morphology of odorant perception.

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Appendices

Appendix A: Supplemental experimental procedures for Chapter 2

A.1 Calculating colour contrast in psychophysical colour spaces

I used a bee colour hexagon model (Chittka, 1992), dipteran categorical and tetrahedral models (Troje, 1993), and a hawkmoth Maxwell triangle model (Johnsen *et al.*, 2006) for comparing the spectra of paper used for cardboard model spiders to those obtained from the corresponding body surface of *N. pilipes* to approximate colour vision of diurnal and nocturnal prey respectively. Chromatic contrasts were computed as the Euclidean distance between stimuli in visual spaces. Achromatic (brightness) contrasts were calculated as the difference in photoreceptor signal of green receptor in bee colour hexagon model (Chittka, 1992), whereas in hawkmoth Maxwell triangle model (Johnsen *et al.*, 2006) and in dipteran categorical and tetrahedral models (Troje, 1993; Yamaguchi, Desplan & Heisenberg, 2010), the achromatic contrasts were computed as the difference of photoreceptor signals between stimuli divided by the sum of photoreceptor signals of stimuli, when the achromatic channel (green-alone receptor for hawkmoth model; R1-6 receptor for dipteran model) is considered exclusively. In the following calculations, I used published reflectance spectra of *N. pilipes* (Tso, Lin & Yang, 2004) and paper measured in this study as stimuli, while the vegetation background reflectance spectrum is that measured by Tso, Lin and Yang (2004).

A.1.1 Bee colour hexagon model

Calculating the quantum catch Q of photons reflected by stimulus n and captured by photoreceptor type i across the given wavelength λ (300-700 nm):

$$Q_i = \int_{\lambda} R_n(\lambda) S_i(\lambda)\, I(\lambda) d(\lambda) \qquad (eq\ 1)$$

where $S_i(\lambda)$ is the sensitivity function of photoreceptor i which stands for UV, Blue or Green as reported by Chittka (1992), $R_n(\lambda)$ is the reflectance spectrum of stimulus n (spider body patches or papers) and $I(\lambda)$ is the illuminant spectrum obtained from Tso, Lin and Yang (2004), in which study the daylight spectrum of the understory forest illumination. Quantum catches q were then normalized by the quantum catch from the

vegetation background to model chromatic adaptation. Following the von Kries transformation:

$$q_{n,i} = \frac{Q_{n,i}}{Q_{back,i}} \qquad (eq\ 2)$$

Photoreceptor signals S were assumed to be nonlinearly related to quantum catches. A nonlinear relationship was modelled using a hyperbolic transformation:

$$S_{n,i} = \frac{q_{n,i}}{1 + q_{n,i}} \qquad (eq\ 3)$$

Neural signals from different photoreceptors can be coded by combining $\mathrm{s}_{n,i}$ from the different photoreceptors. In colour hexagon model, the coordinates are calculated as:

$$\{x_n, y_n\} = \left\{ \sqrt{\frac{3}{2}} \left(s_{n,Green} - S_{n,UV}\right), \qquad S_{n,Blue} - 0.5\left(S_{n,UV} + S_{n,Green}\right) \right\} \qquad (eq\ 4)$$

A.1.2 Hawkmoth Maxwell triangle model

The following equation was used to calculate the quantum catches N of one ommatidium of hawkmoth across the given wavelength λ (350-700 nm):

$$N = 1.13 \left(\frac{\pi}{4}\right) n \Delta\rho^2 D^2 \Delta t \int_{\lambda} \kappa\tau \left(1 - e^{-\mathrm{k}\mathrm{R}_i(\lambda)\mathrm{l}}\right) \mathrm{L}(\lambda)\mathrm{d}\lambda \qquad (eq\ 5)$$

Where n is the effective facets in the superposition, $\Delta\rho$ is the photoreceptor acceptance angle, D is the diameter of a facet lens, Δt is the integration time of a photoreceptor, κ is the quantum efficiency of transduction, τ is the fractional transmission of the eye media, k is the absorption coefficient of the rhabdom, l is the rhabdom length doubled by tapetal reflection, $\mathrm{R}_i(\lambda)$ (i=UV, Blue, Green, or Green alone) are the absorbance spectra of three photoreceptor, and $\mathrm{L}(\lambda)$ is the radiance of stimuli, which is the multiplication of reflectance spectra of stimuli (spider body patches or paper) and full moonlight

illumination. Apart from reflectance spectra, the aforementioned parameters and nocturnal illumination spectrum follow those reported in Johnsen *et al.* (2006). Next, quantum catches $N$are intensity-normalized with von Kries transformation incorporating the sum of quantum catches from the vegetation background.

$$q_{n,i} = \frac{\frac{N_{n,i}}{N_{back,i}}}{\frac{N_{n,UV}}{N_{back,UV}} + \frac{N_{n,Blue}}{N_{back,Blue}} + \frac{N_{n,Green}}{N_{back,Green}}} \qquad (eq\ 6)$$

No further transformation was applied to $q_{n,i}$ assuming photoreceptor signals $S_{n,i}$ are linearly related to quantum catches in Hawkmoth Maxwell triangle model:

$$S_{n,i} = q_{n,i} \qquad (eq\ 7)$$

The coordinates in triangle visual space of each stimulus are calculated using combinations of receptor responses $S_{n,i}$:

$$\{x_n, y_n\} = \left\{\frac{1}{\sqrt{2}}\left(s_{n,Green} - S_{n,Blue}\right), \quad \frac{\sqrt{2}}{\sqrt{3}}\left(S_{n,UV} - \frac{S_{n,Green} + S_{n,Blue}}{2}\right)\right\} \qquad (eq\ 8)$$

A.1.3 Dipteran categorical and tetrahedral models

The quantum catches q was estimated using formula of $eq\ 1$ with normalised relative sensitivity function of the photoreceptor subtypes R7p, R7y, R8p, R8y, and R1-R6 of *Drosophila melanogaster* reported by Yamaguchi, Desplan and Heisenberg (2010).

The quantum catch values were normalised to sum to one, and the coordinates in categorical space of each stimulus are computed as:

$$\{x_n, y_n\} = \{q_{R7p} - q_{R8p};\ q_{R7y} - q_{R8y}\} \qquad (eq\ 9)$$

In tetrahedral space, the quantum catches were log-transformed according to Weber-Fechner's law and then normalised to sum to one, the coordinates were subsequently determined as:

$$\{x_n, y_n, z_n\} = \left\{ \frac{1 - 2q_{R7y} - q_{R8p} - q_{R7p}}{2} \sqrt{\frac{3}{2}}, \frac{-1 + 3q_{R8p} + q_{R7p}}{2\sqrt{2}}, q_{R7p} - \frac{1}{4} \right\} (eq\ 9)$$

A.2 Chromatic properties of dummies

I compared the spectra of papers (Figure A1) with those obtained from body parts of *N. pilipes* (Tso, Lin & Yang, 2004) using achromatic and chromatic colour contrasts in bee colour hexagon model (Chittka, 1992), fruit fly categorical model (Troje, 1993), tetrahedral model (Endler & Mielke, 2005), and hawkmoth Maxwell model (Johnsen *et al.*, 2006). The results are presented in Table A3 and Table A4. For papers that I used for the daytime field manipulation, the chromatic contrast value between the chosen yellow paper and corresponding spider yellow body is significantly lower than the honeybee colour discrimination threshold of 0.11 hexagon units for unconditioned bees (Dyer & Chittka, 2004) (Table A3. One sample t-test: μ=0.11, t=-18.786, df=4, p<0.0001). The chromatic colour contrast of black paper against spider black body parts is significantly lower than the discrimination threshold (Table A3. One sample t-test: $\mu = 0.11,\ t = -5.554,\ df = 4,\ p = 0.003$), while the chosen blue paper has the lowest achromatic contrast value ($MEAN \pm SEM = 0.059 \pm 0.001$) against corresponding spider yellow body parts among all blue paper samples, but its chromatic contrast value ($MEAN \pm SEM = 0.146 \pm 0.001$) is slightly higher than honeybee colour discrimination thresholds (Table S3. One sample t-test: $\mu = 0.11, t = 37.987, df = 4, p < 0.0001$).

The chromatic and achromatic contrast values of blue paper against spider yellow body parts are significantly higher than those of yellow paper (Table A3; two sample equal variance t-test for chromatic contrast comparison in categorical space: $t = -78.473,\ df = 8,\ p < 0.0001$; two sample equal variance t-test for achromatic contrast comparison in categorical space: $t = -53.715,\ df = 8,\ p < 0.0001$; two sample unequal variance t-test for chromatic contrast comparison in tetrahedral space: $t = -25.845,\ df = 4.2631,\ p < 0.0001$; two sample equal variance t-test for achromatic contrast comparison in tetrahedral space: $t = -54.343,\ df = 8,\ p < 0.0001$), suggesting that the blue paper I used in the daytime

experiment is more conspicuous than spider yellow body parts in drosophilid vision. The chromatic contrast in bee hexagon model between yellow and blue paper is significantly higher than the honeybee colour discrimination threshold of 0.11 hexagon units for unconditioned bees (Table S3. One sample t-test: $\mu=0.11,\ t=10.978,\ df=24,\ p<0.0001$), while the achromatic contrast is significantly lower than the threshold value (Table A3. One sample t-test: $\mu = 0.11,\ t = -92.548,\ df = 24,\ p < 0.0001$), suggesting that the blue paper has similar luminance but its chromatic signal is distinct from the yellow paper when viewed by hymenopteran insects. The achromatic contrast of yellow paper against blue paper in fruit fly categorical and tetrahedral space are all negative (Table A3. -0.375 ± 0.002 and -0.05 ± 0.000), suggesting that blue paper is brighter than yellow paper when viewed by dipteran insects using the achromatic channel.

For papers that I used for the night-time field manipulation, the chromatic contrast value between the yellow paper and spider yellow body parts is 0.045 ± 0.001 (MEAN $\pm$ SEM), and -0.003 ± 0.026 (MEAN $\pm$ SEM) between the black paper and spider black body parts (Table S4). The chosen blue paper has the lowest achromatic contrast value (Table A4. MEAN $\pm$ SEM $= 0.087 \pm 0.003$) against corresponding spider yellow body parts among all blue paper samples, while its chromatic contrast value is 0.199 ± 0.001 (MEAN $\pm$ SEM). To date, the colour discrimination thresholds in hawkmoth Maxwell triangle model are not applicable hence I didn't perform statistical tests for these chromatic contrast values against threshold value. The chromatic contrast of yellow paper against blue samples is 0.268 ± 0.001 (Table A4), which is the group which has the highest value in rank order of the means in all pairwise comparisons using different combinations of papers, spider body parts, and vegetation background (TukeyHSD test: Minimum Significant Difference $= 0.005,$ alpha $=$ 0.05), while achromatic contrast derived from this group is closest to 0 in rank order of the means (TukeyHSD test: Minimum Significant Difference $= 0.036,$ alpha $= 0.05$) amongst all comparisons, suggesting that the blue paper has similar brightness to the yellow paper but appear discriminable when viewed by nocturnal moths using the chromatic channel.

Appendix A

Supplemental experimental procedures for Chapter 2

Table A1. Dataset for investigating the association between spider ventral signal and potential for prey luring in the comparative analysis. *Natural history* characterises the ambient light condition of a spider species using information on the macrohabitat (*Ma*), microhabitat (*Mi*), and refuge (*Re*) of the spiders. "S" and "L" represent the degree of potential for prey luring as small or large respectively, while "-" denotes unavailable information. Table 1 further describes these characters. Please refer to the Materials and Methods section for details on categorising spider ventral signal and prey viewing environment.

				Natural history						
Family	Genus	Species	Distribution	*Ma*	*Mi*	*Re*	Potential for prey luring	Yellow and dark mosaic pattern	White and dark mosaic pattern	Source
Agelenidae	*Agelenopsis*	*Agelenopsis aperta*	USA, Mexico	-	L	S	Small	Absent	Absent	Singer and Riechert (1995); Whitman-Zai, Francis, Geick and Cushing (2015)
Agelenidae	*Allagelena*	*Allagelena gracilens*	Central Europe, Mediterranean to Central Asia	-	L	S	Small	Absent	Absent	Roberts (1995)
Agelenidae	*Tegenaria*	*Tegenaria domestica*	Cosmopolitan	S	L	S	Small	Absent	Absent	Yin *et al.* (2012)
Agelenidae	*Textrix*	*Textrix denticulata*	Europe	-	L	S	Small	Absent	Present	Roberts (1995)
Amaurobiidae	*Amaurobius*	*Amaurobius ferox*	Holarctic	S	L	S	Small	Absent	Absent	Almquist (2006)
Amaurobiidae	*Callobius*	*Callobius claustrarius*	Palearctic	S	L	S	Small	Absent	Absent	Roberts (1995)
Amaurobiidae	*Coelotes*	*Coelotes atropos*	Europe	-	S	S	Small	Absent	Absent	Roberts (1995)
Amphinectidae	*Metaltella*	*Metaltella simoni*	Brazil, Uruguay, Argentina (introduced in USA and Canada)	S	S	S	Small	Absent	Absent	Leech (1971); Vetter, Vincent, Berrian and Kempf (2008)
Araneidae	*Acanthepeira*	*Acanthepeira stellata*	Canada to Mexico	L	L	S	Small	Absent	Present	Hentz (1850); Levi (1976)
Araneidae	*Araneus*	*Araneus diadematus*	Holarctic	-	L	S	Small	Present	Absent	Dondale, Redner, Paquin and Levi (2003)
Araneidae	*Araneus*	*Araneus marmoreus*	Holarctic	L	L	S	Small	Present	Present	Dondale, Redner, Paquin and Levi (2003); Kim and Lee (2012)

				Natural history						
Family	Genus	Species	Distribution	*Ma*	*Mi*	*Re*	Potential for prey luring	Yellow and dark mosaic pattern	White and dark mosaic pattern	Source
Araneidae	*Argiope*	*Argiope trifasciata*	Cosmopolitan	L	L	L	Large	Present	Present	Levi (2004)
Araneidae	*Argiope*	*Argiope argentata*	USA to Chile, Argentina	L	L	L	Large	Present	Present	Levi (2004)
Araneidae	*Argiope*	*Argiope submaronica*	Mexico to Bolivia, Brazil	L	L	L	Large	Present	Present	Levi (2004)
Araneidae	*Caerostris*	*Caerostris darwini*	Madagascar	L	L	L	Large	Absent	Present	Kuntner and Agnarsson (2010)
Araneidae	*Cyclosa*	*Cyclosa conica*	Holarctic	L	L	L	Large	Absent	Present	Levi (1977b)
Araneidae	*Cyrtophora*	*Cyrtophora moluccensis*	India to Japan, Indonesia, Papua New Guinea, Australia, Fiji, Tonga	L	L	L	Large	Present	Present	Yin *et al.* (2012)
Araneidae	*Gasteracantha*	*Gasteracantha sauteri*	China, Taiwan, Vietnam	L	L	L	Large	Present	Absent	Feng (1990)
Araneidae	*Larinioides*	*Larinioides cornutus*	Holarctic	L	L	L	Large	Present	Absent	Levi (1974)
Araneidae	*Mangora*	*Mangora acalypha*	Palearctic	L	L	L	Large	Present	Present	Almquist (2005)
Araneidae	*Mecynogea*	*Mecynogea lemniscata*	USA to Argentina	L	L	L	Large	Present	Absent	Levi (1980); Levi (1997)
Araneidae	*Metepeira*	*Metepeira labyrinthea*	North America	L	L	S	Small	Present	Present	Levi (1977a)
Araneidae	*Micrathena*	*Micrathena sagittata*	North, Central America	L	L	L	Large	Present	Absent	Levi (1978); Levi (1985)
Araneidae	*Neoscona*	*Neoscona arabesca*	North, Central America, West Indies	L	L	S	Small	Present	Present	Dondale, Redner, Paquin and Levi (2003)
Araneidae	*Phonognatha*	*Phonognatha graeffei*	Australia	L	L	L	Large	Present	Absent	Dondale (1966)
Araneidae	*Zygiella*	*Zygiella x-notata*	Holarctic, Neotropical	S	L	S	Small	Absent	Absent	Dondale, Redner, Paquin and Levi (2003)
Deinopidae	*Deinopis*	*Deinopis longipes*	Mexico to Panama	-	L	L	Large	Absent	Absent	Chickering (1963); Preston-Mafham (1998)

				Natural history						
Family	Genus	Species	Distribution	*Ma*	*Mi*	*Re*	Potential for prey luring	Yellow and dark mosaic pattern	White and dark mosaic pattern	Source
Desidae	*Badumna*	*Badumna longinqua*	Eastern Australia, New Zealand, Japan, USA, Uruguay, Argentina, Germany	L	S	S	Small	Absent	Present	Marples (1959); Forster (1970)
Dictynidae	*Dictyna*	*Dictyna uncinata*	Palearctic	-	L	S	Small	Absent	Present	Roberts (1995); Harvey, Nellist and Telfer (2002)
Eresidae	*Adonea*	*Adonea fimbriata*	Mediterranean	-	S	S	Small	Absent	Present	Miller *et al.* (2012)
Eresidae	*Dorceus*	*Dorceus fastuosus*	Tunisia, Senegal, Israel	L	S	S	Small	Absent	Absent	Koch and Hahn (1846); Miller *et al.* (2012)
Hahniidae	*Cryphoeca*	*Cryphoeca silvicola*	Palearctic	-	S	S	Small	Absent	Absent	Roberts (1995)
Linyphiidae	*Bolyphantes*	*Bolyphantes alticeps*	Palearctic	-	S	L	Small	Absent	Absent	Roberts (1995)
Linyphiidae	*Diplostyla*	*Diplostyla concolor*	Holarctic	-	S	L	Small	Absent	Absent	Comstock (1940); Locket and Millidge (1953)
Linyphiidae	*Drapetisca*	*Drapetisca socialis*	Palearctic	-	L	S	Small	Absent	Absent	Roberts (1995); Harvey, Nellist and Telfer (2002)
Linyphiidae	*Erigone*	*Erigone arctica*	Holarctic	L	S	L	Small	Absent	Absent	Roberts (1987); Harvey, Nellist and Telfer (2002)
Linyphiidae	*Floronia*	*Floronia bucculenta*	Europe, Russia	L	L	L	Large	Absent	Absent	Roberts (1995)
Linyphiidae	*Frontinella*	*Frontinella communis*	North, Central America	L	L	L	Large	Absent	Absent	Kaston (1948); Harvey, Nellist and Telfer (2002)
Linyphiidae	*Gonatium*	*Gonatium rubellum*	Palearctic	-	S	L	Small	Absent	Absent	Locket and Millidge (1953); Roberts (1995)
Linyphiidae	*Helophora*	*Helophora insignis*	Holarctic	L	L	L	Large	Absent	Absent	Roberts (1995)

Appendix A

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				Natural history						
Family	Genus	Species	Distribution	*Ma*	*Mi*	*Re*	Potential for prey luring	Yellow and dark mosaic pattern	White and dark mosaic pattern	Source
Linyphiidae	*Labulla*	*Labulla thoracica*	Europe, Russia	-	S	L	Small	Absent	Absent	Locket and Millidge (1953); Roberts (1995)
Linyphiidae	*Laetesia*	*Laetesia raveni*	Queensland, New South Wales	L	L	L	Large	Absent	Absent	Hormiga and Scharff (2014)
Linyphiidae	*Lepthyphantes*	*Lepthyphantes minutus*	Holarctic	S	S	L	Small	Absent	Absent	Roberts (1995); Harvey, Nellist and Telfer (2002)
Linyphiidae	*Linyphia*	*Linyphia triangularis*	Palearctic, introduced in USA	L	L	L	Large	Present	Absent	Roberts (1995)
Linyphiidae	*Microlinyphia*	*Microlinyphia pusilla*	Holarctic	L	L	L	Large	Absent	Absent	Roberts (1995); Harvey, Nellist and Telfer (2002)
Linyphiidae	*Neriene*	*Neriene clathrata*	Holarctic	L	S	L	Small	Absent	Absent	Roberts (1995)
Linyphiidae	*Neriene*	*Neriene radiata*	Holarctic	L	S	L	Small	Present	Absent	Roberts (1995)
Linyphiidae	*Ostearius*	*Ostearius melanopygius*	Cosmopolitan	S	S	L	Small	Absent	Absent	Roberts (1995)
Linyphiidae	*Stemonyphantes*	*Stemonyphantes lineatus*	Palearctic	-	S	L	Small	Absent	Absent	Roberts (1995)
Linyphiidae	*Tenuiphantes*	*Tenuiphantes tenuis*	Palearctic (elsewhere, introduced)	-	S	L	Small	Absent	Absent	Roberts (1995)
Mysmenidae	*Trogloneta*	*Trogloneta granulum*	Europe	S	S	L	Small	Absent	Absent	Le Peru (2011)
Nephilidae	*Herennia*	*Herennia multipuncta*	India to China, Borneo, Sulawesi	L	L	S	Small	Absent	Absent	Kuntner (2005)
Nephilidae	*Nephila*	*Nephila clavipes*	USA to Argentina, São Tomé	L	L	L	Large	Present	Absent	Levi (1980)
Nephilidae	*Nephilengys*	*Nephilengys malabarensis*	India to China, Philippines, Japan, Ambon	S	L	S	Small	Present	Absent	Kuntner (2007)
Nesticidae	*Nesticus*	*Nesticus cellulanus*	Holarctic	S	S	L	Small	Absent	Absent	Roberts (1995)
Nicodamidae	*Ambicodamus*	*Ambicodamus marae*	Western Australia	-	S	S	Small	Absent	Absent	Harvey (1995)

				Natural history						
Family	Genus	Species	Distribution	*Ma*	*Mi*	*Re*	Potential for prey luring	Yellow and dark mosaic pattern	White and dark mosaic pattern	Source
Oecobiidae	*Oecobius*	*Oecobius navus*	Cosmopolitan	-	S	L	Small	Absent	Absent	Le Peru (2011)
Oecobiidae	*Uroctea*	*Uroctea durandi*	Mediterranean	-	S	L	Small	Absent	Absent	Le Peru (2011)
Pimoidae	*Pimoa*	*Pimoa rupicola*	France, Italy	S	S	S	Small	Absent	Absent	Hormiga (1994)
Stiphidiidae	*Stiphidion*	*Stiphidion facetum*	Eastern Australia, Tasmania, New Zealand	S	S	L	Small	Absent	Absent	Hickman (1967); Forster and Wilton (1973)
Tetragnathidae	*Leucauge*	*Leucauge argyra*	USA to Brazil	L	L	L	Large	Present	Present	Levi (1980)
Tetragnathidae	*Leucauge*	*Leucauge venusta*	Canada to Brazil	L	L	L	Large	Present	Absent	Dimitrov and Hormiga (2010)
Tetragnathidae	*Meta*	*Meta menardi*	Europe to Korea	S	L	L	Small	Absent	Present	Roberts (1995)
Tetragnathidae	*Meta*	*Meta bourneti*	Europe to Georgia, North Africa	S	L	L	Small	Absent	Absent	Roberts (1995)
Tetragnathidae	*Metellina*	*Metellina segmentata*	Palearctic (Canada, introduced)	L	L	L	Large	Present	Present	Roberts (1995); Dondale, Redner, Paquin and Levi (2003)
Tetragnathidae	*Metellina*	*Metellina merianae*	Europe, Ural, Caucasus, Turkey, Iran	S	L	L	Small	Present	Absent	Roberts (1995)
Tetragnathidae	*Metleucauge*	*Metleucauge chikunii*	China, Korea, Taiwan, Japan	L	L	L	Large	Absent	Present	Kim and Lee (2013)
Tetragnathidae	*Opadometa*	*Opadometa fastigata*	India to Philippines, Sulawesi	L	L	L	Large	Present	Absent	Barrion and Litsinger (1995)
Tetragnathidae	*Pachygnatha*	*Pachygnatha degeeri*	Palearctic	L	L	L	Large	Present	Present	Roberts (1995)
Tetragnathidae	*Tetragnatha*	*Tetragnatha versicolor*	North, Central America, Cuba	L	L	L	Large	Present	Absent	Dondale, Redner, Paquin and Levi (2003)
Tetragnathidae	*Tetragnatha*	*Tetragnatha dearmata*	Holarctic	L	L	L	Large	Absent	Absent	Roberts (1995)
Tetragnathidae	*Tylorida*	*Tylorida striata*	Comoro Is., China to Australia	L	L	L	Large	Present	Absent	Yin *et al.* (2012)

				Natural history						
Family	Genus	Species	Distribution	*Ma*	*Mi*	*Re*	Potential for prey luring	Yellow and dark mosaic pattern	White and dark mosaic pattern	Source
Tetragnathidae	*Tylorida*	*Tylorida ventralis*	India to Taiwan, Japan, New Guinea	L	L	L	Large	Absent	Absent	Harmer and Herberstein (2010); Yin *et al.* (2012)
Theridiidae	*Anelosimus*	*Anelosimus vittatus*	Palearctic	L	L	S	Small	Absent	Absent	Le Peru (2011)
Theridiidae	*Dipoena*	*Dipoena melanogaster*	Europe, North Africa to Azerbaijan, Iran	L	S	L	Small	Absent	Present	Le Peru (2011)
Theridiidae	*Enoplognatha*	*Enoplognatha caricis*	Holarctic	-	S	L	Small	Absent	Absent	Merrett and Snazell (1975); Roberts (1995)
Theridiidae	*Episinus*	*Episinus angulatus*	Europe to Russia	L	L	L	Large	Absent	Absent	Roberts (1995)
Theridiidae	*Latrodectus*	*Latrodectus geometricus*	Cosmopolitan	S	S	S	Small	Absent	Absent	Le Peru (2011)
Theridiidae	*Neottiura*	*Neottiura bimaculata*	Holarctic	S	S	L	Small	Absent	Absent	Roberts (1995)
Theridiidae	*Nesticodes*	*Nesticodes rufipes*	Pantropical, introduced elsewhere	S	L	L	Small	Absent	Absent	Levy and Amitai (1982)
Theridiidae	*Parasteatoda*	*Parasteatoda tepidariorum*	Cosmopolitan	S	L	L	Small	Absent	Present	Almquist (2005)
Theridiidae	*Phycosoma*	*Phycosoma mustelinum*	Russia, China, Korea, Japan, Krakatau	L	S	L	Small	Absent	Absent	Namkung (2003); Yin *et al.* (2012)
Theridiidae	*Platnickina*	*Platnickina nigropunctata*	Mediterranean	S	S	L	Small	Absent	Present	Le Peru (2011)
Theridiidae	*Steatoda*	*Steatoda bipunctata*	Holarctic	S	S	L	Small	Absent	Present	Roberts (1995)
Theridiidae	*Steatoda*	*Steatoda borealis*	USA, Canada, Alaska	-	S	L	Small	Absent	Absent	Levi (1957)
Theridiidae	*Theridion*	*Theridion varians*	Holarctic	L	L	L	Large	Absent	Absent	Le Peru (2011)
Uloboridae	*Hyptiotes*	*Hyptiotes paradoxus*	Palearctic	L	L	L	Large	Absent	Present	Le Peru (2011)
Uloboridae	*Uloborus*	*Uloborus plumipes*	Palearctic	L	L	L	Large	Absent	Present	Roberts (1998); Le Peru (2011)
Uloboridae	*Uloborus*	*Uloborus glomosus*	USA, Canada, Mexico	L	L	L	Large	Absent	Present	Kaston (1948)

				Natural history						
Family	Genus	Species	Distribution	*Ma*	*Mi*	*Re*	Potential for prey luring	Yellow and dark mosaic pattern	White and dark mosaic pattern	Source
Uloboridae	*Zosis*	*Zosis geniculata*	Pantropical	L	L	L	Large	Absent	Present	Barrion and Litsinger (1995); Penney (2009)

Table A2. Dataset for investigating the association between spider ventral signal and ambient light condition in the comparative analysis. *Natural history* characterises the ambient light condition of a spider species using information on the macrohabitat (*Ma*) and microhabitat (*Mi)* of the spiders. "D" and "B" represent the degree of ambient light condition as dim or bright respectively, while "-" denotes unavailable information. Table 1 further describes these characters. Please refer to the Materials and Methods section for details on categorising spider ventral signal and prey viewing environment.

				Natural history					
Family	Genus	Species	Distribution	*Ma*	*Mi*	Ambient light condition	Yellow and dark mosaic pattern	White and dark mosaic pattern	Source
Araneidae	*Argiope*	*Argiope trifasciata*	Cosmopolitan	B	B	Bright	Present	Present	Levi (2004)
Araneidae	*Argiope*	*Argiope argentata*	USA to Chile, Argentina	B	B	Bright	Present	Present	Levi (2004)
Araneidae	*Argiope*	*Argiope submaronica*	Mexico to Bolivia, Brazil	B	B	Bright	Present	Present	Levi (2004)
Araneidae	*Caerostris*	*Caerostris darwini*	Madagascar	B	B	Bright	Absent	Present	Kuntner and Agnarsson (2010)
Araneidae	*Cyclosa*	*Cyclosa conica*	Holarctic	B	B	Bright	Absent	Present	Levi (1977b)
Araneidae	*Cyrtophora*	*Cyrtophora moluccensis*	India to Japan, Indonesia, Papua New Guinea, Australia, Fiji, Tonga	B	B	Bright	Present	Present	Yin *et al.* (2012)
Araneidae	*Gasteracantha*	*Gasteracantha sauteri*	China, Taiwan, Vietnam	B	B	Bright	Present	Absent	Feng (1990)
Araneidae	*Larinioides*	*Larinioides cornutus*	Holarctic	B	B	Bright	Present	Absent	Levi (1974)
Araneidae	*Mangora*	*Mangora acalypha*	Palearctic	B	B	Bright	Present	Present	Almquist (2005)
Araneidae	*Mecynogea*	*Mecynogea lemniscata*	USA to Argentina	B	B	Bright	Present	Absent	Levi (1980); Levi (1997)
Araneidae	*Micrathena*	*Micrathena sagittata*	North, Central America	B	B	Bright	Present	Absent	Levi (1978); Levi (1985)
Araneidae	*Phonognatha*	*Phonognatha graeffei*	Australia	B	B	Bright	Present	Absent	Dondale (1966)
Deinopidae	*Deinopis*	*Deinopis longipes*	Mexico to Panama	-	B	Bright	Absent	Absent	Chickering (1963); Preston-Mafham (1998)

Appendix A

Supplemental experimental procedures for Chapter 2

				Natural history					
Family	Genus	Species	Distribution	*Ma*	*Mi*	Ambient light condition	Yellow and dark mosaic pattern	White and dark mosaic pattern	Source
Linyphiidae	*Bolyphantes*	*Bolyphantes alticeps*	Palearctic	-	D	Dim	Absent	Absent	Roberts (1995)
Linyphiidae	*Diplostyla*	*Diplostyla concolor*	Holarctic	-	D	Dim	Absent	Absent	Comstock (1940); Locket and Millidge (1953)
Linyphiidae	*Erigone*	*Erigone arctica*	Holarctic	B	D	Dim	Absent	Absent	Roberts (1987); Harvey, Nellist and Telfer (2002)
Linyphiidae	*Floronia*	*Floronia bucculenta*	Europe, Russia	B	B	Bright	Absent	Absent	Roberts (1995)
Linyphiidae	*Frontinella*	*Frontinella communis*	North, Central America	B	B	Bright	Absent	Absent	Kaston (1948); Harvey, Nellist and Telfer (2002)
Linyphiidae	*Gonatium*	*Gonatium rubellum*	Palearctic	-	D	Dim	Absent	Absent	Locket and Millidge (1953); Roberts (1995)
Linyphiidae	*Helophora*	*Helophora insignis*	Holarctic	B	B	Bright	Absent	Absent	Roberts (1995)
Linyphiidae	*Labulla*	*Labulla thoracica*	Europe, Russia	-	D	Dim	Absent	Absent	Locket and Millidge (1953); Roberts (1995)
Linyphiidae	*Laetesia*	*Laetesia raveni*	Queensland, New South Wales	B	B	Bright	Absent	Absent	Hormiga and Scharff (2014)
Linyphiidae	*Lepthyphantes*	*Lepthyphantes minutus*	Holarctic	D	D	Dim	Absent	Absent	Roberts (1995); Harvey, Nellist and Telfer (2002)
Linyphiidae	*Linyphia*	*Linyphia triangularis*	Palearctic, introduced in USA	B	B	Bright	Present	Absent	Roberts (1995)
Linyphiidae	*Microlinyphia*	*Microlinyphia pusilla*	Holarctic	B	B	Bright	Absent	Absent	Roberts (1995); Harvey, Nellist and Telfer (2002)
Linyphiidae	*Neriene*	*Neriene clathrata*	Holarctic	B	D	Dim	Absent	Absent	Roberts (1995)
Linyphiidae	*Neriene*	*Neriene radiata*	Holarctic	B	D	Dim	Present	Absent	Roberts (1995)
Linyphiidae	*Ostearius*	*Ostearius melanopygius*	Cosmopolitan	D	D	Dim	Absent	Absent	Roberts (1995)
Linyphiidae	*Stemonyphantes*	*Stemonyphantes lineatus*	Palearctic	-	D	Dim	Absent	Absent	Roberts (1995)
Linyphiidae	*Tenuiphantes*	*Tenuiphantes tenuis*	Palearctic (elsewhere, introduced)	-	D	Dim	Absent	Absent	Roberts (1995)

				Natural history					
Family	Genus	Species	Distribution	*Ma*	*Mi*	Ambient light condition	Yellow and dark mosaic pattern	White and dark mosaic pattern	Source
Mysmenidae	*Trogloneta*	*Trogloneta granulum*	Europe	D	D	Dim	Absent	Absent	Le Peru (2011)
Nephilidae	*Nephila*	*Nephila clavipes*	USA to Argentina, São Tomé	B	B	Bright	Present	Absent	Levi (1980)
Nesticidae	*Nesticus*	*Nesticus cellulanus*	Holarctic	D	D	Dim	Absent	Absent	Roberts (1995)
Oecobiidae	*Oecobius*	*Oecobius navus*	Cosmopolitan	-	D	Dim	Absent	Absent	Le Peru (2011)
Oecobiidae	*Uroctea*	*Uroctea durandi*	Mediterranean	-	D	Dim	Absent	Absent	Le Peru (2011)
Stiphidiidae	*Stiphidion*	*Stiphidion facetum*	Eastern Australia, Tasmania, New Zealand	D	D	Dim	Absent	Absent	Hickman (1967); Forster and Wilton (1973)
Tetragnathidae	*Leucauge*	*Leucauge argyra*	USA to Brazil	B	B	Bright	Present	Present	Levi (1980)
Tetragnathidae	*Leucauge*	*Leucauge venusta*	Canada to Brazil	B	B	Bright	Present	Absent	Dimitrov and Hormiga (2010)
Tetragnathidae	*Meta*	*Meta menardi*	Europe to Korea	D	B	Dim	Absent	Present	Roberts (1995)
Tetragnathidae	*Meta*	*Meta bourneti*	Europe to Georgia, North Africa	D	B	Dim	Absent	Absent	Roberts (1995)
Tetragnathidae	*Metellina*	*Metellina segmentata*	Palearctic (Canada, introduced)	B	B	Bright	Present	Present	Roberts (1995); Dondale, Redner, Paquin and Levi (2003)
Tetragnathidae	*Metellina*	*Metellina merianae*	Europe, Ural, Caucasus, Turkey, Iran	D	B	Dim	Present	Absent	Roberts (1995)
Tetragnathidae	*Metleucauge*	*Metleucauge chikunii*	China, Korea, Taiwan, Japan	B	B	Bright	Absent	Present	Kim and Lee (2013)
Tetragnathidae	*Opadometa*	*Opadometa fastigata*	India to Philippines, Sulawesi	B	B	Bright	Present	Absent	Barrion and Litsinger (1995)
Tetragnathidae	*Pachygnatha*	*Pachygnatha degeeri*	Palearctic	B	B	Bright	Present	Present	Roberts (1995)
Tetragnathidae	*Tetragnatha*	*Tetragnatha versicolor*	North, Central America, Cuba	B	B	Bright	Present	Absent	Dondale, Redner, Paquin and Levi (2003)

				Natural history					
Family	Genus	Species	Distribution	*Ma*	*Mi*	Ambient light condition	Yellow and dark mosaic pattern	White and dark mosaic pattern	Source
Tetragnathidae	*Tetragnatha*	*Tetragnatha dearmata*	Holarctic	B	B	Bright	Absent	Absent	Roberts (1995)
Tetragnathidae	*Tylorida*	*Tylorida striata*	Comoro Is., China to Australia	B	B	Bright	Present	Absent	Yin *et al.* (2012)
Tetragnathidae	*Tylorida*	*Tylorida ventralis*	India to Taiwan, Japan, New Guinea	B	B	Bright	Absent	Absent	Harmer and Herberstein (2010); Yin *et al.* (2012)
Theridiidae	*Dipoena*	*Dipoena melanogaster*	Europe, North Africa to Azerbaijan, Iran	B	D	Dim	Absent	Present	Le Peru (2011)
Theridiidae	*Episinus*	*Episinus angulatus*	Europe to Russia	B	B	Bright	Absent	Absent	Roberts (1995)
Theridiidae	*Neottiura*	*Neottiura bimaculata*	Holarctic	D	D	Dim	Absent	Absent	Roberts (1995)
Theridiidae	*Nesticodes*	*Nesticodes rufipes*	Pantropical, introduced elsewhere	D	B	Dim	Absent	Absent	Levy and Amitai (1982)
Theridiidae	*Parasteatoda*	*Parasteatoda tepidariorum*	Cosmopolitan	D	B	Dim	Absent	Present	Almquist (2005)
Theridiidae	*Phycosoma*	*Phycosoma mustelinum*	Russia, China, Korea, Japan, Krakatau	B	D	Dim	Absent	Absent	Namkung (2003); Yin *et al.* (2012)
Theridiidae	*Platnickina*	*Platnickina nigropunctata*	Mediterranean	D	D	Dim	Absent	Present	Le Peru (2011)
Theridiidae	*Steatoda*	*Steatoda bipunctata*	Holarctic	D	D	Dim	Absent	Present	Roberts (1995)
Theridiidae	*Steatoda*	*Steatoda borealis*	USA, Canada, Alaska	-	D	Dim	Absent	Absent	Levi (1957)
Theridiidae	*Theridion*	*Theridion varians*	Holarctic	B	B	Bright	Absent	Absent	Le Peru (2011)
Uloboridae	*Hyptiotes*	*Hyptiotes paradoxus*	Palearctic	B	B	Bright	Absent	Present	Le Peru (2011)
Uloboridae	*Uloborus*	*Uloborus plumipes*	Palearctic	B	B	Bright	Absent	Present	Roberts (1998); Le Peru (2011)
Uloboridae	*Uloborus*	*Uloborus glomosus*	USA, Canada, Mexico	B	B	Bright	Absent	Present	Kaston (1948)

				Natural history					
Family	Genus	Species	Distribution	*Ma*	*Mi*	Ambient light condition	Yellow and dark mosaic pattern	White and dark mosaic pattern	Source
Uloboridae	*Zosis*	*Zosis geniculata*	Pantropical	B	B	Bright	Absent	Present	Barrion and Litsinger (1995); Penney (2009)

Table A3. Achromatic and chromatic contrast values (MEAN ± SEM) of paper used for cardboard spiders in the daytime experiment: (a) against corresponding spider body parts; (b) against vegetation background; (c) against black paper; (d) yellow paper against blue paper.

	Subject	Against object	Honeybee chromatic contrast	Honeybee achromatic contrast	Fruit fly chromatic contrast (categorical space)	Fruit fly achromatic contrast (categorical space)	Fruit fly chromatic contrast (tetrahedral space)	Fruit fly achromatic contrast (tetrahedral space)
(a)	Yellow paper	Spider yellow	0.048±0.003	0.019±0.001	0.026±0.002	0.069±0.006	0.012±0.001	0.009±0.001
	Blue paper	Spider yellow	0.146±0.001	0.059±0.001	0.245±0.001	0.433±0.002	0.03±0.000	0.09±0.000
	Black paper	Spider black	0.075±0.006	0.082±0.009	0.130±0.014	-0.115±0.112	0.0246±0.001	-0.025±0.023
(b)	Yellow paper	Vegetation background	0.076±0.021	0.312±0.029	0.080±0.005	-0.300±0.006	0.029±0.001	-0.040±0.001
	Blue paper	Vegetation background	0.071±0.011	0.289±0.011	0.304±0.001	0.085±0.003	0.047±0.000	0.010±0.000
	Black paper	Vegetation background	0.109±0.009	0.320±0.033	0.316±0.014	-0.914±0.023	0.025±0.002	-0.248±0.021
(c)	Yellow paper	Black paper	0.156±0.004	0.616±0.015	0.250±0.006	0.847±0.016	0.011±0.000	0.211±0.009
	Blue paper	Black paper	0.072±0.002	0.656±0.015	0.096±0.002	-0.927±0.008	0.024±0.001	-0.258±0.009
(d)	Yellow paper	Blue paper	0.133±0.002	0.040±0.001	0.257±0.003	-0.375±0.002	0.028±0.000	-0.05±0.000

Table A4. Achromatic and chromatic contrast values (MEAN ± SEM) of paper used for cardboard spiders in the night-time experiment: (a) against corresponding spider body parts; (b) against vegetation background; (c) against black paper; (d) yellow paper against blue paper. The superscript letters indicate the rank order in the means of Tukey HSD test across all listed comparisons using different combinations of papers, spider body parts, and vegetation background.

	Subject	Against object	Hawkmoth chromatic contrast	Hawkmoth achromatic contrast
(a)	Yellow paper	Spider yellow	0.045±0.001^{f}	0.022±0.001^{c}
	Blue paper	Spider yellow	0.199±0.001^{b}	0.046±0.001^{c}
	Black paper	Spider black	0.035±0.002^{g}	-0.003±0.026^{c}
(b)	Yellow paper	Vegetation background	0.098±0.001^{d}	0.521±0.001^{b}
	Blue paper	Vegetation background	0.141±0.001^{c}	0.538±0.001^{b}
	Black paper	Vegetation background	0.094±0.002^{d}	-0.509±0.019^{b}
(c)	Yellow paper	Black paper	0.202±0.001^{b}	0.814±0.001^{a}
	Blue paper	Black paper	0.060±0.001^{e}	0.821±0.000^{a}
(d)	Yellow paper	Blue paper	0.268±0.001^{a}	-0.023±0.001^{c}

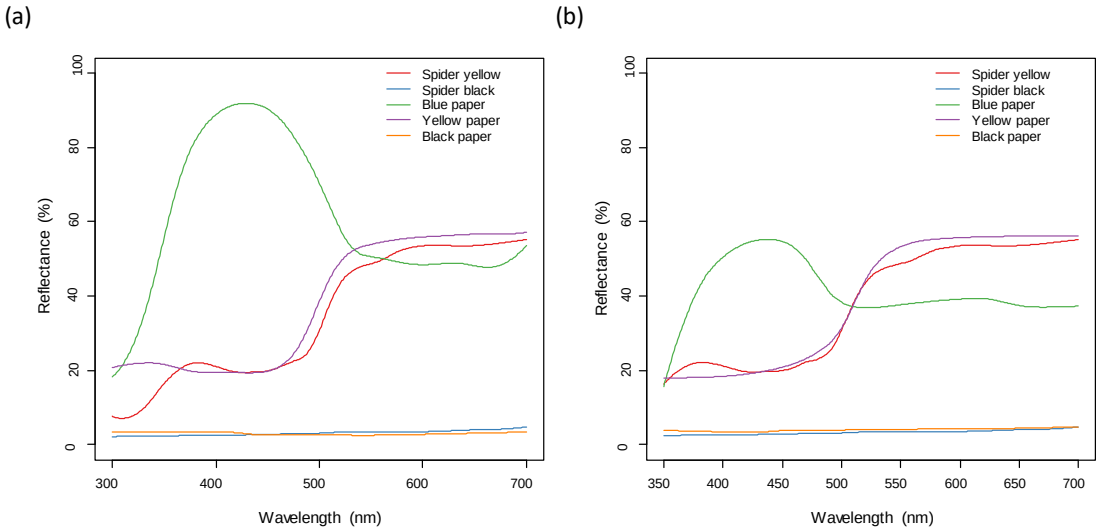

Figure A1. Mean reflectance spectra of spider yellow body parts, spider black body parts, yellow, blue, and black paper used for (a) daytime and (b) night-time field experiment. Given wavelength is reported as colour contrast calculations in bee colour hexagon mode (300-700 nm)l and Hawkmoth Maxwell triangle model (350-700 nm) in $eq\ 1$ and $eq\ 5$ separately (see Appendix A: A1 for details).

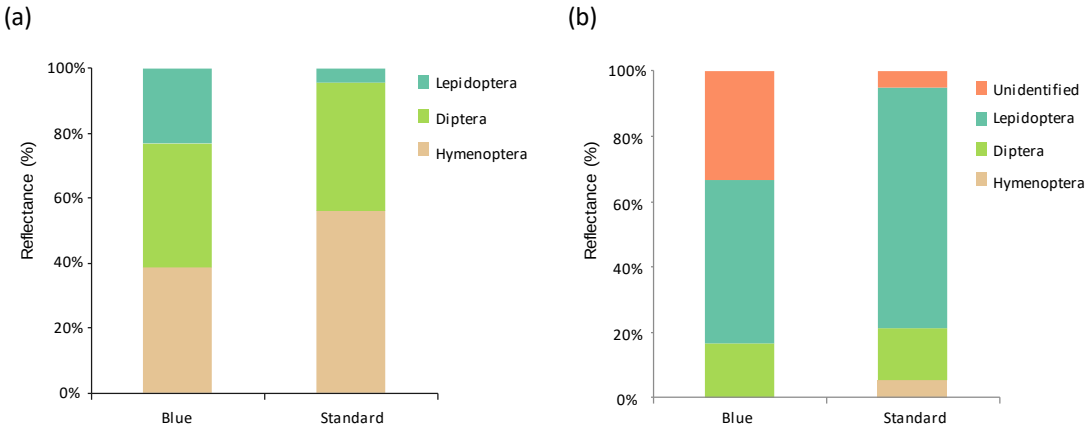

Figure A2. The proportions of each insect prey taxon attracted by blue and standard cardboard model spiders in (a) day and (b) night.

Appendix B: Supplemental information for Chapter 4

B.1 Population genetics analyses

B.1.1 Taxon sampling

I collected 48 specimens of *Argyrodes* specimens: 19 and 29 individuals collected from Japan (*A. kumadai*) and Taiwan (*A. fissifrons*) respectively. Freshly collected specimens were preserved in 95% ethanol at −20℃ until they were used in DNA extraction.

B.1.2 DNA extraction

I extracted DNA from legs and prosoma of the specimens using Maxwell RC blood DNA Extraction Kit (Promega Corporation, USA). The tissue was homogenised in lysis buffer and incubated at 56℃ for 24h. I then followed the standard protocol of Maxwell RC automatic DNA/RNA Extraction for extraction of genomic DNA from each specimen. The average quantity of DNA per sample was 32.37 ± 27.64 ng/µl.

B.1.3 Library preparation of dual-indexed restriction-site associated DNA sequencing (RAD-seq) method

DNA samples were normalised to 50ng/µl and digested for 1.5 hrs with Tru1I (MseI) restriction enzyme in TurboCycler Thermal Cycler (Blue-Ray Biotech Corporation, Taiwan). I then ligated the unique barcoded adaptors (Welgene Taiwan) to the samples for 3 hrs at 16℃. The barcoded fragments were pooled into one tube, purified with magnetic beads after isopropanol precipitation, and re-suspended in TE buffer. I selected the fragments between 350 and 450 bp using pippin Prep (Sage Science, USA) and enriched these using Phusion High Fidelity PCR kit (New England Bio, USA) with FC1.1 and FC2 oligonucleotide primers. To optimise the sequence capture, the amplified molecules were scaled using KAPA Pure Beads (Roche, Switzerland). I applied one paired-end lane (PE-150) of high- throughput Illumina Nova-seq 6000 sequencing and standard Illumina True-seq system to prepare RAD-seq libraries.

B.1.4 Reads assembly

I concatenated sequences using iPyrad (Eaton & Overcast, 2016) with a pre-assembled *Argyrodes miniaceus* genome as a reference. Reads were trimmed of barcodes and adapters

and quality filtered using a q-score threshold of 33, with bases below this score converted to Ns and any reads with more than 5 Ns removed. Reads were mapped to the concatenated reference genomes based on sequence similarity using the default setting. With the collected reads, similar clusters of reads were identified using a threshold of 80% of similarity and were aligned. I then removed consensus sequences with greater than 5 Ns per end of paired-end reads. Reads of each sample were then clustered and aligned to consensus sequences. Finally, I filtered the data set according to maximum 20 indels allowed per read end, maximum 40 SNPs per locus, maximum 0.5 percent of shared heterozygous sites per locus, and minimum 6 samples per locus.

B.1.5 Loci filtering

The initial raw data set in which the number of samples is 48 and the number of SNPs is 31852 was filtered using the following criteria: (1) SNPs with less than 99% reproducibility; (2) SNPs with average number of reads (allelic depth) for reference allele and alternate is less than 3; (4) SNPs with more than 10% missing data were removed; (5) individuals with more than 15% missing data; (6) SNPs with minor allele frequency greater than 0.05. After these steps, loci without conformity to expectations of Hardy–Weinberg equilibrium were filtered. Specifically, I applied locality-based tests of Hardy Weinberg equilibrium, and loci were removed if it had a *P* value of less than 0.05 in at least 25% of the populations (Taiwan and Japan origins). The final filtered dataset which contains 42 samples (numbers of individuals retained: 17 and 25 for *A. kumadai* and *A. fissifrons* respectively) with 5735 SNPs was then imported into R for downstream analyses.

B.1.6 Interference of population structures

The Discriminant Analysis of Principal Components (DAPC) implemented in the R package adegenet (Jombart, 2008) was used to describe the population structure of *A. fissifrons* and *A. kumadai*. This multivariate method does not assume a particular population genetics model and is free of assumptions on linkage equilibrium (Jombart, Devillard & Balloux, 2010). To avoid over-fitting of the discriminate functions (Jombart, Devillard & Balloux, 2010), I determined the optimal number of principal components to retain based on the a-score (Figure S1A; optimal number of PCs: 6). Genetic differentiation between species was tested by hierarchical analysis of molecular variance (AMOVA) in R package

poppr (Kamvar, Tabima & Grünwald, 2014). The significance of genetic differentiation between species was assessed using a permutation test with 9,999 permutations.

B.2 Supplemental Results

The results of DAPC showed little separation between samples (Figure B1), and AMOVA test further revealed that there was no significant genetic division between species (P = 0.066), where the genetic divergence between samples within species is the largest source of genetic variation (82.4%) rather than between species (3.81%).

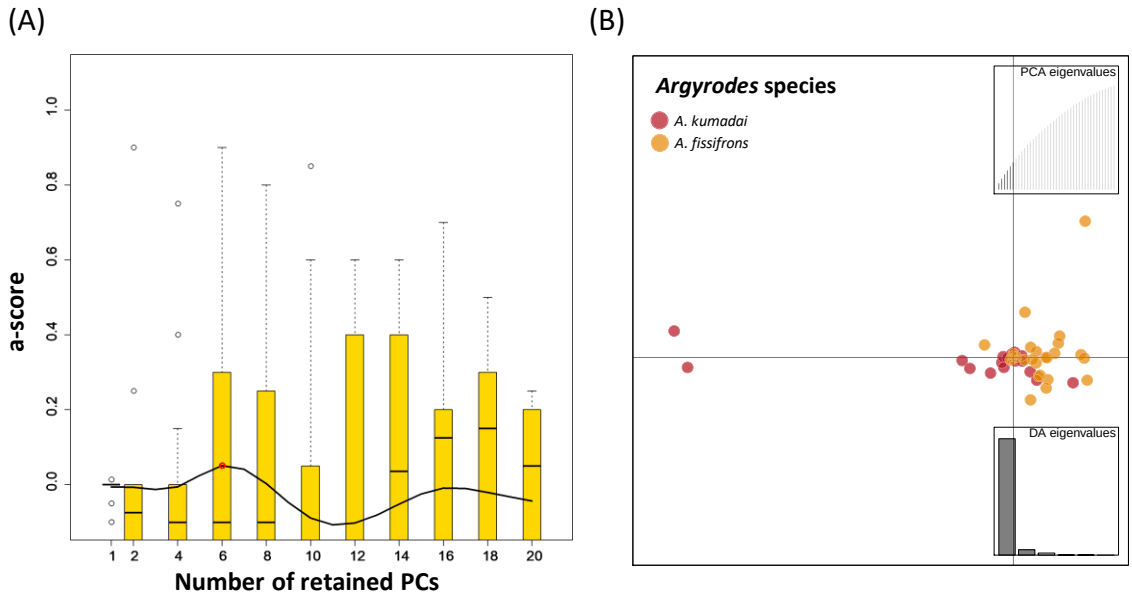

Figure B1. The results of Discriminant Analysis of Principal Components (DAPC). (A) Optimal number of informative principal components (red dot), suggested by the optimisation of the spline interpolation of the a-scores. (b) Scatterplot of the DAPC performed on SNPs datasets for *A. kumadai* and *A. fissifrons* specimens; axes represent the first two linear discriminants; the insets indicate the amount of variation contained in the different principal components (top right) and relative contribution of the eigenvalues to the discriminant analysis (bottom right).