

**The fig wasps associated with *Ficus microcarpa*, an
invasive fig tree**

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Abstract

Fig trees and their pollinating fig wasps represent one of the most species-specific mutualistic systems with a long history of co-evolution. Besides the pollinators, figs are also exploited by numerous non-pollinating fig wasps (NPFW). A few fig trees have become established outside their native ranges, and *Ficus microcarpa*, a monoecious fig tree, has become widely invasive, due to the widespread introduction of its specific pollinator, *Eupristina verticillata*. In this thesis, a global study was carried out to unravel the distribution and community structure of fig wasps associated with *F. microcarpa*. The work also examined which NPFW are potential bio-control agents and whether the plant is invasive due to reduced seed and pollinator predation in its introduced range (the 'enemy release' hypothesis).

At least 43 fig wasp species utilize *F. microcarpa* figs with more than 20 species present in the plant's introduced range. In newly established NPFW populations, a lack of male fig wasps at low population densities can cause Allee-like effects for fig wasps, but inter-specific facilitation is able to mitigate them. Generally, parasitoids were far less diverse than phytophages in the tree's introduced range with significant latitudinal effects on species richness. We unraveled the food web of fig wasps where sycoryctines (Pteromalidae) were parasitoids of agaonids, and eurytomids were parasitoids of epichrysomallines. A large galler species, *Meselatus bicolor*, is independent of the pollinator and can suppress both male and female reproductive successes of figs via competition for nutrients and preventing pollinators from entering figs. It may be an ideal bio-control agent. Enemy release in the introduced range failed to increase the plant's seed production but benefited the pollinator, and the greater survival of pollinator larvae in more peripheral galls emphasises the role of parasitoids in maintaining the fig-pollinator mutualism in monoecious figs.

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Chapter 1 General introduction

1.1 Biological invasion

Species are increasingly being transported outside their natural ranges via either deliberate introductions for horticulture and agriculture or unintentional transit accompanied with international commerce (Mack et al. 2000; Hulme 2009; Catford et al. 2012). Many non-indigenous species have become established or even invasive. These invasive species can heavily suppress native species, alter soil nutrient cycling and hydrology, thereby threatening endemic species diversity and ecosystem functions and even contributing to the global change pattern (Mack et al. 2000; Ehrenfeld 2010; Simberloff 2011; Catford et al. 2012; Simberloff et al. 2013). In addition, invasive species also have triggered severe socio-economic problems such as health and food insecurity (Hancock et al. 2010; Gluckman et al. 2011), and Pimentel (2005) estimated that the global annual cost of invasive species could reach US\$ 1.4 trillion.

Initially, small populations of invasive species must overcome low genetic diversity induced by founder effect (Kolbe et al. 2004) and Allele effect (positive density dependence of individual fitness) (Tobin et al. 2011), and the success of biological invasion depends on the characters of both non-indigenous species and the invulnerable communities. Generally, species with high efficiency in resource utilization, i.e. more competitive (Vitousek 1990) or allelopathic capacities (He et al. 2009), and those that have generalist mutualists (e.g. seed dispersers and pollinators) (Richardson et al. 2000; Mack et al. 2000) are more likely to be invasive outside their native ranges. On the other hand, communities that were unsaturated with species tend to be more invulnerable because many unexploited niches are available there (Sax et al. 2007; Catford et al. 2012). In addition, species composition also highly relates to invulnerability, and normally a community where the specific natural enemies of non-indigenous species are absent is anticipated to create an 'enemy release' environment, facilitating their invasion,

despite that generalist predators may be present there (Keane & Crawley 2002; Müller-Schärer & Schaffner 2008; Pearson et al. 2011). Actually, full occupancy of niches is uncommon in natural communities (Hawkins & Compton 1992; Witman et al. 2004), and 'enemy release' hypothesis has been widely recognized as a pervasive mechanism resulting in the vulnerability of a community to some non-indigenous species (Keane & Crawley 2002).

Underlying these ecological causes, biological invasion is driven by a series of evolutionary factors including both preadaptation of non-indigenous species and fast evolution after their introduction (Carroll 2011). Prior to long-distance transport, phylogenetic history has largely determined whether a species has characters to efficiently use resources, outperform native species or escape from potential threats like grazing, parasitism and predation in a novel environment (Dietz & Edwards 2006; Schlaepfer et al. 2010). For instance, the red alga *Bonnemaisonia hamifera* produces the metabolite 1,3,3-tetrabromo-2-heptanone, which can help to resist common generalist herbivores, stimulating its invasion (Enge et al. 2012). After entering a novel community, many non-indigenous species also experience rapid adaptive evolution mainly through the following three ways: (1) gene introgression, non-indigenous species may acquire new genes, enhancing their performance in the novel environment via hybridizing with genetically-related natives (Arnaud et al. 2010; Carroll 2011) or gene engineered crops (Sasua et al. 2003); (2) multiple introductions, the populations of some non-indigenous species may have accepted multiple waves of immigrants from different parts of their native ranges, leading to high population genetic diversity that enables adaptation (Kolbe et al. 2004; Taylor & Keller 2007; Simberloff 2009); (3) genotype-by-environment interaction, the direction of selection may be altered in new environments where some rare and formerly inferior alleles are favored (Ghalambor et al. 2007; Barrett et al. 2008). In addition, some epistatic genes are likely to be absent in small founder populations, causing the dominance of additive genes, and the resulting strengthened phenotypes may cause fast adaptive evolution in some specific

environments (Gomulkiewicz et al. 2010).

1.2 Control of invasive species

Reducing the threats of invasive species involves both management of species immigration and control of the species that have invaded. Efforts have focused on preventing entry of non-indigenous species that have potential to be invasive, which mainly depends on the quarantine by customs with the help of risk assessment (Mack et al. 2000). Normally, a risk assessment model estimates the invasibility of a non-indigenous species based on a large series of attributes that were generated based on the inventory of invasive species (Pheloung et al. 1999; Pyšek et al. 2012). Nevertheless, the prevention of non-indigenous species also needs the reinforcement of policies and laws considering the pros and cons of species introduction (Mack et al. 2000; McGeoch et al. 2010).

The most straightforward way to get rid of invasive species is eradication, but it is costly, and most successful cases are only reported at the early stage of invasion or on islands (Mack et al. 2000; Ramsey et al. 2008). Instead of eradication, Carroll (2011) proposed conciliation biology which attempted to establish a new balance between the invaders and natives via co-evolution because some merits of invasive species are essential for human. For the purpose of either eradication or conciliation biology, the target species needs to be controlled under an acceptable density (Navntoft et al. 2009; Carroll 2011; Wilson et al. 2011), and control of invasive species mainly includes three measures: chemical, mechanical and biological control (Mack et al. 2000). The application of chemical materials such as DDT is usually a great threat to the local environment, native species and human health (Mack et al. 2000), and mechanical approaches suffer from the high expense and difficulty of finding targets (Mack et al. 2000; Simberloff 2003). In contrast, biological control aims to suppress the target species through introducing its specific natural enemies based on 'enemy release' hypothesis, and has been recognized as a promising way to manage invasive species

(Keane & Crawley 2002; Wilson et al. 2011; van Lenteren 2012), but the selection of bio-control agents must be with caution because an introduction of a generalist predator, in a sense, equals triggering a new biological invasion, and even if the selected agent is the species-specific natural enemy in its native range, its performance in the novel community usually remains unknown (Civeyrel & Simberloff 1996; Mack et al. 2000). As to invasive plants, host specific herbivores including those that are detrimental to pollination and seed production have been frequently selected as the bio-control agents (Garren & Strauss 2009; Baraibar et al. 2011; Wilson et al. 2011). Though the success of biological control depends on to what extent the agents can reduce seed output (Knochel et al. 2010), overall species-specific seed predators could exhaust the seed banks of target plants, greatly interfering their population recruitment and expansion (Navntoft et al. 2009; Wilson et al. 2011).

1.3 Mutualism between fig trees and their pollinating fig wasps

The genus *Ficus* (fig trees, Moraceae) is of great ecological importance because not only is this genus one of the most diverse genera, composing of at least 750 species, but also numerous vertebrates either inhabit fig trees or feed on ripe figs, leading to many fig species being keystone species, especially in tropical and subtropical forests (Shanahan et al. 2001; Herre et al. 2008). Most fig trees have tropical or sub-tropical distributions, and just a few species are exclusively temperate (Berg & Corner 2005). Factors determining their native distribution ranges may be linked to the trees themselves, or the limited environmental tolerances of their pollinators (Zhang et al. in press).

The obligate mutualism between fig trees and their pollinating fig wasps (Chalcidoidea, Agaonidae) is highly species-specific with all fig species pollinated by one or only a few fig wasps and pollinating fig wasps only reproducing inside the syconia (figs) of their specific host fig trees (Wiebes 1979; Cook & Rasplus 2003; Molbo et al. 2003; Herre et al. 2008; Chen et al. 2012). Related fig trees tend to be pollinated by related fig

wasps, suggesting a long history of co-evolution, though some host switching between lineages has also taken place (Cook & Segar 2010; Cruaud et al. 2012).

Fig trees produce flowers that are enclosed in figs that exclude generalist pollinators like bees (Cook & Rasplus 2003). A hollow space is formed inside a fig, and tiny staminate and pistillate flowers line the inner side of the fig wall. Pollen is carried into the figs when female agaonids enter through a narrow ostiole that opens temporarily when the flowers are ready to be pollinated. The pollinator females enter the figs in order to lay their eggs inside the ovules, which they also gall. Often, they only enter one fig and do not re-emerge. Oviposition is achieved by the agaonids inserting their ovipositors along the styles, which vary in length depending on where the associated ovule is located, relative to the fig wall. Ovules towards the centre of the fig are more likely to produce agaonid offspring than those located closer to the fig wall, which in turn are more likely to develop seeds (Dunn et al. 2008a). Pollination is either active, with the females unloading pollen that they had collected previously into thoracic pouches, or passive, from pollen attached to their bodies (Cook & Rasplus 2003). Adult male pollinator offspring are responsible for releasing female pollinator offspring from both galls and figs. Adult agaonids do not feed, and females only survive outside figs for one or two days (Ahmed et al. 2009). Almost all pollinator fig wasps are host tree specific. Specificity is maintained by pollinator attraction to the host and developmental stage specific chemical cues associated with receptive figs (Ware & Compton 1994; Wang et al. 2013a) and physical constraints imposed by host figs (van Noort & Compton, 1996).

The population dynamics of pollinator fig wasps is driven by the fruiting phenology of their individual host *Ficus*. Pollination rates however can be independent of crop size (Jauharlina 2014), but not all suitable figs are found by pollinators, and these un-utilised figs eventually abort and fall to the ground. Individuals of most monoecious fig trees (where each fig can support the development of both seeds and pollinator larvae) generally produce synchronised crops of figs. This ensures outbreeding,

because pollinator females must disperse to other trees to find figs that are suitable for oviposition (Herre et al. 2008). Pollinator populations are maintained because different trees support crops at different stages of development (McKey 1989). However, in strongly seasonal environments, fig trees usually produce small crops of figs during winter, which develop slowly and the release of pollinators also slows, or stops (Yang et al. 2013; Zhang et al. in press). Dioecious fig trees (with figs that either support pollinator offspring or develop seeds, but not both) have diverse fruiting phenologies, which can vary between different sexes of the same species (Wang et al. 2009; Suleman et al. 2011). Some dioecious fig trees have exceptional fruiting phenologies that are adapted for temperate climates with long winter periods, e.g. *Ficus pumila* (Chen et al. 2012).

Many fig tree species have been planted outside their native ranges, some of which are capable of setting seeds, or even become invasive, after the arrival of their associated pollinators via either deliberate introduction or unintentional long-distance transport (McKey 1989; Starr et al. 2003; Miao et al. 2011; Caughlin et al. 2012; van Noort et al. 2013). Relative to the highly complicated seed dispersal system (Shanahan et al. 2001), the mating system of fig trees is clearly more vulnerable and thereby can be used for biological control. Some animals such as ants, nematodes and gall midges can adversely influence both male (female pollinator abundance) and female (seed production) reproductive functions of figs (Compton & Robertson 1988; Herre 1993; Miao et al. 2011), but they are generalists with potential threats to other plants and therefore are not anticipated to be appropriate bio-control agents.

1.4 Non-pollinating fig wasps

Figs are also utilised by a large number of non-pollinating fig wasps (NPFW) mainly belonging to families of Chalcidoidea other than Agaonidae (Cook & Rasplus 2003; Cook & Segar 2010; Segar & Cook 2012), though non-pollinating agaonids have been recorded (e.g. Compton et al. 1991; Peng et al. 2008). Most fig trees especially

dioecious figs support only a few NPFW species, but more than 30 non-pollinating fig wasps (NPFW) species have been recorded from a single *Ficus* species (Compton & Hawkins 1992; Cook & Rasplus 2003). Most NPFW oviposit from the outside of figs, entirely preventing them from pollinating, and their eggs are consequently often concentrated in the ovules close to the fig wall (Dunn et al. 2008a). Like pollinators, NPFW generally develop inside galled ovules except that some gall in the outer wall of their host figs. In addition, some NPFW can be independent of pollinators because they are able to develop in unpollinated figs. Some lineages of NPFW are highly host specific, but others contain species that exploit more than one fig species (Cook & Segar 2010). Relative to pollinating agaonids, adults of some NPFW species can feed and hence have far longer longevities (approximate several weeks) (Compton 1993a). Large crops are likely to contain more NPFW, but individual figs often support only a subset of the local fig wasp fauna, particularly when it is diverse (Compton & Hawkins 1992).

Details of larval feeding biology are only available for a tiny number of NPFW species, but generally NPFW have diverse feeding behaviours including seed predators (Pereira et al. 2007), primary gallers utilizing either ovules or fig walls (Segar & Cook 2012), secondary gallers that enlarge the galls induced by primary gallers (Chen et al. 2013), primary parasitoids of primary gallers (most of which also feed on some plant tissue) (Segar & Cook 2012), and hyper-parasitoids (Compton et al. 2009). The specific relationships between parasitoid NPFW and primary gallers are also rarely documented (Cook & Segar 2010; Segar et al. 2013), but shared niches such as spatial location of galls and body size and co-evolutionary history provide informative clues to judge the specific parasitoids of gall-formers (Compton 1993b; West et al. 1996; Dunn et al. 2008a). Therefore, it is only possible to classify species within a particular fig wasp community into either exclusive phytophages including both primary gallers and seed predators, and parasitoids that kill larvae of other fig wasps.

It has been frequently reported that pollinators are detrimentally impacted by their

parasitoids that mainly belong to subfamily Sycoryctinae (family Pteromalidae), but their presence may also benefit the seed production of monoecious figs because they also prevent pollinators from depriving all the ovules in a fig, leaving a certain part of female flowers available for pollination (Dunn et al. 2008a; Segar & Cook 2012; Yu & Compton 2012; Suleman et al. 2013). Besides, density-dependent predation of parasitoids also restricts its influence on the pollinators (Suleman et al. 2013). In addition to parasitoids, some large gallers are also able to cause significant costs on both male and female reproductive successes of figs probably due to their priority in nutrition acquisition (e.g. Kobbi et al. 1996). Phytophagous NPFW therefore provide a promising species pool for the selection of bio-control agents.

1.5 Balance between pollinators and seeds in monoecious figs

In dioecious figs, seed production is independent of pollinator offspring since they develop in functionally female and male figs respectively, but the pistillate flowers in monoecious figs can either set seeds or support fig wasps, routinely contributing to both male and female reproductive successes of figs (Cook & Rasplus 2003; Herre et al. 2008). Generally, the pedicel and style lengths of ovules are reciprocally correlated, and the style length, an indicator of the spatial position of ovules, is normally continuous in a monoecious fig (Compton & Nefdt 1990; Nefdt & Compton 1996). Further, the pollinator foundresses prefer to oviposit in the ovules with short styles in monoecious figs though they can oviposit even in the ovule with the longest style, and seeds are more likely to be located in the periphery of a fig, therefore forming a spatial stratification between these two fig contents (Compton & Nefdt 1990; Dunn et al. 2008a).

The mechanisms underlying the balance between the two sexual reproductive functions of monoecious figs have not been confirmed though many hypotheses have been proposed. However, some of these hypotheses are not reliable, such as short ovipositors of female pollinators that prevent them laying eggs in long-style ovules, because female

pollinator foundresses can lay eggs into the ovule with longest style (Nefdt & Compton 1996; Dunn et al. 2008a), and insufficient eggs carried by adult female pollinators (Dunn et al. 2008a). Besides, almost all ovules inside a fig are able to be utilized by female pollinator foundresses, contradicting the 'unbeatable seeds' hypothesis which argues that a certain part of ovules in a fig are only available for pollination (Nefdt & Compton 1996). However, recent research has detected a higher mortality of pollinator offspring in the outer ovules, supporting 'unbeatable seeds' (Wang et al. 2013b).

Nevertheless, there are also some more promising explanations to why pollinators normally do not occupy all female flowers inside monoecious figs: (1) life-span constraint, the pollinator foundresses are supposed to spend less handling time on ovipositing in the short-styled ovules, and such high oviposition efficiency may be preferred by pollinators given their extremely limited life span (Dunn et al. 2008b); (2) less suitable ovules, female pollinator offspring in the galls close to the fig wall are less likely to be visited and released by adult male pollinators, and the lack of intra-species facilitation decreases the oviposition value of outer ovules (Dunn et al. 2008b); (3) 'optimal foraging' hypothesis, the oviposition behavior of pollinator foundresses, i.e. the preference of short-styled female flowers, is determined by both the offspring fitness and oviposition handling time (Yu et al. 2004); and (4) effects of parasitoids, the parasitoids of pollinators tend to kill the pollinator offspring whose galls are located in the periphery of figs, inducing a strong selection that forces pollinator foundresses to leave a part of female flowers for seeds (Dunn et al. 2008a).

Invasion of fig trees creates a splendid chance to assess the contribution of parasitoids of pollinators to the co-existence of pollinators and seeds. If the parasitoids are critical to the fig-pollinator mutualism, then 'Enemy release' among fig trees outside their natural range would bias the balance towards the pollinator, leading to higher ratio of pollinator offspring to seeds, otherwise this ratio should be consistent across both native and introduced populations of monoecious figs.

1.6 *Ficus microcarpa* and its associated fig wasps

F. microcarpa L. is a monoecious fig tree native to South and South-east Asia extending to North Australia, where it grows from rocks or as a strangler fig (Berg & Corner 2005). *F. microcarpa* has been widely introduced as an ornamental tree into many tropical and subtropical areas (Nadel et al. 1992; Figueiredo et al. 1995; Kobbi et al. 1996; Beardsley 1998; Burrows & Burrows 2003; Starr et al. 2003; van Noort et al. 2013). The figs of this species contain tiny seeds that are mainly dispersed by frugivorous birds, with ants also serving as secondary dispersal agents (Kaufmann et al. 1991; Shanahan et al. 2001). Its seedlings can cause damage to buildings and infrastructures and it is increasingly reported from semi-natural vegetation, where it can become invasive especially on islands like Hawaii and Bermuda (McKey 1989; Beardsley 1998; Starr et al. 2003; Corlett 2006; Caughlin et al. 2012). In the areas dominated by seasonal climates, the tree produces fewer crops in winter, but they are sufficient to maintain fig wasp populations, which recover rapidly in spring (Compton 1989; Lo Verde et al. 1991; 2007; Yang et al. 2013).

The pollinator of *F. microcarpa* is *Eupristina verticillata* Waterston, a taxon that, based on molecular data, consists of a complex of several cryptic species (Sun et al. 2011; A. Cruaud, J-Y. Rasplus, S.G. Compton & R. Wang, unpublished), and *F. microcarpa* is the only known host of its pollinator. In addition, the figs of *F. microcarpa* are also utilized by a ‘cheater’ *Eupristina* species that does not actively pollinate due to its reduced pollen pockets, but its distribution is extremely restricted in South-western China (J-Y. Rasplus, S.G. Compton & R. Wang, unpublished). *E. verticillata* has been recorded not only throughout the plant's native range but also in most sites where *F. microcarpa* has been introduced (Stange & Knight 1987; Bouček 1993; Kobbi et al. 1996; Beardsley 1998; Chen et al. 1999; van Noort & Rasplus 2010; Doğanlar 2012; Mifsud et al. 2012). Molecular study has also detected only one of the genetically-distinguishable taxa within *E. verticillata* outside the tree's natural range (A. Cruaud, J-Y. Rasplus, S.G. Compton & R. Wang, unpublished).

Besides the cheater agaonid, at least 26 NPFW species have been recorded from the figs of *F. microcarpa* in the plant's native range, belonging to the families Pteromalidae, Eurytomidae and Ormyridae (Chen et al. 1999; Zhang & Xiao 2008; Feng & Huang 2010; Li et al. 2013). Like agaonids, each larva of these NPFW develops in a single ovule (gall), but these NPFW do not enter figs and instead lay eggs through the outer walls of figs using their long ovipositors (Galil and Copland 1981; Bouček 1988; van Noort et al. 2013). Most NPFW associated with *F. microcarpa* are also host-specific, but a small number of NPFW have also been found in some related fig trees (Zhou et al. 2012). *F. microcarpa* also supports a special fig wasp (*Josephiella microcarpae* Beardsley & Rasplus) which galls in the plant's leaves (Beardsley & Rasplus 2001). The food web of fig wasps associated with *F. microcarpa* has not been described, but species belonging to subfamilies Epichrysomallinae and Otitesellinae (family Pteromalidae) are expected to be phytophages (Bouček 1988; Compton 1993b), and species in families Eurytomidae and Ormyridae and subfamily Sycoryctinae (family Pteromalidae) are normally considered as parasitoids (Compton 1993b; Segar & Cook 2012; Suleman et al. 2013). In addition, *Sirovena costallifera* Li, Xiao & Huang (Pireninae, Pteromalidae) may be a parasitoid of Cecidomyiidae species, but this is unconfirmed because of its rarity (Li et al. 2013).

1.7 Objectives of our study

Here, we carried out a global study to describe the fig wasp fauna associated with *F. microcarpa* figs, and then tried to select competent bio-control agents among NPFW to achieve biological control of the invasive fig tree and unravel the underlying mechanisms that facilitate its invasion.

The aims of the present study are:

(1) to describe the global distribution of fig wasp species associated with *F. microcarpa* and unravel the trend of immigration of these fig wasps into the plant's introduced range (Chapter 2).

(2) to test whether Allee effects negatively affected the establishment of newly colonised NPFW populations in South Africa where the pollinator was not present and whether inter-specific facilitation could mitigate such detrimental influence (Chapter 3).

(3) to test the effects of latitude and climatic factors on community composition in a fig wasp fauna extending from the plant's native range to its introduced range in south-west China and whether species feeding at different trophic levels responded differentially to latitude and its associated climate factors (Chapter 4).

(4) to test whether *Philotrypesis taiwanensis* Chen, a species that was found to emerge from seeds of *F. microcarpa*, is an obligate seed predator rather than a parasitoid like other species in the genus *Philotrypesis* (Chapter 5).

(5) to describe the fig wasp community in the Mediterranean and test the effects of two common large galler NPFW, *Odontofrogattia galili* Wiebes and *Meselatus bicolor* Chen, on both the male and female reproductive functions of figs (Chapter 6).

(6) to unravel the food web of fig wasps associated with *F. microcarpa* and test which characteristics of fig wasps such as gall size and spatial locations of galls inside figs contribute to the compartments of the food web (Chapter 7).

(7) to test whether the absence of parasitoids of the pollinator *E. verticillata* resulted in an increase in the sexual reproductive successes of figs and a change in the fig-pollinator mutualism in the introduced populations of *F. microcarpa* (Chapter 8).

1.8 Statistical appendix

Linear models (LMs) are a widely used approach to quantify and test the linear relationship between independent variable(s) and response variable(s) (Crawley 2007; Bolker et al. 2009). This statistical method assumes that the residuals of response variable(s) (the detrended data) ought to be randomly distributed (independent) and of constant variance (homoscedasticity). When residuals do not follow normal distribution, data in response variable(s) need to be transformed, usually by natural

logarithm or square-root.

Sometimes, residuals of data are intrinsically not able to meet the assumptions of LMs, e.g. presence/absence data, and curvilinear relationships that can be transformed into linearity are predicted between independent variable(s) and response variable(s). Under these circumstances, generalized linear models (GLMs) are the optimal choice. Here, a large number of distributions are available for fitting the distributions of residuals (Bolker et al. 2009). Generally, two distributions are used: a binomial distribution that is specific to presence/absence data with a logit link function and the Poisson distribution, designed for count data including many zeros with a logarithm link function. The dispersion parameter (the ratio of residual deviance to residual degrees of freedom) is applied to indicate whether the selected GLM provides an appropriate fit to the data (if this parameter is close to 1, then the model can be considered as correct). If overdispersion is present, it is necessary to adopt a quasi-likelihood model that can adjust the expected dispersion parameter for model fitting based on the data.

The results from LMs and GLMs may however be influenced by pseudoreplication, which is mainly caused by spatial and temporal autocorrelations (hierarchical grouping ('nesting') of samples) (Bolker et al. 2009; Stone et al. 2011). Analyses therefore must consider sampling scales as random effects (factors whose effects vary among samples, unlike fixed effects), and use linear (LMMs) or generalized linear mixed models (GLMMs), where both fixed and random effects can be defined (Bates et al. 2013).

In addition to hierarchical sampling issues, insufficient sampling effort (the sample size cannot reflect the actual species pool at a site) is also likely to bias the results, and hence sampling effort has been incorporated in some GLMMs (Stone et al. 2011). Describing a species accumulation curve provides a straightforward approach to test sampling effort, and sufficient sampling effort is proved if accumulated species richness reached asymptotes within the range of sample sizes. Besides direct

accumulation, many algorithms that estimate the species richness of local species pool and delineate accumulation curve have been developed. They consider the probability of rare species not being sampled, such as the abundance-based coverage estimator (Chao & Lee 1992) and the first-order jackknife estimator (Burnham & Overton 1978; Heltshe & Forrester 1983). The estimation of parameters is highly sensitive to the initial data. The jackknife method therefore has been frequently adopted to avoid the potential bias induced by some unusual initial data (of either extremely large or small value) by using each sample as the initial value and averaging all estimates.

In the present study, all statistical analyses except those relevant to sampling effort were carried out in R version 2.14.2 (R Development Core Team 2012). When using R, data were analysed by either LMMs in R package nlme version 3.1 (Pinheiro et al. 2013) or GLMMs in R package lme4 version 1.0-5 (Bates et al. 2013) in all results chapters (Chapter 2-8) except Chapter 3 and Chapter 4. In Chapter 3, LMMs and GLMMs were implemented in R package Language R version 1.4.1 (Baayen 2011) and MCMCglmm version 2.17 (Hadfield 2012) respectively because the estimates of maximum likelihood in some analyses failed to converge using the Laplace Approximation algorithm in R package lme4, while it converged using Markov chain Monte Carlo (MCMC) methods after setting its length as 10000 repetitions. In Chapter 4, convergence of maximum likelihood in some GLMMs could not be achieved even with an extremely long length (100000 repetitions) of MCMC, probably because of strong overdispersion of the data (Bolker et al. 2009). We therefore only carried out analyses using LMs and GLMs assuming either quasibinomial or quasiPoisson distribution of residuals, in this Chapter. A likelihood ratio test that calculates significance by comparing the likelihood ratios in the models with/without a particular fixed effect was used to test a fixed effect, and then multiple tests with Bonferroni corrections were adopted in the pairwise comparisons between different levels within a fixed effect. When using LMs and LMMs, data were rescaled by either natural logarithm or squareroot transformation where necessary.

In the analyses relevant to fig contents and composition of fig wasp community, crop identity of *F. microcarpa* was set as the random effect in all result chapters except the final one, Chapter 8, where crop identity nested in sampling sites was considered as the random effect. This was because only a few sites were involved in the other results chapters, but the combined data at global scale were analysed in Chapter 8 with 27 sample sites and thereby neither levels of hierarchical sampling, i.e. crop identity and sampling site, could be ignored. For the same reason, fig identity, the higher sampling level of ovules, and fig identity nested in crops were taken as the random effects in the analyses concerning gall size and spatial stratification of fig contents in Chapters 2-7 and Chapter 8 respectively.

In Chapter 2, we tested whether we had detected most or all of fig wasp species in their local species pools (sampling effort at each site) by delineating species accumulation curves with increasing sample size using both direct accumulation and the first order jackknife algorithm (Burnham & Overton 1978; Heltshe & Forrester 1983), in SDR version 4.1.2 (Seaby & Henderson 2006). The jackknife method was used in case of bias resulting from taking some outliers as initial values for parameter estimation.

Chapter 2 Global distribution of fig wasps associated with the widely-introduced fig tree *Ficus microcarpa*

2.1 Abstract

Fig trees are a species-rich group of largely tropical and subtropical plants, characterized by their unusual inflorescences (figs). Each species is pollinated by one or a few species of host-specific fig wasps. Their larvae develop inside galled ovules, which are also exploited by numerous non-pollinating fig wasps (NPFW) that are also phytophagous or are parasitoids. *Ficus microcarpa* has an extensive natural distribution in Asia and Australasia and is also a widely-planted ornamental tree. Introductions of its pollinator fig wasp have allowed the tree to reproduce and it is increasingly invasive. Here, we combine previously published records of the distributions of fig wasps associated with *F. microcarpa* with the results of our extensive surveys across much of its introduced and native range. At least 43 morpho-species of fig wasps are associated with figs of *F. microcarpa*, most of which have only been recorded from this host. Limited molecular screening shows that additional ‘cryptic’ species are also present. 21 NPFW have established outside their natural ranges and there has been limited colonization of introduced figs by locally-native fig wasps. The pollinator fig wasp is the most widely-introduced species, and the most wide-spread NPFW are phytophages that are independent of the pollinator. Fig wasp communities in the introduced range are less species-rich and have a lower proportion of parasitoids.

2.2 Introduction

The genus *Ficus* (fig trees) is one of the most diverse plant genera, containing at least 800 species distributed mainly in the tropics and subtropics (Harrison 2005; Herre et al. 2008). The genus is characterized by their figs (syconia) – unique enclosed inflorescences that prevent all but highly specialized insects from reaching their

flowers (Cook & Rasplus 2003; Herre et al. 2008). When ripe, figs act as food for numerous fruit-eating birds and mammals, supporting more frugivorous species than any other plants (Shanahan et al. 2001).

The hundreds or thousands of female flowers that line the inner surfaces of figs are only pollinated by female pollinating fig wasps (Agaonidae) that enter the figs in order to lay their eggs - a mutualism that has persisted for around 60 million years (Compton et al. 2010). Each fig tree species only has one or a small number of host-specific pollinator species (Molbo et al. 2003). Pollinator fig wasp larvae develop singly inside galled ovules, producing adults that mate inside the figs before the females actively or passively collect pollen and depart via exit tunnels drilled through the fig wall by their males (Suleman et al., 2012). Figs also support numerous non-pollinating fig wasps (NPFWs) belonging to families Agaonidae, Eurytomidae, Ormyridae, Pteromalidae and Torymidae (Bouček 1988; Cook & Segar 2010). Most NPFW also display strict host specificity, though a few utilize more than one host fig tree species (Cook & Segar 2010; Zhou et al. 2012). Fig wasp communities can be complex, with 30 or more fig wasp species associated with some host species, though only a subset develop inside any one fig (Bouček et al. 1981; Hawkins and Compton 1992). Fig wasp communities show some convergence in ecological characters across continents, despite differences in their taxonomic composition (Segar et al. 2013) but vary in size in response to factors such as fig size, host plant breeding system and latitude (Compton and Hawkins 1992; Hawkins and Compton 1992; Mcleish et al. 2011; R. Wang unpublished).

NPFW can be grouped into two major trophic groups - those species with larvae that feed only on plant tissue, most commonly utilising ovules that they gall (seed predators are rare, Periera et al. 2007), and those species whose offspring kill the larvae of other fig wasps (but may also feed on plant tissues). The latter include a diversity of feeding styles, including secondary galls (Chen et al. 2013), parasitoids and inquilines (Segar & Cook 2012), and obligate hyper-parasitoids (Compton et al. 2009). NPFW can have negative impacts on the reproductive success of their host fig trees by either reducing

the numbers of pollen-carrying agaonid fig wasp offspring in the figs or reducing seed production (Kerdelhué & Rasplus 1996; Segar & Cook 2012). Parasitoids of the pollinator influence only the male component of the plant's reproductive success, but phytophages can reduce both seed and pollinator offspring numbers by competing with pollinator foundresses for oviposition sites and reducing the number of ovules that can set seed. Consequently, parasitoids that target other NPFW, rather than pollinator offspring, indirectly benefit their host plants (Compton et al. 2010).

Fig trees are widely-grown as pot plants and are also popular amenity and street trees. They are often planted outside their native range, where any figs they produce initially fail to contain any seeds, due to the absence of their specific pollinators. However, when suitable pollinators are introduced the trees can start to produce figs that are then attractive to a wide range of seed dispersers, and the tree start to become established (McKey 1989). This occurs mainly in urban environments, where the trees were planted, but one species, *F. microcarpa* L. has now also invaded natural habitats, where it is viewed as a significant threat to biodiversity (Stange & Knight 1987; Miao et al. 2011; Caughlin et al. 2012).

F. microcarpa L. is a monoecious fig tree native to South and South-east Asia extending to Australia, where it grows from rocks or as a strangler fig (Berg & Corner 2005). It has been introduced as an ornamental tree into many tropical, subtropical and warm temperate areas around the world (Nadel et al. 1992; Figueiredo et al. 1995; Kobbi et al. 1996; Beardsley 1998; Burrows & Burrows 2003; Starr et al. 2003; van Noort et al. 2013). The figs of this species contain tiny seeds that are mainly dispersed by frugivorous birds, with ants also serving as secondary dispersal agents (Kaufmann et al. 1991; Shanahan et al. 2001). Its seedlings can cause damage to buildings and it is increasingly reported from semi-natural vegetation, where it can become invasive (McKey 1989; Beardsley 1998; Starr et al. 2003; Corlett 2006; Caughlin et al. 2012, Mifsud, 2014). *F. microcarpa* is often planted in coastal towns, because it is salt tolerant, and the tree is particularly invasive on islands such as Hawaii and Bermuda (Beardsley

1998; Starr et al. 2003). *F. microcarpa* can also support fig wasp populations when planted in areas with seasonal climates such as Italy and Greece, as well as further north than the tree's natural range in China (Compton 1989; Lo Verde et al. 1991; 2007). The tree produces fewer crops in winter, but they are sufficient to maintain fig wasp populations, which recover rapidly in spring and may be aided by long distance dispersal of the fig wasps from more equitable sites (Yang et al. 2013).

The recorded pollinator of *F. microcarpa* is *Eupristina verticillata* Waterston, a taxon that, based on molecular data, consists of a complex of several morphologically-similar species (Sun et al. 2011; A. Cruaud and J-Y. Rasplus, Pers. Comm.). In addition, the figs of *F. microcarpa* are also host to an undescribed agaonid 'cheater' (*Eupristina* sp.) that does not actively pollinate its host figs and can be distinguished visually by its reduced pollen pockets (J-Y. Rasplus, S.G. Compton & R. Wang, unpublished). *E. verticillata* has been recorded throughout the plant's native range (Waterston 1921; Baltazar 1966; Hill 1967; Chen et al. 1999; Priyadarsanan 2000; McPherson 2005; van Noort & Rasplus 2010; Ramya 2011; Sun et al. 2011), and was deliberately introduced from the Philippines to Hawaii in the 1930s (Pemberlake 1939; Stange & Knight 1987; Beardsley 1998). During the last fifty years it has also successfully colonized most of the areas where *F. microcarpa* has been introduced, including Bermuda, Brazil, California, Canary Islands, Colombia, El Salvador, Florida, Honduras, Italy, Ivory Coast, Madeira, Malta, Mexico, Morocco, Puerto Rico, Spain, Tunisia, Turkey and United Arab Emirates (UAE) (Stange & Knight 1987; Lo Verde et al. 1991; Bouček 1993; Gill 1994; Figueiredo et al. 1995; Kobbi et al. 1996; Baez 1998; Ramirez & Montero 1998; Otero & Ackerman 2002; Koponen & Askew 2002; van Noort & Rasplus 2010; Doğanlar 2012; Mifsud et al. 2012; J-Y. Rasplus, pers. comm.). Molecular screening has detected only one of the genetically-distinguishable taxa within *E. verticillata* outside the tree's natural range, and the 'cheater' *Eupristina* species has also not been found elsewhere (R. Wang, A. Cruaud and J-Y. Rasplus, unpublished).

F. microcarpa is the only known host of its pollinator (*E. verticillata*) and most of its associated NPFW, but a small number of the NPFWs have also been reared from related fig trees (Zhou et al., 2012; Wang et al., unpublished). At least 26 NPFW species have been recorded from the figs of *F. microcarpa* in the plant's native range, belonging to the families Pteromalidae, Eurytomidae and Ormyridae (Grandi 1926; Ishii 1934; Wiebes 1980; Bouček 1988; Yokoyama & Iwatsuki 1998; Chen et al. 1999; Zhang & Xiao 2008; Feng & Huang 2010; Li et al. 2013). Unlike the agaonids, these species have adult females that do not enter the figs to oviposit. They utilise their long ovipositors to lay their eggs through the outer walls of the figs (Galil and Copland, 1981). *F. microcarpa* also supports a leaf-galling wasp (*Josephiella microcarpae* Beardsley & Rasplus) that is taxonomically related to fig wasps and that has been introduced into the Mediterranean area (Beardsley & Rasplus 2001; Mifsud et al. 2012).

Details of the biology of most of species of NPFW are poorly known (Chen et al., 2013), but our extensive studies of the species associated with *F. microcarpa* have provided indications of the biology of most of the species (Wang et al., in preparation). All known agaonid larvae develop inside galled ovules. The larvae of all the NPFW associated with *F. microcarpa* also develop in female flowers (rather than, for example, in galls within the fig wall). The flowers may or may not have been galled by other fig wasps. Species belonging to the subfamilies Epichrysomallinae and Otitesellinae (Pteromalidae) may all be gallers (Bouček 1988), whereas species in the families Eurytomidae and Ormyridae and subfamily Sycoryctinae of Pteromalidae are mainly parasitoids of gall formers (Compton 1993; Segar & Cook 2012; Suleman et al. 2013). *Philotrypesis taiwanensis* Chen (Sycoryctinae) is exceptional in that its larvae develop in seeds (Wang et al., in press). The rarely-encountered pteromalid *Sirovena costallifera* Li, Xiao & Huang (Pireninae) may be a parasitoid, but this is unconfirmed (Li et al. 2013). There are no confirmed obligate hyper-parasitoids (Compton et al. 2009) among the NPFW associated with *F. microcarpa*, but some parasitoids mainly or

entirely develop at the expense of gall-forming NPFW (Wang et al. unpublished).

Outside the native range of *F. microcarpa*, its associated phytophagous NPFW have been introduced into tropical, sub-tropical and temperate countries wherever their host plant is available. These include Bermuda, Brazil, California, Cayman Islands, Florida, Greece, Hawaii, Israel, Italy, Madeira, Malta, South Africa, Tunisia, Turkey and UAE (Wiebes 1980; Stange & Knight 1987; Ramirez & Montero 1988; Compton 1989; Hilburn et al. 1990; Lo Verde et al. 1991; Bouček 1993; Gill 1994; Kobbi et al. 1996; Beardsley 1998; Lo Verde et al. 2007; Farache et al. 2009; van Noort & Rasplus 2010; Doğanlar 2012; Mifsud et al. 2012; van Noort et al. 2013; J. Cook, pers. comm.). Putative parasitoid NPFW have become established less commonly, but are recorded from Brazil, Florida, California, Hawaii, Italy, and Turkey (Stange & Knight 1987; Bouček 1993; Gill 1994; Figueiredo et al. 1995; Beardsley 1998; Farache et al. 2009; Mifsud et al. 2012). The pollinator of *F. microcarpa* has been recorded from most of the countries where NPFW are present, except South Africa, though it is not always the first fig wasp to be introduced (Compton 1989). Interestingly, several putative galler and parasitoid species indigenous to two African fig trees have been detected in *F. microcarpa* figs in South Africa, though at extremely low densities (van Noort et al. 2013).

Hawaii is the only location where pollinators were deliberately introduced as part of an officially sanctioned policy (Stange & Knight 1987; Beardsley 1998), using figs collected from the Philippines in 1921. Hawaii acquired a diverse NPFW fauna very quickly, suggesting some may have arrived as contamination when the pollinators were introduced, though this has been disputed (Beardsley 1998). The mode of transfer of fig wasps between other countries is unknown. It may be an accidental product of the widespread international trade in *F. microcarpa*, though most trade is likely to involve small plants that would not have figs present. Adult fig wasps can emerge from figs collected several days earlier, which suggests the transport of detached figs by air, in order to make use of the seeds they contain, may have been a more frequent source of

international transfer. Pollinator fig wasps, at least, are also excellent long distance migrants (Ahmed et al. 2009), and secondary within-continental and between-island movements may often have been achieved without further human intervention (Compton et al. 1988). Pollinators have sometimes been the first fig wasps to be recorded, but *Odontofroggattia galili* Wiebes and *Walkerella microcarpae* Bouček were present before the first pollinators in the Mediterranean, and both species are present in South Africa, where the pollinator is still absent (Compton 1989; van Noort et al. 2013). Here, we collate previously published information on the distribution of fig wasps associated with figs of *F. microcarpa* and describe the results of fresh surveys in Asia and elsewhere. Here, we hypothesized that: (1) many fig wasp species utilize *F. microcarpa* figs; (2) high host specificity is present in the fig wasps associated with the plant; (3) only a few fig wasp species have become established outside their native range; (4) parasitoids are less likely to become established than phytophages; (5) large phytophages that are independent of the pollinator are more likely to disperse outside their native ranges; and (6) species are increasing immigrating into the introduced range of *F. microcarpa*.

2.3 Materials and methods

2.3.1 Published records

Literature relevant to fig wasps and *F. microcarpa* was searched via Web of Science, Google and citations in other papers. Some additional records were obtained via personal communications. Together with locations, the year before when each fig wasp species was first collected was noted, or if this was not provided, the year before when the record was published was noted. Synonymies were checked using the Universal Chalcidoidea Database, accessed March 2014 (<http://www.nhm.ac.uk/research-curation/research/projects/chalcidoids/database/>). Some published records do not specify the species concerned, but provide descriptions that are sufficient for

provisional assignment to species. These are indicated within the tables. *Philotrypesis okinavensis* and *P. emeryi* are closely related species, with females and smaller males that are hard to separate. We have segregated these species on the basis of lines of hairs at both sides of the head in males of *P. okinavensis*, which are considered to be absent in *P. emeryi* males. We have applied names to the two taxa *sensu* Chen et al. (1999), but not Bouček (1993). There appears to be some confusion over their identification in the literature and many listed records of *P. emeryi* probably refer to *P. okinavensis sensu* Chen. In the absence of confirmatory specimens we have retained the identifications used in the original publications, but these should be considered as provisional. Current higher taxonomic classifications of fig wasps are based on Rasplus et al. (1998), Campbell et al. (2000), Cruaud et al. (2010) and Heraty et al. (2013), as summarized in figweb (<http://www.figweb.org>).

2.3.2 Sample sites

A uniform sampling regime was adopted at sites in the introduced and native ranges of *F. microcarpa* (Table 2.1). In the plant's native range, figs were sampled from trees growing in climates that ranged from the more seasonal, with colder winters (mainland China), the seasonal tropics and subtropics with pronounced wet and dry seasons (Taiwan, Thailand), and the humid tropics (The Philippines). As elsewhere, the figs came from plants were almost all planted, rather than in natural forests. We also collected figs from 20 areas outside the plant's native range. These included areas in south-central China and eastern Australia, two countries where *F. microcarpa* grows naturally at lower latitudes than the introduced-range sample sites. Further from the native range, sample sites were located in Brazil, the Caribbean, southern USA, the Mediterranean and South Africa. Their climates vary considerably but tended to be more seasonal than those in the native range of *F. microcarpa*.

Table 2.1 Collection data for *F. microcarpa* figs in the present study. Note that only figs that contained fig wasps are indicated.

Area	Site (abbreviation)	Location	Year(s)	N crops	N figs
Native range					
China mainland	Guangzhou (GZ)	N 23°11', E 113°22'	2011-2012	12	169
China mainland	Xishuangbanna (XS)	N 22°00', E 100°48'	2010-2013	17	279
Philippines	Manila (MN)	N 14°40', E 121°04'	2012	2	40
Taiwan	Taipei (TB)	N 25°01', E 121°33'	2012	11	220
Thailand	Bangkok (BK)	N 13°44', E 100°33'	2012-2013	4	39
Thailand	Chiang Mai (CM)	N 18°46', E 98°59'	2012	4	47
Thailand	Kanchanaburi (KC)	N 14°04', E 99°32'	2012-2013	6	63
Total	--	--	--	56	857
Introduced range					
Australia	Brisbane (BR)	S 27°29', E 153°06'	2012	2	40
Brazil	Rio de Janeiro (RJ)	S 22°53', W 43°34'	2012	6	130
Canary Islands	Tenerife (TN)	N 28°29', W 16°19'	2013	1	30
China mainland	Chengdu (CD)	N 30°40', E 104°06'	2012	3	20
China mainland	Kunming (KM)	N 24°53', E 102°50'	2010-2011	6	48
China mainland	Mianyang (MY)	N 31°28', E 104°41'	2012	5	33
China mainland	Panzhuhua (PZ)	N 26°35', E 101°43'	2012	10	136
China mainland	Sanming (SM)	N 26°16', E 117°38'	2013	4	71
China mainland	Xichang (XC)	N 27°53', E 102°17'	2012	12	181
Florida	Davie (DV)	N 26°04', W 80°14'	2012	4	100
Greece	Rhodes (RD)	N 36°10', E 27°58'	2011-2012	27	294
Greece	Symi (SY)	N 36°35', E 27°50'	2012	4	37
Italy	Sicily (SC)	N 38°07', E 13°22'	2012	10	99
Libya	Tripoli (TP)	N 32°51', E 13°12'	2011-2012	7	96
Malta	Malta (MT)	N 35°56', E 14°23'	2011	9	130
Puerto Rico	Puerto Rico (PR)	N 18°23', W 66°04'	2013	7	47
South Africa	Grahamstown (GH)	S 33°19', E 26°31'	2011	7	140
South Africa	Port Elizabeth (PE)	S 33°58', E 25°37'	2011	5	81
Spain	Majorca (MJ)	N 39°35', E 2°40'	2012	6	101
Turkey	Marmaris (MM)	N 36°51', E 28°15'	2012	1	10
Total	--	--	--	136	1824
Grand total	--	--	--	192	2681

2.3.3 Fig wasp faunas

F. microcarpa crops were sampled between December 2010 and July 2013. Each sample comprised ten or more mature figs collected haphazardly from each crop (late C or early D phase, *sensu* Galil & Eisikowitch 1968). Figs at this stage contain adult fig wasps that are about to emerge from the figs. Any figs that had exit holes were rejected. The figs were stored in 70% ethanol.

The contents of each fig were recorded after they were sliced into quarters and softened by being soaked in water for more than ten minutes. Using a binocular microscope, all fig wasps inside our sampled figs were identified using mainly Chen et al. (1999) and Feng & Huang (2010), with additional morpho-species coded where necessary. Note that fig wasp species were distinguished on the basis of their morphology. Molecular studies suggest that complexes of closely related fig wasp species may also be present (Li et al. 2010; Sun et al. 2011; Zhou et al. 2012). The fig wasps were stored in 95% ethanol for long-term preservation, and are deposited at University of Leeds, UK.

The fig wasp species were divided into two trophic groups: phytophages including the pollinator (an ovule galler), NPFW that induce ovule galls (species in subfamilies Agaoninae, Epichrysomallinae, Otitesellinae) and the seed predator *Philotrypesis taiwanensis*, and parasitoids with larva grow at the expense of galler offspring (species in families Eurytomidae and Ormyridae and most species of Pteromalidae subfamily Sycoryctinae and (tentatively) subfamily Pireninae). Prevalence at each trophic level and of individual species were calculated as the proportion of figs where they were present compared with the total number of figs. Any figs that contained no figs were excluded from any analyses.

2.3.4 Changes over time in fig wasp faunas outside the plant's native range

In the plant's introduced range, we extracted previously published distribution records of individual taxa and combined them with the results of our surveys to generate species richness estimates. We also charted the expansions in distribution of the most

widely introduced species, including the pollinator, the two major NPFW phytophages (*Odontofroggattia galili* Wiebes and *Walkerella microcarpae* Bouček) and the most widely distributed parasitoids (*Philotrypesis emeryi* Grandi and *Philotrypesis okinavensis* Ishii). We combined records for these two closely related species in some analyses because of confusion with their identification in previous publications.

2.3.5 Statistical analyses

In the present study, differences between the native and introduced ranges of *F. microcarpa* in prevalence (the proportion of figs occupied) of the pollinator, phytophagous NPFW and parasitoids were tested using Generalized Linear Models (GLMs) in R 3.0.0 (R Development Core Team, 2013), assuming quasi-binomial distributions of residuals.

In each sample site, sampling effort was tested by comparing curves of accumulated species richness with increasing sample size using both direct accumulation and first order jackknife algorithms (Burnham & Overton 1978; Heltshe & Forrester 1983), in SDR version 4.1.2 (Seaby & Henderson 2006). When analysing, each fig was selected as the initial data to estimate the coefficients of curves (jackknife method), and the final curves at a site were shaped by the mean values of coefficients from all estimations in case of the bias resulting from setting some outliers as initial values.

2.4 Results

2.4.1 Published records

E. verticillata was described as the pollinator of *F. microcarpa* in 1921 (Waterston, 1921) and the first of its NPFW associates were described in 1926 (Grandi 1926). Published records and personal communications concerning the fig wasps associated with figs of *F. microcarpa* have provided information from 35 areas (10 in the plant's native range and 25 in its introduced range) (Tables 2.2 and 2.3). A total of 26 fig wasp

species were recorded from *F. microcarpa* figs in the plant's native range, comprising 16 putative phytophages and 10 putative parasitoids (Table 2.4). The highest recorded species richness was in Taiwan (20 species; Tables 2.2).

The introduction of *E. verticillata* to Hawaii in 1938 was recorded by Pemberlake (1939), but the un-planned spread of this species outside the native range was not recorded to have occurred before 1986 in the New World (Stange & Knight 1987; Ramírez & Montero 1988) and 1989 in Europe (Lo Verde et al. 1991). Prior to that, the first NPFW (*O. galili*) was recorded from Israel prior to 1980 (Wiebes 1980). Since then the pollinator and several NPFW have been recorded almost throughout the introduced range of *F. microcarpa*. Published records from 44% of the 25 areas where the plant was introduced referred to only a single species, usually the pollinator (Table 2.3). The published records give an indication of the richness of the fig wasp fauna associated with *F. microcarpa* in areas of its native range where faunal studies were conducted, the surprisingly rich fauna associated with the plant in Hawaii, and the preponderance of three species (*E. verticillata*, *W. microcarpae* and *O. galili*) throughout the introduced range of the plant. The two NPFW were both described originally from their introduced ranges.

E. verticillata was the most prevalent species appearing in all areas in both the plant's native and introduced ranges except Cayman Islands, Greece, Israel and South Africa. Putative NPFW phytophages emerged from figs in 80% and 68% of areas in the plant's native and introduced ranges, and *O. galili* and *W. microcarpae* were the most wide-spread putative NPFW phytophages, being recorded in 60% and 40% of areas in the plant's native range, and 44% and 40% of areas in the plant's introduced range (Tables 2.2 & 2.4). Putative parasitoids were seldomly recorded in both ranges with *P. emeryi* (combining with *P. okinavensis*) being the most prevalent species, emerging in 30% and 16% of areas in the plant's native and introduced ranges (Table 2.4).

Expansion of host ranges among figs wasps that normally utilize other fig tree hosts has only been detected in South Africa, where adult offspring of three phytophages (two of

them are pollinators) and two parasitoids of local fig wasps have been collected in small numbers (Table 2.3). It may not be coincidental that the pollinator has not been introduced to South Africa, and the absence of the pollinator is likely to leave sufficient unused niches for host-shifts (van Noort et al. 2013).

Table 2.2 Published records of the distributions of fig wasps associated with *F. microcarpa* in its native range.

Note that *Eupristina verticillata* is a complex of ‘cryptic’ species and that the name *Parapristina verticillata* Waterston, used in older literature, is a synonym *Eupristina verticillata* Waterston. *Walkerella yashiroi* Ishii is listed as a synonym of *Micranisa yashiroi* Ishii in the Universal Chalcidoidea Database. All Australian species were recorded from the plant's native range in north Australia.

Area	Species	Reference
Australia	<i>Eupristina verticillata</i> Waterston	McPherson 2005
	<i>Odontofroggatia galili</i> Wiebes	Bouček 1988
	<i>Walkerella kurandensis</i> Bouček	Bouček 1988
China mainland	<i>Eupristina verticillata</i> Waterston	Sun et al. 2011
	<i>Acophila quinata</i> Zhang & Xiao	Zhang & Xiao 2008
	<i>Odontofroggatia corneri</i> Wiebes	Feng & Huang 2010
	<i>Odontofroggatia gajimaru</i> Wiebes	Feng & Huang 2010
	<i>Odontofroggatia galili</i> Wiebes	Feng & Huang 2010
	<i>Odontofroggatia ishii</i> Wiebes	Feng & Huang 2010
	<i>Odontofroggatia quinifuniculus</i> Feng & Huang	Feng & Huang 2010
	<i>Walkerella microcarpae</i> Bouček	S. G. Compton, pers. comm.
India	<i>Sirovena costallifera</i> Li, Xiao & Huang	Li et al. 2013
	<i>Eupristina verticillata</i> Waterston	Priyadarsanan 2000; Ramya 2011
	<i>Walkerella kurandensis</i> Bouček	Bouček 1988
Indonesia	<i>Eupristina verticillata</i> Waterston	Baltazar 1966
	<i>Philotrypesis emeryi</i> Grandi	Grandi 1926
Japan	<i>Eupristina verticillata</i> Waterston	Hill 1967
	<i>Eufroggattisca okinavensis</i> Ishii	Ishii 1934

	<i>Odontofroggatia galili</i> Wiebes	Yokoyama & Iwatsuki 1998
	<i>Odontofroggatia gajimaru</i> Wiebes	Ishii 1934
	<i>Micranisa yashiroi</i> Ishii (<i>Walkerella yashiroi</i> Ishii)	Ishii 1934; Yokoyama & Iwatsuki 1998
	<i>Walkerella microcarpae</i> Bouček	Beardsley 1998
	<i>Philotrypesis emeryi</i> Grandi	Chen et al. 1999
	<i>Philotrypesis okinavensis</i> Ishii	Ishii 1934
	<i>Sycoscapter gajimaru</i> Ishii	Ishii 1934
Malaysia	<i>Eupristina verticillata</i> Waterston	Waterston 1921
	<i>Odontofroggatia corneri</i> Wiebes	Wiebes 1980
	<i>Odontofroggatia gajimaru</i> Wiebes	van Noort & Rasplus 2010
	<i>Odontofroggatia galili</i> Wiebes	Wiebes 1980
	<i>Odontofroggatia ishii</i> Wiebes	Wiebes 1980
	<i>Walkerella microcarpae</i> Bouček	S. van Noort, pers. comm.
	<i>Sycoscapter gajimaru</i> Ishii	S. van Noort, pers. comm.
Papua New Guinea	<i>Eupristina verticillata</i> Waterston	van Noort & Rasplus 2010
	<i>Epichrysomalla</i> sp.	Bouček 1988
	<i>Odontofroggatia corneri</i> Wiebes	Bouček 1988
	<i>Odontofroggatia galili</i> Wiebes	Bouček 1988
	<i>Odontofroggatia ishii</i> Wiebes	Bouček 1988
	<i>Walkerella kurandensis</i> Bouček	Bouček 1988
	<i>Walkerella microcarpae</i> Bouček	S. van Noort, pers. comm.
Solomon Islands	<i>Eupristina verticillata</i> Waterston	van Noort & Rasplus 2010
	<i>Odontofroggatia corneri</i> Wiebes	Wiebes 1980
Taiwan	<i>Eupristina verticillata</i> Waterston	Chen et al. 1999
	<i>Acophila microcarpae</i> Chen	Chen et al. 1999

	<i>Eufroggattisca okinavensis</i> Ishii	Chen et al. 1999
	<i>Meselatus bicolor</i> Chen	Chen et al. 1999
	<i>Odontofroggatia corneri</i> Wiebes	Chen et al. 1999
	<i>Odontofroggatia gajimaru</i> Wiebes	Chen et al. 1999
	<i>Odontofroggatia galili</i> Wiebes	Chen et al. 1999
	<i>Odontofroggatia ishii</i> Wiebes	Chen et al. 1999
	<i>Micranisa degastris</i> Chen	Chen et al. 1999
	<i>Walkerella kurandensis</i> Bouček	Chen et al. 1999
	<i>Bruchophagus sensoriae</i> Chen	Chen et al. 1999
	<i>Sycophila curta</i> Chen	Chen et al. 1999
	<i>Sycophila maculifacies</i> Chen	Chen et al. 1999
	<i>Sycophila petiolata</i> Chen	Chen et al. 1999
	<i>Ormyrus lini</i> Chen	Chen et al. 1999
	<i>Philotrypesis emeryi</i> Grandi	Chen et al. 1999
	<i>Philotrypesis okinavensis</i> Ishii	Chen et al. 1999
	<i>Philotrypesis taiwanensis</i> Chen	Chen et al. 1999
	<i>Sycoryctes moneres</i> Chen	Chen et al. 1999
	<i>Sycoscapter gajimaru</i> Ishii	Chen et al. 1999
The Philippines	<i>Eupristina verticillata</i> Waterston	Baltazar 1966

Table 2.3. Published records of the distributions of fig wasps associated with *F. microcarpa* outside its native range, as summarized in previous publications.

The latest possible date of colonization of a fig wasp species in each area is given as either the year before specimens were first collected (where collection dates are provided) or as the year before publication if collection dates were not provided. Note that the study site in Australia (Brisbane) is to the south of the plant's native range, and we therefore included it in the introduced areas of the plant.

Area	Species	Reference	Arrived before
Australia	<i>Eupristina verticillata</i> Waterston	McPherson 2005	2003
	<i>Odontofroggatia galili</i> Wiebes	McPherson 2005	2003
Bermuda	<i>Eupristina verticillata</i> Waterston	Stange & Knight 1987	1986
	<i>Odontofroggatia galili</i> Wiebes	Hilburn et al. 1990	1989
	<i>Walkerella microcarpae</i> Bouček	Bouček 1993	1985
Brazil	<i>Eupristina verticillata</i> Waterston	Figueiredo et al. 1995	1994
	<i>Odontofroggatia ishii</i> Wiebes	Farache et al. 2009	2005
	<i>Walkerella microcarpae</i> Bouček	Ramírez & Montero 1988; Bouček 1993	1982
	<i>Philotrypesis emeryi</i> Grandi	Figueiredo et al. 1995; Farache et al. 2009	1987
California	<i>Philotrypesis taiwanensis</i> Chen	Farache et al. 2009	2005
	<i>Eupristina verticillata</i> Waterston	Gill 1994	1993
	<i>Odontofroggatia galili</i> Wiebes	Bouček 1993	1975
	<i>Odontofroggatia ishii</i> Wiebes	Gill 1994	1993
	<i>Walkerella microcarpae</i> Bouček	Gill 1994	1993
Canary Islands	<i>Sycophila</i> sp.	Gill 1994	1993
	<i>Eupristina verticillata</i> Waterston	Baez 1998	1997

Cayman Islands	<i>Walkerella microcarpae</i> Bouček	Bouček 1993	1985
Colombia	<i>Eupristina verticillata</i> Waterston	Ramírez & Montero 1988	1987
El Salvador	<i>Eupristina verticillata</i> Waterston	Ramírez & Montero 1988	1985
Florida	<i>Eupristina verticillata</i> Waterston	Stange & Knight 1987; Bouček 1993	1985
	<i>Odontofroggatia galili</i> Wiebes	Stange & Knight 1987; Bouček 1993	1986
	<i>Odontofroggatia ishii</i> Wiebes	Bouček 1993	1991
	<i>Micranisa yashiroi</i> Ishii (<i>Walkerella yashiroi</i> Ishii)	Stange & Knight 1987; Bouček 1993	1986
	<i>Walkerella microcarpae</i> Bouček	Stange & Knight 1987; Bouček 1993	1986
	<i>Philotrypesis emeryi</i> Grandi (<i>P. okinavensis sensu</i> Chen et al. (1999))	Stange & Knight 1987; Bouček 1993	1986
	Greece	<i>Odontofroggatia galili</i> Wiebes	Compton 1989
Hawaii	<i>Eupristina verticillata</i> Waterston	Pemberlake 1939; Stange & Knight 1987; Beardsley 1998	Introduced in 1938
	<i>Odontofroggatia galili</i> Wiebes	Beardsley 1998	1974
	<i>Odontofroggatia quinifuniculus</i> Feng & Huang (based on description)	Beardsley 1998	1974
	<i>Micranisa yashiroi</i> Ishii	Beardsley 1998	1970
	<i>Walkerella microcarpae</i> Bouček	Beardsley 1998	1988
	<i>Sycophila maculafacies</i> Chen (based on description)	Beardsley 1998	1988
	<i>Sycophila petiolata</i> Chen (based on description)	Beardsley 1998	1975
	<i>Philotrypesis emeryi</i> Grandi	Beardsley 1998	1988
	<i>Philotrypesis taiwanensis</i> Chen (based on description)	Beardsley 1998	1993
Honduras	<i>Eupristina verticillata</i> Waterston	Ramírez & Montero 1988	1985
Israel	<i>Odontofroggatia galili</i> Wiebes	Wiebes 1980	1978
Italy	<i>Eupristina verticillata</i> Waterston	Lo Verde et al. 1991	1989
	<i>Odontofroggatia galili</i> Wiebes	Lo Verde et al. 1991	1989

	<i>Walkerella microcarpae</i> Bouček	Lo Verde et al. 2007	2006
	<i>Philotrypesis emeryi</i> Grandi	Lo Verde et al. 2007	2006
Ivory Coast	<i>Eupristina verticillata</i> Waterston	J-Y. Rasplus, pers. comm.	2010
Madeira	<i>Eupristina verticillata</i> Waterston	Koponen & Askew 2002	2001
	<i>Walkerella microcarpae</i> Bouček	J. Cook, pers. comm.	2010
Malta	<i>Eupristina verticillata</i> Waterston	Mifsud et al. 2012	2010
	<i>Odontofroggatia galili</i> Wiebes	Lo Verde & Porcelli 2010	2008
Mexico	<i>Eupristina verticillata</i> Waterston	Ramírez & Montero 1988	1984
Morocco	<i>Eupristina verticillata</i> Waterston	J-Y. Rasplus, pers. comm.	2010
Puerto Rico	<i>Eupristina verticillata</i> Waterston	Otero & Ackerman 2002	1998
	<i>Micranisa degastris</i> Chen (based on description)	Otero & Ackerman 2002	1998
South Africa	<i>Elisabethiella baijnathi</i> Wiebes	van Noort et al. 2013 (indigenous host: <i>Ficus burtt-davyi</i>)	2011
	<i>Elisabethiella stueckenbergi</i> Grandi	van Noort et al. 2013 (indigenous host: <i>Ficus burkei</i>)	2012
	<i>Odontofroggatia corneri</i> Wiebes	van Noort et al. 2013	2011
	<i>Odontofroggatia galili</i> Wiebes	van Noort et al. 2013	2009
	<i>Otitesella uluzi</i> van Noort	van Noort et al. 2013 (indigenous host: <i>Ficus burtt-davyi</i>)	2011
	<i>Walkerella microcarpae</i> Bouček	van Noort et al. 2013	2009
	<i>Sycophila punctum</i> Bouček	van Noort et al. 2013 (indigenous host: <i>Ficus burkei</i>)	2012
	<i>Sycoryctes</i> sp.	van Noort et al. 2013 (indigenous host: <i>Ficus burtt-davyi</i>)	2011
Spain	<i>Eupristina verticillata</i> Waterston	J-Y. Rasplus, pers. comm.	2010
Tunisia	<i>Eupristina verticillata</i> Waterston	Kobbi et al. 1996	1985
	<i>Odontofroggatia galili</i> Wiebes	Kobbi et al. 1996	1991
Turkey	<i>Eupristina verticillata</i> Waterston	Doğanlar 2012	2010
	<i>Odontofroggatia ishii</i> Wiebes	Doğanlar 2012	2010
	<i>Walkerella microcarpae</i> Bouček	Doğanlar 2012	2010

	<i>Philotrypesis emeryi</i> Grandi	Doğanlar 2012	2010
	<i>Philotrypesis taiwanensis</i> Chen	Doğanlar 2012	2010
United Arab Emirates	<i>Eupristina verticillata</i> Waterston	van Noort & Rasplus 2010	2004
	<i>Acophila microcarpae</i> Chen	van Noort & Rasplus 2010	2004

Table 2.4 Summary of published records of fig wasps associated with figs of *F. microcarpa* (see Tables 2.2 and 2.3 for full citations).

Trophic level	Wasp taxon	Native range (N areas recorded)	Introduced range (N areas recorded)
Putative phyophages			
Agaonidae, Agaoninae			
	<i>Eupristina verticillata</i> Waterston	10	21
Pteromalidae, Epichrysoallinae			
	<i>Acophila microcarpae</i> Chen	1	1
	<i>Acophila quinata</i> Zhang & Xiao	1	0
	<i>Epichrysoalla</i> sp.	1	0
	<i>Eufroggattisca okinavensis</i> Ishii	2	0
	<i>Meselatus bicolor</i> Chen	1	
	<i>Odontofroggattia corneri</i> Wiebes	5	1
	<i>Odontofroggattia gajimaru</i> Wiebes	4	0
	<i>Odontofroggattia galili</i> Wiebes	6	11
	<i>Odontofroggattia ishii</i> Wiebes	4	4
	<i>Odontofroggattia quinifuniculus</i> Feng & Huang	1	1
Pteromalidae, Otitesellinae			
	<i>Micranisa degastris</i> Chen	1	1
	<i>Micranisa yashiroi</i> Ishii	1	2
	<i>Walkerella kurandensis</i> Bouček	4	0
	<i>Walkerella microcarpae</i> Bouček	4	10
Pteromalidae, Sycoryctinae			
	<i>Philotrypesis taiwanensis</i> Chen	1	3
Putative parasitoids			
Eurytomidae			
	<i>Bruchophagus sensoriae</i> Chen	1	0
	<i>Sycophila curta</i> Chen	1	0
	<i>Sycophila maculifacies</i> Chen	1	1
	<i>Sycophila petiolata</i> Chen	1	1
Ormyridae			
	<i>Ormyrus lini</i> Chen	1	0
Pteromalidae, Pireninae			
	<i>Sirovena costallifera</i> Li, Xiao & Huang	1	0
Pteromalidae, Sycoryctinae			
	<i>Philotrypesis emeryi</i> Grandi	3	4
	<i>Philotrypesis okinavensis</i> Ishii	2	1
	<i>Sycoryctes moneres</i> Chen	1	0
	<i>Sycoscapter gajimaru</i> Ishii	3	0

2.4.2 Current fig wasp faunal surveys

We dissected 2681 figs with fig wasps from 192 crops (Table 2.1), and recorded a total of 99038 fig wasps. We identified a total of 32 fig wasp morpho-species including 14 putative phytophages and 18 putative parasitoids (Table 2.5). Among those species, we recorded 11 taxa for the first time from *F. microcarpa* figs, including 10 new morpho-species belonging to the genera *Eupristina* (one species, a ‘cheater’ that fails to actively pollinate), *Sycobia* (one species), *Walkerella* (one species), *Sycophila* (two species), *Ormyrus* (one species), *Philotrypesis* (three species), *Sycoryctes* (one species) and *Sycoscapter* (one species). One species, *Walkerella nigrabdomina* Ma & Yang, has been recorded previously from another host (Ma et al. 2013). Each of these newly-recorded species was recorded from no more than two sample sites, with low prevalence, suggesting a highly restricted distribution pattern and the possibility of other *Ficus* species as their major hosts. The fig wasp fauna we recorded in the plant's native range did not include *Sycobia* sp., *Bruchophagus sensoriae* Chen and *Ormyrus* sp., all of which were only recorded from planted trees located further north in China. We however failed to detect 6 species that have been previously recorded in the plant's native range (*Acophila microcarpae* Chen, *Epichrysomalla* sp., *Eufroggattisca okinavensis* Ishii, *Odontofroggattia gajimaru* Wiebes, *Micranisa yashiroi* Ishii and *Walkerella kurandensis* Bouček). Together with the five species indigenous to African fig trees in South Africa, the total number of morphologically distinguishable fig wasp species recorded from the figs of *F. microcarpa* stands at 43, probably more than for any other fig tree.

Species richness at a sample site reached up to 18 (in Xishuangbanna and Taibei; Table 2.6), though not all these were recorded from a single crop. Fig wasp communities in the introduced range of *F. microcarpa* were generally less species rich than in the native range, with the highest species richness (12) appearing in Panzhihua (Table 2.6). Most (86%) of the putative phytophagous species, but just less than half the putative parasitoid species were recorded from outside the plant's native range (Tables 2.5 &

2.6). Fig wasp communities at all sample sites were composed of at least two species, except for Marmaris, where sampling was relatively limited (Table 2.6).

Both direct accumulation and first order jackknife methods suggested that species richness reached asymptotes within our range of sample sizes at every sample site (Fig. 2.1 & 2.2), and estimates of the size of the local species pools based on our sample size using first order jackknife algorithms indicated that we had recorded most but not all of the species predicted to be present at some sites (Table 2.6).

The pollinating agaonid was the dominant fig wasp species in both the native and introduced ranges, but it was entirely absent from four sample sites (Grahamstown, Kunming, Mianyang and Port Elizabeth; Table 2.6) outside the native range. It was also present in a higher proportion of the figs in the plant's native range (GLM: pollinator prevalence (quasi-binomial): $df=1$, $LR=148.020$, $p<0.001$; Table 2.5).

NPFW were also common throughout the native and introduced ranges of *F. microcarpa*, occupying 84% of the figs overall. Some phytophagous NPFW appear to be more tolerant of seasonal climates than the pollinator, and the prevalence of NPFW phytophages (75% and 82% of the figs in the native and introduced ranges) and parasitoids (53% and 18% in the native and introduced ranges) were different in the two ranges, with NPFW phytophages more prevalent in the plant's introduced range (GLM: NPFW phytophages prevalence (quasi-binomial): $df=1$, $LR=15.238$, $p<0.001$) whereas parasitoids were at a much lower frequency there (GLM: parasitoids prevalence (quasi-binomial): $df=1$, $LR=341.180$, $p<0.001$; Tables 2.5 & 2.6). Further, putative parasitoids were entirely absent from 45% of the sample sites in the introduced range of the plant (Table 2.6). *O. galili* and *W. microcarpae* were the most prevalent putative phytophages, with both recorded in more than 42% of sample sites and occupying more than 12% of figs in both ranges (Table 2.5). Among putative parasitoids, *Sycophila maculafacies* Chen and *Philotrypesis okinavensis* Ishii were widespread, both being recorded from at least 30% of sites and more than 6% of the figs in both ranges (Table 2.5).

Table 2.5 Fig wasps recorded from figs of *F. microcarpa* in the present study.

Calculations of prevalence (the proportion of figs where each species was recorded) are based only on figs where fig wasps were present.

Taxonomic level	Wasp taxon	Native range		Introduced range	
		N sites present	Prevalence (%)	N sites present	Prevalence (%)
Putative phytophages					
Agaonidae, Agaoninae					
<i>Ev</i>	<i>Eupristina verticillata</i> Waterston	7	66.6	16	41.6
<i>Es</i>	<i>Eupristina</i> sp. ('Cheater')	1	21.0	1	1.0
Pteromalidae, Epichrysomallinae					
<i>Aq</i>	<i>Acophila quinata</i> Zhang & Xiao	1	0.1	1	0.1
<i>Mb</i>	<i>Meselatus bicolor</i> Chen	2	1.2	7	14.6
<i>Oc</i>	<i>Odontofroggatia corneri</i> Wiebes	6	10.2	6	11.2
<i>Og</i>	<i>Odontofroggatia galili</i> Wiebes	3	24.0	15	37.8
<i>Oi</i>	<i>Odontofroggatia ishii</i> Wiebes	5	8.4	4	2.0
<i>Oq</i>	<i>Odontofroggatia quinifuniculus</i> Feng & Huang	4	2.9	1	0.4
<i>Sbs</i>	<i>Sycobia</i> sp.	0	0	2	0.8
Pteromalidae, Otitesellinae					
<i>Md</i>	<i>Micranisa degastris</i> Chen	5	6.8	6	7.5
<i>Wm</i>	<i>Walkerella microcarpae</i> Bouček	5	11.6	12	23.7
<i>Wn</i>	<i>Walkerella nigrabdomina</i> Ma & Yang	2	2.0	0	0
<i>Ws</i>	<i>Walkerella</i> sp.	1	1.5	0	0
Pteromalidae, Sycoryctinae					
<i>Pt</i>	<i>Philotrypesis taiwanensis</i> Chen	7	23.1	4	3.3

Putative parasitoids

Eurytomidae

<i>Bs</i>	<i>Bruchophagus sensoriae</i> Chen	0	0	2	1.2
<i>Sc</i>	<i>Sycophila curta</i> Chen	1	0.2	0	0
<i>Sm</i>	<i>Sycophila maculifacies</i> Chen	4	15.4	6	6.3
<i>Smp</i>	<i>Sycophila maculifacies</i> ('pale')	2	2.5	3	1.3
<i>Sp</i>	<i>Sycophila petiolata</i> Chen	3	17.0	2	0.9
<i>Ss</i>	<i>Sycophila</i> sp.	1	0.6	0	0

Ormyridae

<i>Ol</i>	<i>Ormyrus lini</i> Chen	2	0.8	0	0
<i>Os</i>	<i>Ormyrus</i> sp.	0	0	1	0.1

Pteromalidae, Pireninae

<i>Sic</i>	<i>Sirovena costallifera</i> Li, Xiao & Huang	1	0.1	0	0
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Pteromalidae, Sycoryctinae

<i>Pe</i>	<i>Philotrypesis emeryi</i> Grandi	5	7.7	5	0.7
<i>Po</i>	<i>Philotrypesis okinavensis</i> Ishii	5	8.3	6	6.8
<i>Ps1</i>	<i>Philotrypesis</i> sp.1	2	0.6	0	0
<i>Ps2</i>	<i>Philotrypesis</i> sp.2	1	0.1	0	0
<i>Ps3</i>	<i>Philotrypesis</i> sp.3	1	0.5	0	0
<i>Srm</i>	<i>Sycoryctes moneres</i> Chen	6	13.1	0	0
<i>Srs</i>	<i>Sycoryctes</i> sp.	1	2.8	0	0
<i>Scg</i>	<i>Sycoscapter gajimaru</i> Ishii	5	15.9	1	2.6
<i>Scs</i>	<i>Sycoscapter</i> sp.	1	0.5	0	0

Table 2.6 Global distributions and prevalence of fig wasp species recorded from figs of *F. microcarpa* in the present study.

Order and abbreviations of sample sites are as in Table 2.1; Order and abbreviations of species are as in Table 2.5. **S_g**: species richness of putative phytophages; **S_p**: species richness of putative parasitoids; **S_t**: total fig wasp species richness; **S_e**: size of local species pool estimated by first order jackknife algorithms; Prevalence at each site (proportion of occupied figs) was calculated using only figs containing fig wasps.

Site	Putative phytophages														Putative parasitoids											S _t	S _e										
	Ev	Es	Aq	Mb	Oc	Og	Oi	Oq	Sbs	Md	Wm	Wn	Ws	Pt	S _g	Bs	Sc	Sm	Smp	Sp	Ss	Ol	Os	Sic	Pe			Po	Ps1	Ps2	Ps3	Srm	Srs	Scg	Scs	S _p	
Native range																																					
GZ	75				2	28	12	6		2	24			36	8		20		8		1			4	28				20		16		7	15	15		
XS	70	65	0.4		11	0.4	6	4		9	14	5		21	11	1	3							15	6			4	9	19		7	18	20			
TB	45				2	72	9	1		10	3			21	8		39	7	58	2	2		3	3			10		22	2	10	18	19				
BK	46			21	21		10	8		3	5		33	18	9								3	13	3	10					4	13	15				
CM	100				2							9		2	4													11		13		2	6	7			
KC	68			3	24		17			10	14			35	7		6	10	8				8	2	2	2	6	16		3	10	17	20				
MN	100													10	2													80			1	3	3				
Introduced range																																					
BR	95					43		20						3			33	15														2	5	5			
RJ	78						19				38			25	4									1	44							2	6	7			
TN	100										37			2																		0	2	2			
CD	30			1	1	65					5			5																		0	5	7			
KM		38	2	8	52	31			10	33				7	6		21					2			35						4	11	13				
MY				94	15									2																		0	2	2			
PZ	12			20	29	27			7		61			1	7	14		7	4	4					25						5	12	13				
SM	100				6	4	4			1	6			6											1						1	7	9				

XC	2	2	66	62	3	27	6	30	6	6				3	9	9	
DV	75			84	91		19	4	3			5		48	3	7	7
RD	23	60		26		1		4	9			2			2	6	6
SY	24	65		16	3			4				8			1	5	6
SC	88			63		1		3				1			1	4	5
TP	78			76				2							0	2	2
MT	44			95				2							0	2	2
PR	94				17	49	11	17	5			6	2		2	7	7
GH				18			93		2						0	2	2
PE				2			100		2						0	2	2
MJ	69			40		5	10		4						0	4	4
MM	100								1						0	1	1

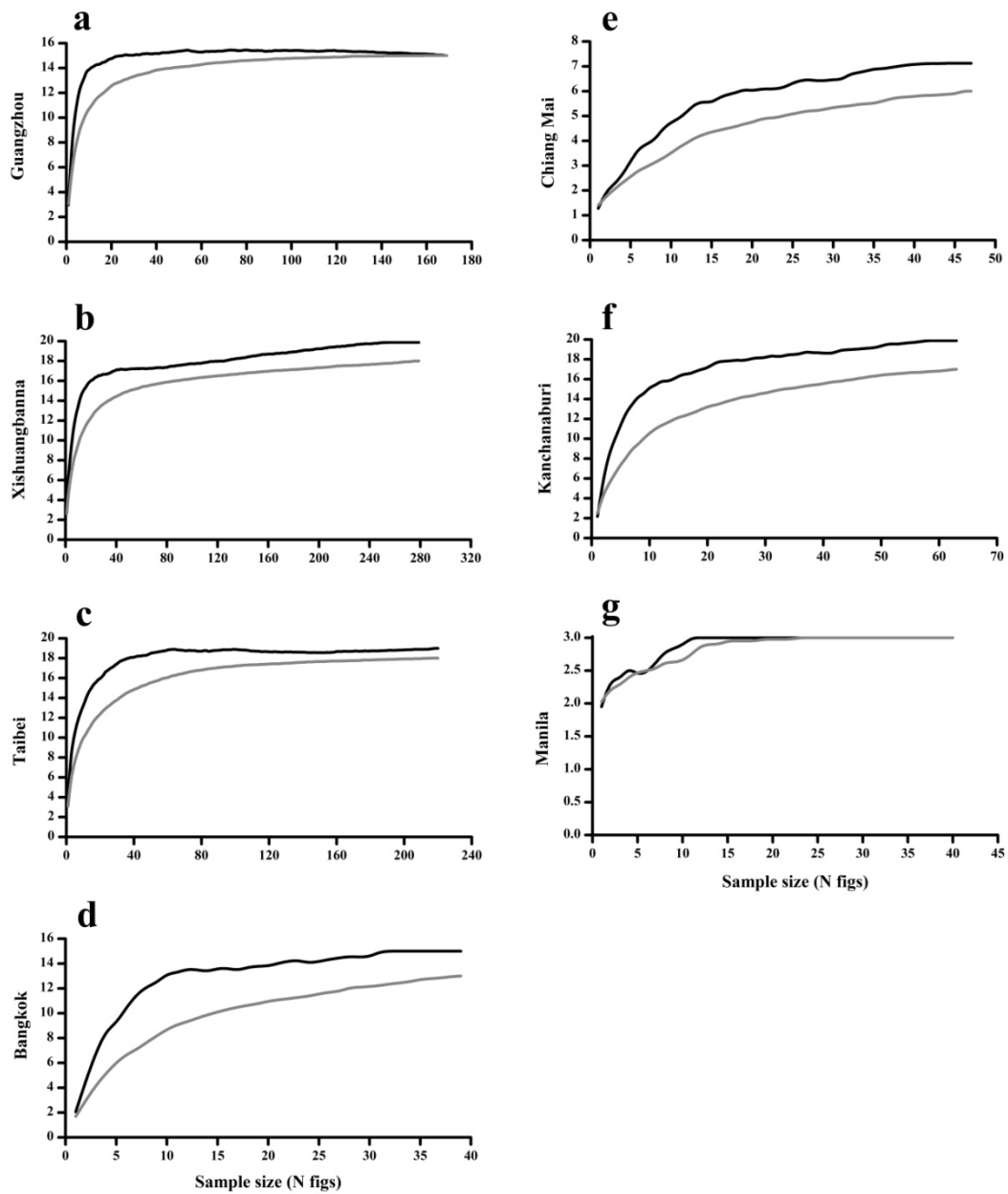
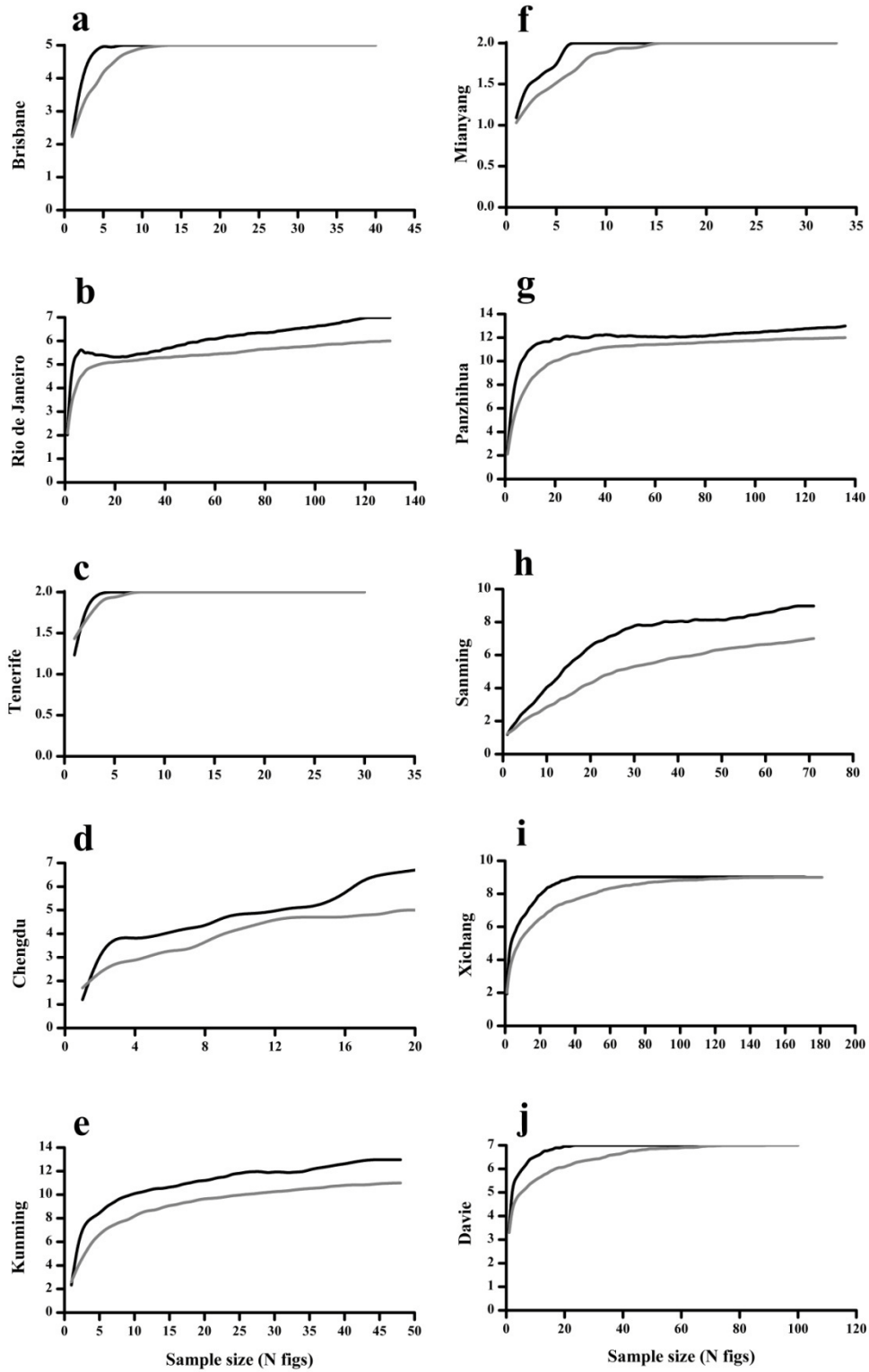


Fig. 2.1 Curves of estimated *Ficus microcarpa* fig wasp regional species richness in relation to sample size at sites in the plant's native range.

Black lines indicated estimated species accumulation using a first order jack-knife algorithm, while grey lines showed observed values. Note the variation in axis scales.



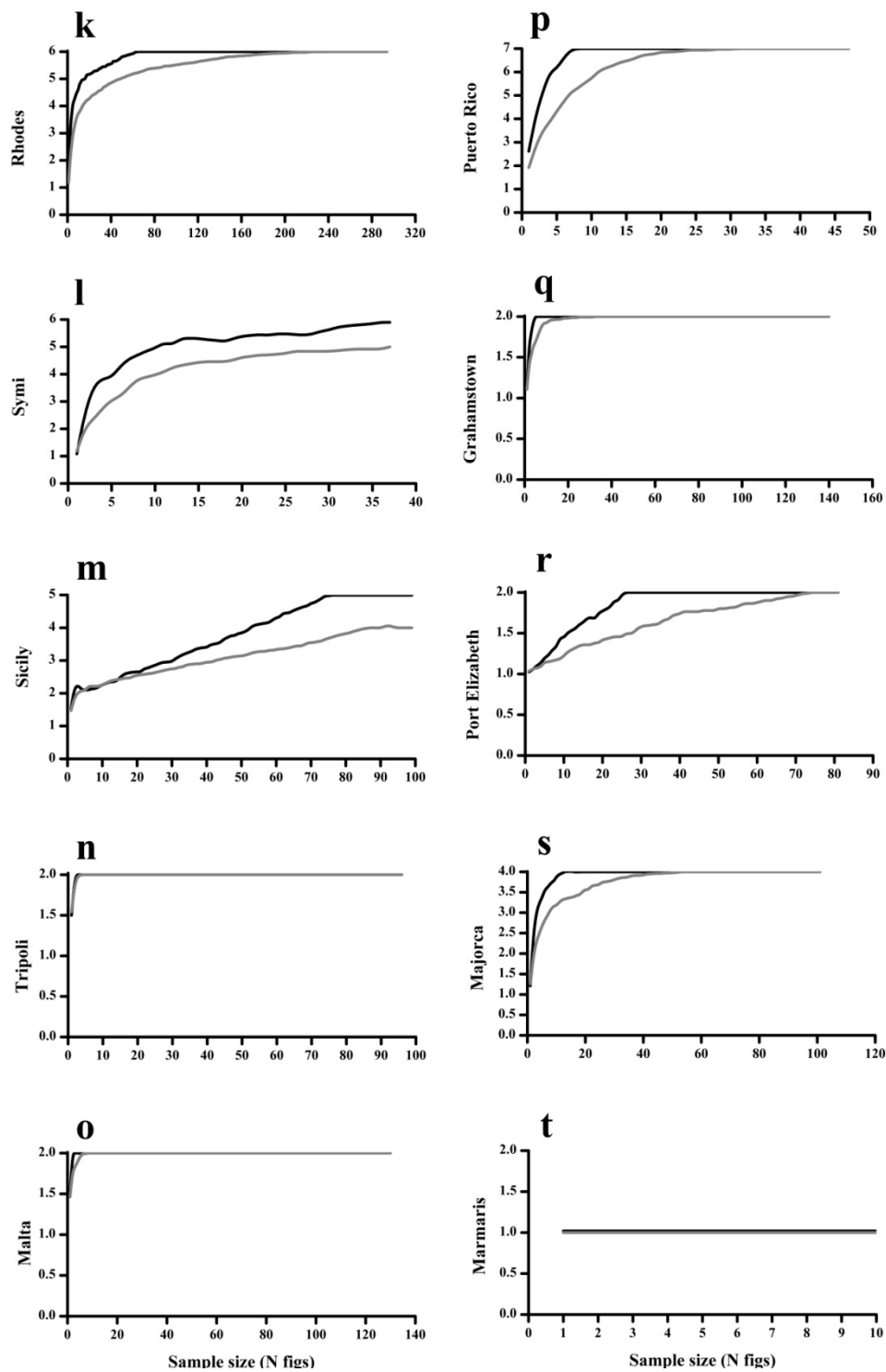


Fig. 2.2 Curves of estimated *Ficus microcarpa* fig wasp regional species richness in relation to sample size at sites in the plant's native range.

Black lines indicated estimated species accumulation using a first order jack-knife algorithm, while grey lines showed observed values. Note the variation in axis scales.

2.4.3 Changes over time in fig wasp faunas outside the plant's native range

Based on combined datasets from previous publications and our present study, in the plant's introduced range, figs from the Chinese mainland contained more fig wasp species (17 species) than elsewhere, with fig wasp community in 30% sampling areas being composed of only one species in the non-Chinese introduced range (Fig. 2.3). The pollinator was the most widely distributed species, but was absent from 3 out of 27 study areas at the times when they were sampled (Cayman Islands, Israel and South Africa). Parasitoids are far less common than phytophages, with much lower species richness, and only occurring in 37% of the sample sites in the plant's introduced range (Fig. 2.3). Species richness of phytophages was always more than that of parasitoids over time (Fig. 2.4). A total of 22 species (21 NPFWs) including 14 phytophages (13 NPFW phytophages) and 8 parasitoids are currently established outside the plant's native range. The dominant phytophages (*E. verticillata*, *O. galili* and *W. microcarpae*) and the major parasitoids of the pollinators (*P. emeryi* and *P. okinavensis*) are continuing to expand their distribution outside the plant's native range (Fig. 2.5).

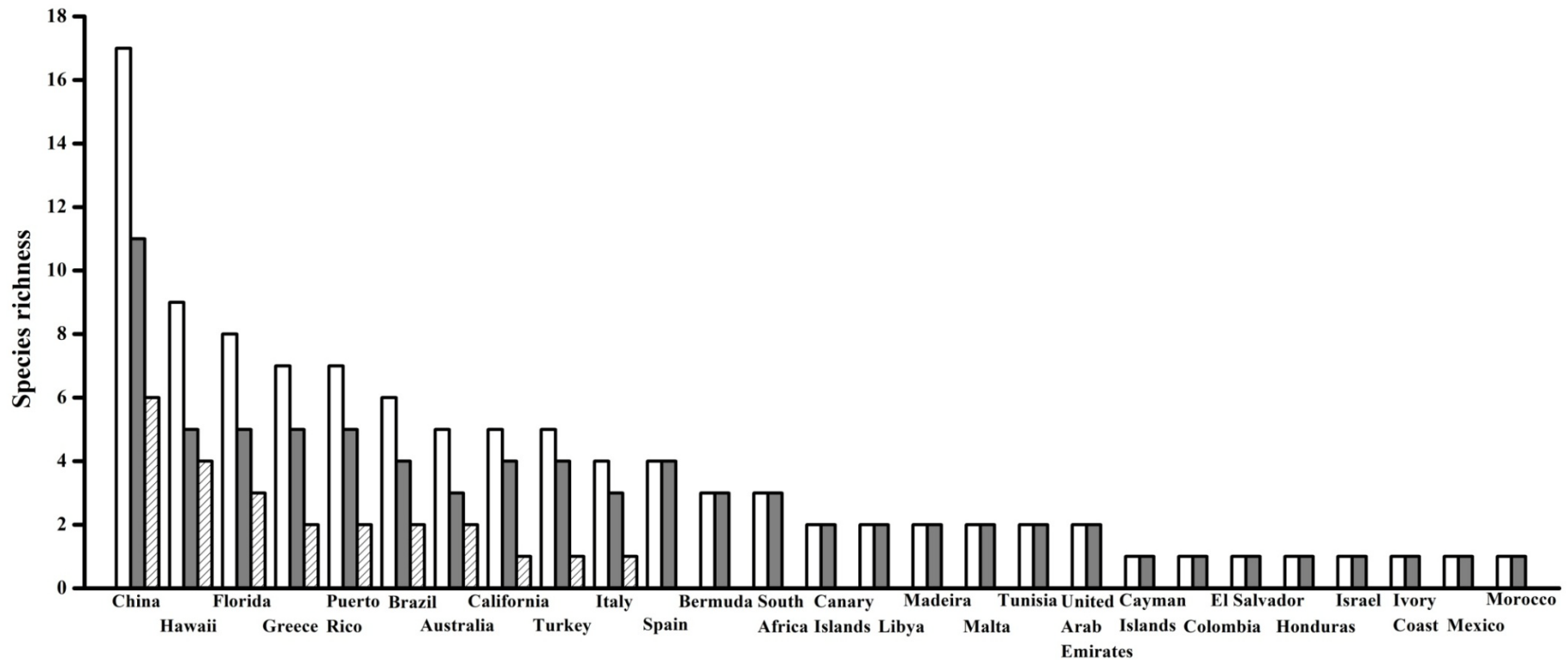


Fig. 2.3 Species richness of fig wasp in sampling areas where *F. microcarpa* has been introduced.

Open, filled and hatched bars represent total, phytophage and parasitoid species richness respectively. Data were extracted from both previous publications and our present study.

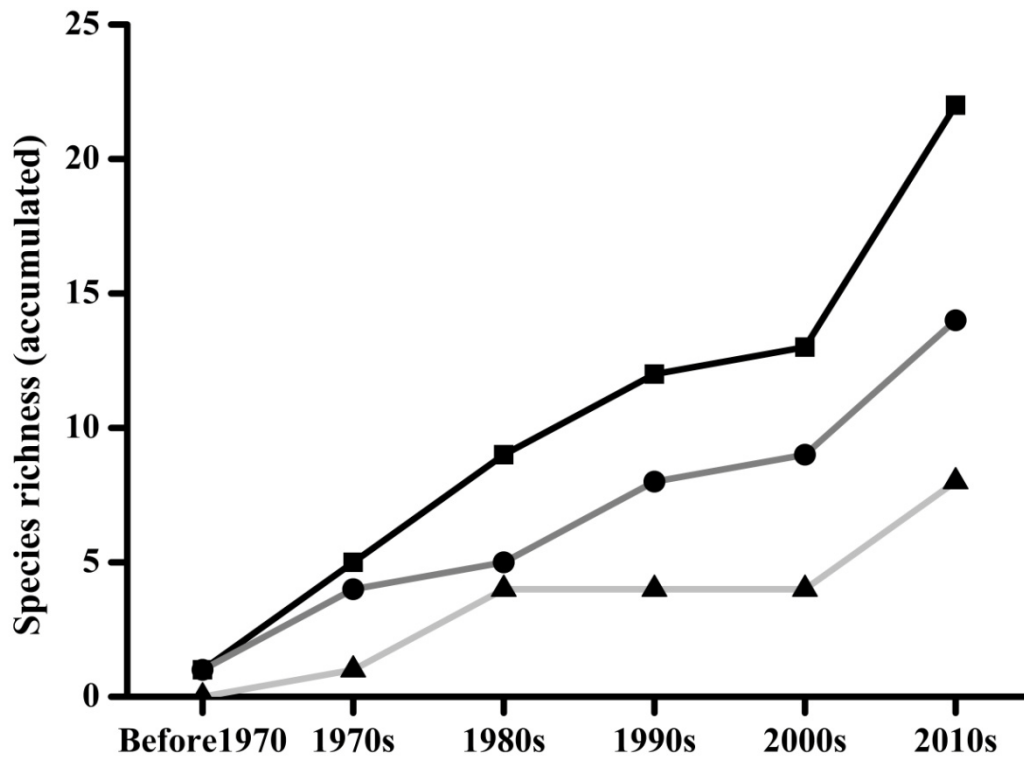


Fig. 2.4 Accumulated species richness of fig wasps in the introduced range of *F. microcarpa* over time.

Squares (black line), circles (grey line) and triangles (light grey line) represent total, phytophage and parasitoid species richness respectively. Data were extracted from both previous publications and our present study. Pre-1970s data is largely assumed because of lack of data.

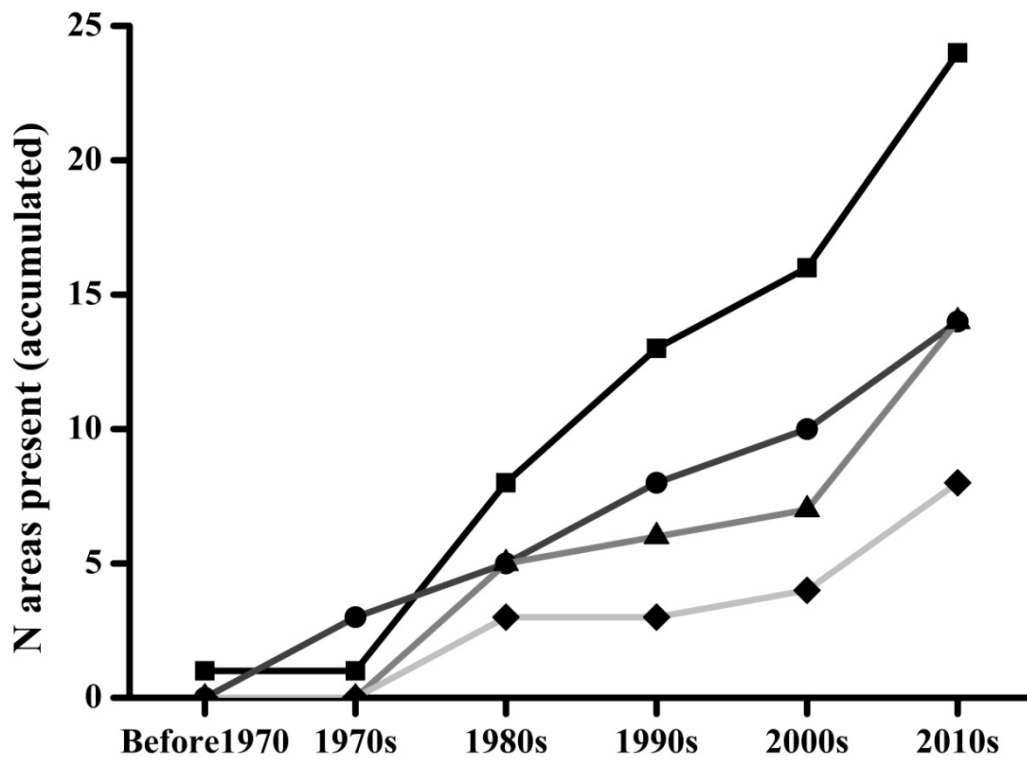


Fig. 2.5 Accumulated changes in time of areas where *E. verticillata*, *O. galili*, *W. microcarpae* and *P. emeryi* (mixed with *P. okinavensis*) were present in the introduced range of *F. microcarpa*.

Squares (black line), circles (dark grey line), triangles (grey line) and diamonds (light grey line) represent *E. verticillata*, *O. galili*, *W. microcarpae* and *P. emeryi* mixed with *P. okinavensis* respectively. Data were extracted from both previous publications and our present study. Pre-1970s data is largely assumed because of a lack of data.

2.5 Discussion

Many fig trees have wide natural distributions, within which local fig wasp communities may vary among locations (Compton & Hawkins 1992; Hawkins & Compton 1992; Segar et al. in press). Furthermore, not all the species of fig wasps may be host plant specific, and only use a particular tree intermittently, resulting in some NPFW being rare or highly local even in the native range of their host plants. Consequently, extensive widespread sampling is necessary and the complete fig wasp fauna associated with a single fig tree is not necessarily fixed. *F. microcarpa* is a fig tree with a wide native range covering tropical and sub-tropical areas in Asia-pacific region, so it is not surprising that its figs support a large fig wasp fauna (Compton & Hawkins 1992; Mcleish et al. 2011). The presence of some species in China that were only recorded from planted trees north of the tree's native range, sometimes in large numbers, suggests that they prefer seasonal climates and are sufficiently rare further south for our surveys to fail to detect them. Elsewhere, in Africa, clearly native species have colonized the figs of *F. microcarpa* (van Noort et al. 2013). This is the only area where we failed to detect pollinators in our samples, and their absence may somehow facilitate rare colonization events, an observation of significance for the evolution of the mutualism because it suggests a mechanism for host plant shifts and eventual speciation.

The structure of a fig wasp community reflects both niche partitioning and host shifts (Cook & Segar 2010; Segar et al. 2013). Spatial stratification of galls has been detected among different fig wasp groups, associated with *F. microcarpa*, suggesting some niche partitioning (R. Wang & S.G. Compton, unpublished data). The more abundant fig wasps found in the plant's native range may generally be specific to *F. microcarpa*, although *Walkerella nigrabdomina* Ma & Yang is also associated with *Ficus pisocarpa* (Ma et al. 2013) and molecular data has indicated that *Philotrypes emeryi* Grandi and *P. taiwanensis* also use hosts in figs of *Ficus benjamina*, a species

closely related to *F. microcarpa* (Zhou et al. 2012).

Outside the plant's native range, only 22 species were found, and fig wasp communities were usually much less diverse. Phytophagous fig wasps are more numerous and more widespread than parasitoid NPFW and NPFW phytophages occupied a higher proportion of the figs than in the native range. This is possible because most do not require pollinated figs and so are independent of the pollinators (the seed predator is an exception) and their greater prevalence outside the native range suggests an element of competitor release (there are fewer pollinators colonizing figs) and possibly reduced parasitoid pressure, because parasitoids were less frequent in the plant's introduced range. The contrasting distribution patterns between species feeding at the two trophic levels suggests that there is enemy release in the plant's introduced range, which may contribute to the invasion of *F. microcarpa* if enemy release favours pollinators, or could increase damage to the plant, if phytophagous NPFW are able to reach higher numbers than in the native range, because there are fewer parasitoids present. Generally, we had recorded most fig wasp species that were predicted at sites in both ranges of *F. microcarpa*, suggesting sufficient sample efforts in the present study.

The pollinator and two NPFW phytophages (*O. galili* and *W. microcarpae*) have been recorded widely outside the plant's native range, but no parasitoid is as widely distributed. It therefore seems that species at the lower trophic level are more likely to be the pioneer species, so long as the NPFW are independent of the pollinator. Pollinators often have long-distance movement ability (e.g. Ahmed et al. 2009), but their world-wide spread, and that of the NPFW has clearly been favored by human-mediated transportation (van Noort et al. 2013). The widespread introduction of the three species mirrors their wide distribution in the native range of *F. microcarpa*, suggesting they exhibit wide environmental tolerances that have facilitated their colonization of new areas, which often have relatively harsh and seasonal climates, rather than tropical ones. In addition, being widespread made it more likely that they

would happen to be present in the first figs that were moved overseas, and they will be more likely to be present in figs that are collected and moved between countries now, if the process is continuing. There is also some inter-species facilitation of emergence by females, so these combinations of species benefit from being in shared figs (R. Wang & S.G. Compton, unpublished). Several factors may explain the reduced colonization rates among parasitoid NPFW. The lower abundance of species at the higher trophic level may reduce their chance of being occasionally transferred by human, their very long ovipositors (e.g. Sycoryctinae species) may also to some extent restrict their dispersal ranges, and their particular hosts must already be established.

Combining our present study with previous studies, we detected an apparent ongoing wave of species immigration of both phytophages and parasitoids into areas where the plant is non-indigenous, though the piecemeal sampling in the past makes detailed assessments, especially of the timing of arrivals, difficult. The Greek islands provide an example, where only one species (*O. galili*) was recorded in Symi in the late 1980s (Compton 1989), while 25 years later, five additional species including 3 phytophages and 2 parasitoids are present. Such migration is consistent with the generally increasing number of exotic species around the world, which is tightly linked with globalization and more frequent international trade (Mack et al. 2000; van Noort et al. 2013).

At a global scale, we have reviewed and described the extremely diverse fig wasp fauna associated with *F. microcarpa* figs for the first time. All species however were identified based on their morphological characters, and just like in other fig wasp faunas (e.g. Molbo et al. 2003; Li et al. 2010; Zhou et al. 2012), cryptic species are likely to be detected using molecular data. At present, the only relevant study is Sun et al. (2011), in which some cryptic species of *E. verticillata* had been revealed, but unpublished data shows that there are even more species present (A. Cruaud & J-Y Rasplus, unpublished). Nevertheless, the large pool of NPFW, especially the large

gallers (subfamily *Epichrysomallinae*) and the natural enemies of the pollinator (subfamily *Sycoryctinae*), offer a great chance for selecting biological control agents that can be deliberately used to reduce both male and female reproductive success of their invasive host plant.

Chapter 3 Strong Allee effects among introduced fig wasps in the absence of pollinators, despite inter-specific facilitation

3.1 Abstract

Non-indigenous species are usually vulnerable to Allee effect at early stage of their colonisations due to low population densities. In fig wasps, this Allee effect may result in a certain part of male-free figs, preventing the emergence of females from those figs. *Ficus microcarpa* is a widely-established invasive fig tree from SE Asia. Its pollinator is absent in South Africa, so the tree cannot reproduce, but two non-pollinating fig wasps (NPFW) *Walkerella microcarpae* and *Odontofroggattia galili* occupy its figs. We compared abundance patterns of the two NPFW and proportion of male-free figs in South Africa with Spain, where the pollinator of *F. microcarpa* has arrived, and within/near the tree's native range (south China), where its fig wasp community is much more diverse. The two NPFW were present at lower densities, and there was a far higher proportion of male free figs where female fig wasps were expected to be trapped in South Africa. A clear pattern of Allee effect was revealed that low population density contributed to the failure of female dispersal. In South Africa, strong inter-specific facilitation however helped the escape of most *O. galili* females in the absence of conspecific males, but this phenomenon did not occur on *W. microcarpae*, the dominant species there. Existence of pollinators that are always with males can help release NPFW and thereby maintain the relatively high densities of their populations, while selection pressures in South Africa currently favour greater aggregation of fig wasp offspring in smaller numbers of figs, a preference for shared figs and less female-biased offspring sex ratios.

3.2 Introduction

Species are increasingly dispersing into novel environments, where the mix of species with which they interact may be a sub-set of those within their natural range, or entirely different (Tylianakis et al. 2008). Although most non-indigenous species fail to become established, those that do include a small proportion that eventually becomes economic pests or invasive aliens, sometimes because they have benefited by escaping from their natural enemies (Keane & Crawley 2002). Generalist species are expected to be able to establish more easily in novel environments, because they are not dependent on specific resources or co-habitants, whereas some specialist herbivores, for example, are entirely dependent on the presence of their particular food plant (Richardson et al. 2000). Initially, non-indigenous species are also usually present at low densities, preventing demographic ‘rescue effects’ from adjacent populations and increasing the probability that they will fail to establish long term. Low initial densities may also make introduced species vulnerable to Allee effects.

The Allee effect refers to a causal positive relationship between individual fitness and population density (Tobin et al. 2011). The causes of Allee effects in low-density populations include inbreeding depression, reduced foraging or mating efficiency, reduced resistance to natural enemies and a reduced ability to circumvent host defences (Courchamp et al. 2008), but from the outset, intraspecific cooperation have been seen as mechanisms likely to generate this phenomenon (Allee et al. 1949). Despite density-dependent threat, species may benefit from species facilitation, a positive interaction with other species through mutualism or commensalism, which frequently enables the survival and establishment of non-indigenous species at the early stage of their colonisation (Richardson et al. 2000).

Ficus (Moraceae) is one of the most diverse plant genera in tropical and sub-tropical forests, with over 800 species (Harrison 2005). Its obligate mutualism with pollinating fig wasps (Agaonidae) (Wiebes 1979) means that each fig tree species is dependent for sexual reproduction on one or a small number of host-specific insects

whose larvae develop inside galled ovules within the plants' unique inflorescences - figs. In addition to the pollinators, figs are also exploited by many non-pollinating fig wasp (NPFW) species that can also be host-plant specific. Often several NPFW species share individual figs, where they have a negative effect on their host plants by killing or competing with pollinators and also reducing seed production (Kerdelhue & Rasplus 1996). Many fig tree species have been introduced outside their native range, and when their particular pollinators are also introduced there is the potential for the trees to reproduce, and even become invasive (Starr et al. 2003; Miao et al. 2011).

The unusual breeding site and associated behaviour of pollinating fig wasps makes them heavily rely on their males that are normally responsible for production of the exit holes used by female fig wasps sharing the same figs. This is in contrast to more typical large galls with multiple chambers, where adult male and female wasps independently release themselves through holes they chew themselves (László et al. 2008). The likelihood that there will be no adult male pollinators in a fig is increased by their female-biased sex ratios and the often high mortality rates caused by NPFW. 'Insurance males' may be one response to this problem (Heimpel 1994; Moore et al. 2005), and larval male pollinators are also sometimes located towards the centre of figs, where NPFW impacts are reduced (Yu & Compton 2012).

NPFWs often depend on pollinator males to generate the exit holes that allow them to escape from their natal figs, though males of some NPFW can independently produce their own exit holes. Their effectiveness is reduced however, because NPFW species are often present in low densities within individual figs, inducing Allee effect that makes sex ratios extremely female-biased and males of any one species may be entirely absent (Fellowes et al. 1999). Consequently, female fig wasps that develop in figs that contain no male fig wasps, especially male pollinators, run the risk of remaining trapped in their natal figs, and dying there. The increase of fig wasp species however can reduce the random probability of a fig only with females, facilitating the release of fig wasps via sharing males. In the present study, we therefore hypothesized

that in the absence of the pollinators, females of newly colonised NPFW species with low density are expected to suffer from a high likelihood of being trapped in a fig without any males due to strong Allee effect on sex ratio, but inter-specific facilitation may relieve such a negative impact.

F. microcarpa L. is a monoecious fig tree species with a wide natural distribution that extends from India to China and Australia (Berg & Corner 2005). It has also been introduced into many tropical and subtropical areas around the world, including the Mediterranean and Caribbean, mainland USA and Hawaii, Brazil and South Africa, where they became invasive (Bouček 1988; Nadel et al. 1992; Figueiredo et al. 1995; Kobbi et al. 1996; Beardsley 1998; Burrows & Burrows 2003; Starr et al. 2003; Caughlin et al. 2012). Frugivorous vertebrates, especially birds, are the main dispersers of the tiny seeds, and ants can act as secondary seed dispersal agents (Kaufmann et al. 1991; Nadel et al. 1992; Shanahan et al. 2001). The host-specific pollinators of *F. microcarpa* comprise a species complex currently grouped within *Eupristina verticillata* Waterston (Sun et al. 2011). They actively pollinate the fig flowers while ovipositing in and galling some of the ovules. As with fig wasps in general, a single offspring develops within each galled ovule. Adult male offspring are wingless. After mating inside the figs, they chew an exit hole through the fig wall at the ostiole, which allows their females, and also NPFWs, to emerge.

At least 20 species of NPFW develop inside the figs of *F. microcarpa* (Beardsley 1998; Bouček 1988; Chen et al. 1999; Feng & Huang 2010). Some of these are ovule-gallers that can complete their development in figs that have not been entered by pollinators. Like pollinator females, female NPFWs usually depend upon male fig wasps to chew an exit hole through the ostiole of their natal figs, which allows them to escape and disperse to find figs elsewhere that are suitable for oviposition. Males of some of the NPFW can independently chew these exit-holes, though they may not be efficient at exit-hole production (Suleman et al. 2012). Populations of two such species (*Walkerella microcarpae* Bouček and *Odontofroggattia galili* Wiebes) have been

established in South Africa since at least 2007, although *E. verticillata* is not present (S. van Noort, unpublished). In the absence of pollinator males, females of these two species must depend on NPFW males (both con- and heterospecific) to aid their escape from natal figs, and any females that develop in figs that lack males are likely to remain unmated and die there.

In the present study we hypothesized that: (1) the two NPFW were less abundant and proportion of male-free figs was lower in South Africa than those in Spain (where they have been introduced together with the tree's pollinator) and in southern China (where most area are within or near the plant's native range); (2) Allee effects could result in females failing to emerge from their natal figs; and (3) there were some inter-specific facilitations that mitigate the restriction on female dispersal.

3.3 Materials and methods

3.3.1 Study NPFW species

Walkerella microcarpae Bouček (Pteromalidae, Otitesellinae) is a NPFW with larvae that develop inside galled ovules, like those of the pollinator, but females lay their eggs from the external surface of the figs and fail to pollinate the plant. The species displays strong sexual dimorphism, with fully winged females and wingless males that have large jaws that they use for fighting with each other. *W. microcarpae* can develop successfully in figs that contain no other fig wasp species, with mating taking place within and outside the figs (Figueiredo & Motta 1993). It is only known from figs of *F. microcarpa*. Native range records include mainland China and Papua New Guinea (R. Wang et al., unpublished and S. van Noort, Pers. Comm.), but it has also been introduced into many of the countries where *F. microcarpa* is grown.

Odontofroggattia galili Wiebes (Epichrysomallinae, a sub-family that is currently un-placed taxonomically) has been recorded from figs of *F. microcarpa* and the closely-related *F. prasinicarpa* Elmer and has a wide natural distribution extending

from China to Australia (Bouček 1988). During the last 40 years it has also been recorded from many introduced populations of *F. microcarpa* in countries around the Mediterranean and elsewhere (Galil & Copland 1981; Stange & Knight 1987; Compton 1989). It is a larger species than *W. microcarpae*, but has a generally similar biology and can also develop successfully in figs that lack the other species (Galil & Copland 1981). Both males and females have winged adults, and most mating probably takes place outside their natal figs.

Both *W. microcarpae* and *O. galili* are gall makers and potentially compete with *E. verticillata* for oviposition sites (ovules) and nutrient resources within shared figs, thereby reducing the host plant's male reproductive success (Kobbi et al. 1996). Both species nonetheless tend to occupy more peripheral ovules, rather than those containing *E. verticillata* larvae (S.G. Compton et al., unpublished), and by preventing ovules from developing into seeds may also reduce the female reproductive success of *F. microcarpa*. The males of both NPFW are capable of producing exit holes through the fig wall, thereby allowing conspecific females to escape and disperse. *O. galili* is noticeably larger than *W. microcarpae*, so exit holes produced by the former are clearly suitable for females of the latter species. Whether *O. galili* females can make use of exit holes generated by *W. microcarpae* males is unclear, but to do so the holes will often need to be widened by the females. *O. galili* is expected to be more out-bred than *W. microcarpae*, because most of its matings are outside natal figs and are more likely to be between individuals that developed in different figs.

3.3.2 Sample sites

F. microcarpa is planted as an ornamental tree on roadsides and shopping malls in Port Elisabeth and Grahamstown in South Africa. Port Elisabeth is a coastal city and Grahamstown is approximately 43 km inland (Table 3.1). They are located approximately 130 km apart. They have a semi-arid Mediterranean climate, with

unpredictable rainfall that averages about 460 mm annually. *F. microcarpa* is widely planted on roadsides and in small parks in Palma, on the coast of Majorca (Spain). This is the largest island in the Balearic archipelago and has a typical Mediterranean climate with mild and relatively rainy winters and hot, dry summers. Annual rainfall is about 330 mm. The native range of *F. microcarpa* in southern China includes Xishuangbanna and Guangzhou, but not Kunming. In figs from Kunming, we however detected high species richness of fig wasps similar to that in Xishuangbanna. The trees we sampled in Xishuangbanna were planted in the Xishuangbanna Tropical Botanical Garden, in Kunming we sampled roadside trees and in Guangzhou we sampled a mixture of roadside and parkland trees. Xishuangbanna and Kunming are located in southwest China, about 380 km apart and more than 1000 km from Guangzhou. Xishuangbanna has a tropical monsoonal climate, whereas Kunming is further north and at a higher elevation, resulting in a noticeably milder climate, with colder winters. Annual rainfall levels in Xishuangbanna and Kunming are broadly similar, with about 1200 mm and 1000 mm respectively. Guangzhou is located near the southeast coast of China and has a humid subtropical climate with hot and wet summers, mild, relatively dry winters and an annual rainfall of c. 2000 mm.

Table 3.1 Locations of samples of mature figs of *F. microcarpa*.

Country	Province	City	Co-ordinates	N crops	N figs
South Africa	Eastern Cape	Grahamstown	S 33°56', E 25°34'	7	140
South Africa	Eastern Cape	Port Elizabeth	S 33°19', E 26°31'	5	81
Spain	Majorca	Palma	N 39°38', E 3°02'	6	101
China	Guangdong	Guangzhou	N 23°08', E 113°16'	6	49
China	Yunnan	Kunming	N 25°02', E 102°43'	5	40
China	Yunnan	Xishuangbanna	N 22°01', E 100°48'	12	161

3.3.3 Sampling protocols

We haphazardly collected mature figs without fig wasp exit holes (early D phase *sensu* Galil & Eisikowi (1968) from *F. microcarpa* trees and stored them in 70-100% ethanol (Table 3.1). In August 2012, we also collected fallen figs from the ground below 10 *F. microcarpa* growing in Grahamstown. These figs were divided into quarters and soaked in water for at least 10 minutes to soften the galled ovules before the figs were examined under a dissecting microscope. In all the figs, each flower was examined and sorted into the following categories: male flowers, seeds, unfertilized female flowers, galled ovules containing wasps and empty galls. Empty galls ('bladders') were hollow or only contained the remains of dead larvae. The fig wasps were extracted from their galls and stored in 95% ethanol. They were identified using mainly (Chen et al. 1999; Feng & Huang 2010). We also checked whether the females of either NPFW species can release themselves without the help of males by identifying any adult wasps that were present in the fallen figs collected from Grahamstown and recording gall number and whether an exit hole had been created in those figs. Only figs containing fig wasps were included in the present study.

3.3.4 Statistical analysis

All statistical analyses were carried out using R version 2.14.2 (R Development Core Team 2012). Generalized linear mixed models (GLMM) were implemented in the MCMCglmm package (Hadfield 2012) and assumed a binomial distribution of residuals. *P*-values were then estimated based on the Markov Chain Monte Carlo (MCMC) method with default settings. Linear mixed models (LMM) were run using the lme4 package (Bates et al. 2013) with the maximum likelihood method. Post-hoc comparisons were carried out using 10000 MCMC with the help of the package Language R (Baayen 2011). Multiple tests with Bonferroni correction were used in pair-wise comparisons. Crop was included as a random effect in all analyses involving either of those mixed models. The proportions of female-only figs on the

ground and still on the trees were compared using ANOVA. Dependent variables were transformed where necessary for LMM and ANOVA.

3.4 Results

3.4.1 Fig wasp community

Between December 2010 and August 2012, samples of 250, 221 and 101 mature figs that contained fig wasps were collected from several crops in China, South Africa and Spain respectively (Table 3.1).

The fig wasp faunas in the Chinese *F. microcarpa* figs were diverse, comprising the pollinator (*E. verticillata*), a second, undescribed, *Eupristina* species and a further 19 NPFW species, whilst only four fig wasp species were recorded outside China, its pollinator *E. verticillata* (Spain only) and three NPFW species that gall the ovules: *Micranisa degastris* Chen (Pteromalidae, Otitesellinae) (Spain only), *W. microcarpae* (both countries) and *O. galili* (both countries). Reflecting the contrast in faunal composition among countries, fig wasp species richness within individual figs in China was significantly higher than that in South Africa and Spain, where similar species richness were detected (LMM: South Africa vs. China: $t=8.01$, $p<0.001$; Spain vs. China: $t=5.16$, $p<0.001$; South Africa vs. Spain: $t=1.05$, $p=0.236$; Table 3.2). The fig wasp community in *F. microcarpa* figs was dominated by *E. verticillata* in Spain and China, where it was present in about 70% of the figs and comprised at least 60% of the fig wasp individuals (Table 3.3). In its absence, *W. microcarpae* was the more common species in South Africa, where it occupied about 95% of the figs that contained fig wasps, and comprised 94% of the individual fig wasps, whereas *O. galili* was present in 13% of the figs and contributed the remaining 6% of individuals there (Table 3.3). Many figs in South Africa contained no fig wasps.

Table 3.2 The contents of mature *F. microcarpa* figs (mean \pm SE) in the three sampling countries.

Note that female flower numbers were not recorded for all figs.

Country	Crops	Species richness (N figs)	Fig wasp abundance (N figs)	Female flowers (N figs)	Pollinators prevalence (%) (N figs)	Total male fig wasps (N figs)	Figs with no male fig wasps (%) (N figs)
South Africa	12	1.08 \pm 0.02 (221)	6.5 \pm 0.4 (221)	180.1 \pm 3.3 (181)	0 (221)	1.95 \pm 0.15 (221)	19.9 (221)
Spain	6	1.24 \pm 0.04 (101)	16.1 \pm 1.0 (101)	199.3 \pm 4.0 (101)	69.3 (101)	4.14 \pm 0.35 (78)	2.5 (79)
China	23	2.34 \pm 0.08 (250)	67.7 \pm 2.8 (250)	198.4 \pm 3.4 (250)	80.4 (250)	10.17 \pm 0.58 (250)	0.4 (250)

Table 3.3 Prevalence, abundance (mean \pm SE) and sex ratio of the three major fig wasps in the three sampling countries.

Note that sexes were not assessed in all the figs, so some sample sizes differ from abundance counts. Fig wasp abundance was calculated only for figs where the particular species was present.

Country	Prevalence (%) (N crops)	Fig wasp abundance (N figs)	Sex ratio (N figs)
South Africa			
<i>W. microcarpae</i>	94.4 \pm 2.7 (12)	6.3 \pm 0.4 (211)	0.33 \pm 0.02 (211)
<i>O. galili</i>	12.7 \pm 4.1 (12)	3.3 \pm 0.6 (27)	0.38 \pm 0.07 (27)
Spain			
<i>E. verticillata</i>	69.1 \pm 10.5 (6)	13.9 \pm 1.2 (70)	0.21 \pm 0.01 (66)
<i>W. microcarpae</i>	9.8 \pm 3.3 (6)	9.4 \pm 1.2 (10)	0.37 \pm 0.05 (10)
<i>O. galili</i>	39.7 \pm 13.6 (6)	12.5 \pm 1.3 (40)	0.33 \pm 0.03 (21)
China			
<i>E. verticillata</i>	70.4 \pm 8.5 (23)	70.4 \pm 3.1 (201)	0.12 \pm 0.01 (201)
<i>W. microcarpae</i>	19.5 \pm 5.9 (23)	9.8 \pm 1.8 (34)	0.36 \pm 0.04 (34)
<i>O. galili</i>	11.8 \pm 5.8 (23)	16.6 \pm 2.6 (28)	0.49 \pm 0.06 (28)

3.4.2 Fig wasp density

The numbers of fig wasp individuals (all species combined) within the figs were far higher in the Chinese *F. microcarpa* figs than elsewhere (Table 3.2). Mean numbers of female flowers (potential oviposition sites) within the *F. microcarpa* figs varied from about 180 to 200 (Table 3.2). Less than 5% of the female flowers in the figs from South Africa contained an adult fig wasp (Fig. 3.1), whereas occupancy rates were about 10% in Spain, and over 30% in China (LMM: South Africa vs. China: $t=8.78$, $p<0.001$; Spain vs. China: $t=4.88$, $p<0.001$, South Africa vs. Spain: $t=1.82$, $p=0.010$).

Densities of the three fig wasps (*E. verticillata*, *W. microcarpae* and *O. galili*) within those figs where they were present varied among countries, with the lowest densities in South Africa and the highest in China (Table 3.3). The variation in *W. microcarpae* densities was relatively small, but it was significantly less abundant in the South African figs that it occupied (LMM: South Africa vs. Spain: $t=2.48$, $p=0.012$; South Africa vs. China: $t=2.30$, $p=0.022$), whereas its densities in occupied figs from Spain and China were similar (LMM: Spain vs. China: $t=0.88$, $p=0.378$). Densities of *O. galili* in the figs it occupied were considerably lower in South Africa than elsewhere (LMM: South Africa vs. Spain: $t=4.59$, $p<0.001$; South Africa vs. China: $t=3.12$, $p=0.002$). *O. galili* were equally abundant in figs from China and Spain (LMM: $t=1.15$, $p=0.321$; Table 3.3). Densities of *E. verticillata* in the figs it occupied were also significantly higher in China than Spain, despite the numerous putative parasitoids and competitors sharing the Chinese figs (LMM: $t=4.05$, $p<0.001$; Table 3.3).

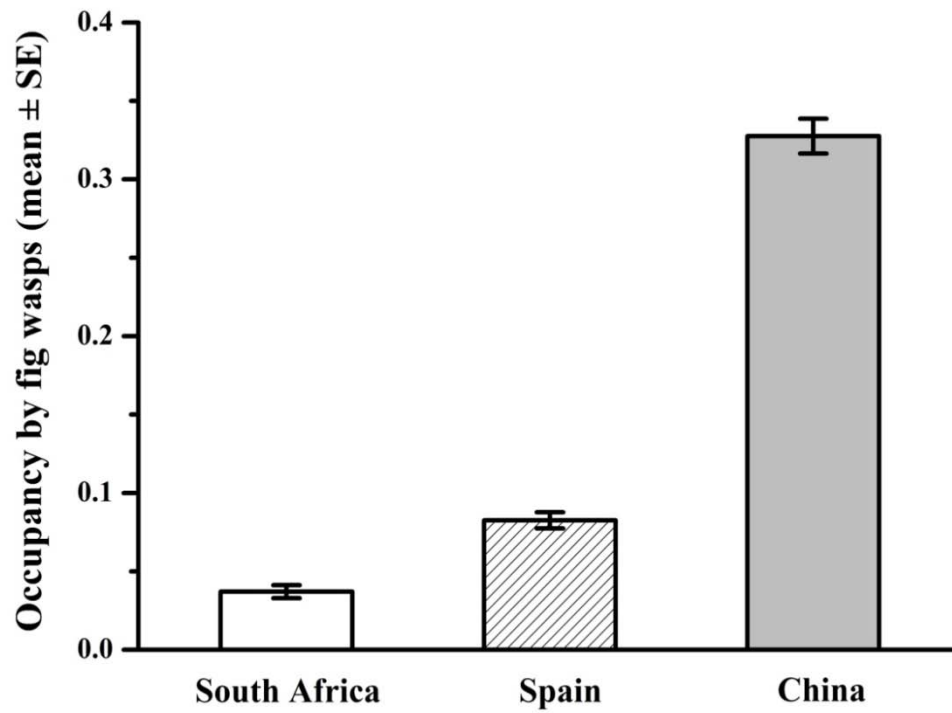


Fig. 3.1 The proportion of female flowers in figs of *F. microcarpa* that contained adult fig wasps (all species combined) in South Africa, Spain and China.

3.4.3 Trapped female fig wasps and male-free figs

The fallen figs collected beneath 10 *F. microcarpa* trees in Grahamstown, South Africa included 148 figs that contained ovules galled by fig wasps. Fig wasps had emerged from most of these fallen figs, but $14.5\% \pm 3.2\%$ and $3.0\% \pm 1.3\%$ (means \pm SE) were male-free figs only with trapped females of *W. microcarpae* and *O. galili* (respectively). These proportions were at the same level with the proportions of figs that contained females of either species but no males of any species (*W. microcarpae*: $18.9\% \pm 7.7\%$; *O. galili*: $1.4\% \pm 1.0\%$) from the 140 figs collected on trees in Grahamstown (ANOVA: *W. microcarpae*: $F=0.35$; $df=1$; $p=0.561$; Kruskal-Wallis test: *O. galili*: $\chi^2=0.47$; $df=1$; $p=0.493$), indicating that females of those two NPFW can't escape from their natal figs without the aid of males.

Only two (2.5%) and one (0.4%) figs without male fig wasps (of any species) were recorded in Spain and China respectively (Table 3.1), while there were 44 (19.9%) male-free figs in South Africa, leading to significant differences in the likelihood that female fig wasps failed to disperse (GLMM: South Africa vs. Spain: $p=0.004$; South Africa vs. China: $p<0.001$).

3.4.4 Allee effect on sex ratio

Generally, there was a significant positive relationship between the probability that a fig contained males of either species and the density of that species (GLMM: *W. microcarpae*: β (mean (95 confidence interval))= 0.602 ($0.571, 0.634$), $p<0.001$; *O. galili*: $\beta=0.672$ ($0.449, 0.842$), $p<0.001$; Fig. 3.2), indicating Allee effect on sex ratio, and the probability of a fig without conspecific males of either species increased rapidly with the decreasing density of that species when the density of that species was lower than 4 (in *O. galili*) and 5 (in *W. microcarpae*) individuals per fig. In neither species, such relationship varied significantly among different countries (GLMM: interaction: *W. microcarpae*: South Africa vs. Spain: $p=0.105$, South Africa vs. China: $p=0.093$, Spain vs. China: $p=0.556$; *O. galili*: South Africa vs. Spain: $p=0.072$, South Africa vs. China: $p=0.223$, Spain vs. China: $p=0.597$). The proportions of figs containing females of either species, but no conspecific males were resultantly the highest in South Africa where population densities of the two NPFW were extremely low, and this phenomenon occurred in very few figs in Spain and China (GLMM: *W. microcarpae*: South Africa vs. Spain: $p=0.204$, South Africa vs. China: $p<0.001$, Spain vs. China: $p=0.175$; *O. galili*:

South Africa vs. Spain: $p=0.004$, South Africa vs. China: $p=0.006$, Spain vs. China: $p=0.948$; Table 3.4).

Table 3.4 Proportions of figs containing *W. microcarpae* or *O. galili* females but no conspecific males and those of figs containing females of either species but no males of any fig wasp species in the three sampling countries.

Countries	Figs with female but no male <i>W. microcarpae</i> (%)	Figs with female but no male <i>O. galili</i> (%)	Figs with female <i>W.</i> <i>microcarpae</i> but no male fig wasps (%)	Figs with female <i>O. galili</i> but no male fig wasps (%)
	(N figs)	(N figs)	(N figs)	(N figs)
South Africa	21.8 (211)	29.6 (27)	20.4 (211)	7.4 (27)
Spain	10.0 (10)	4.8 (21)	0 (10)	0 (21)
China	0 (34)	3.6 (28)	0 (34)	0 (28)

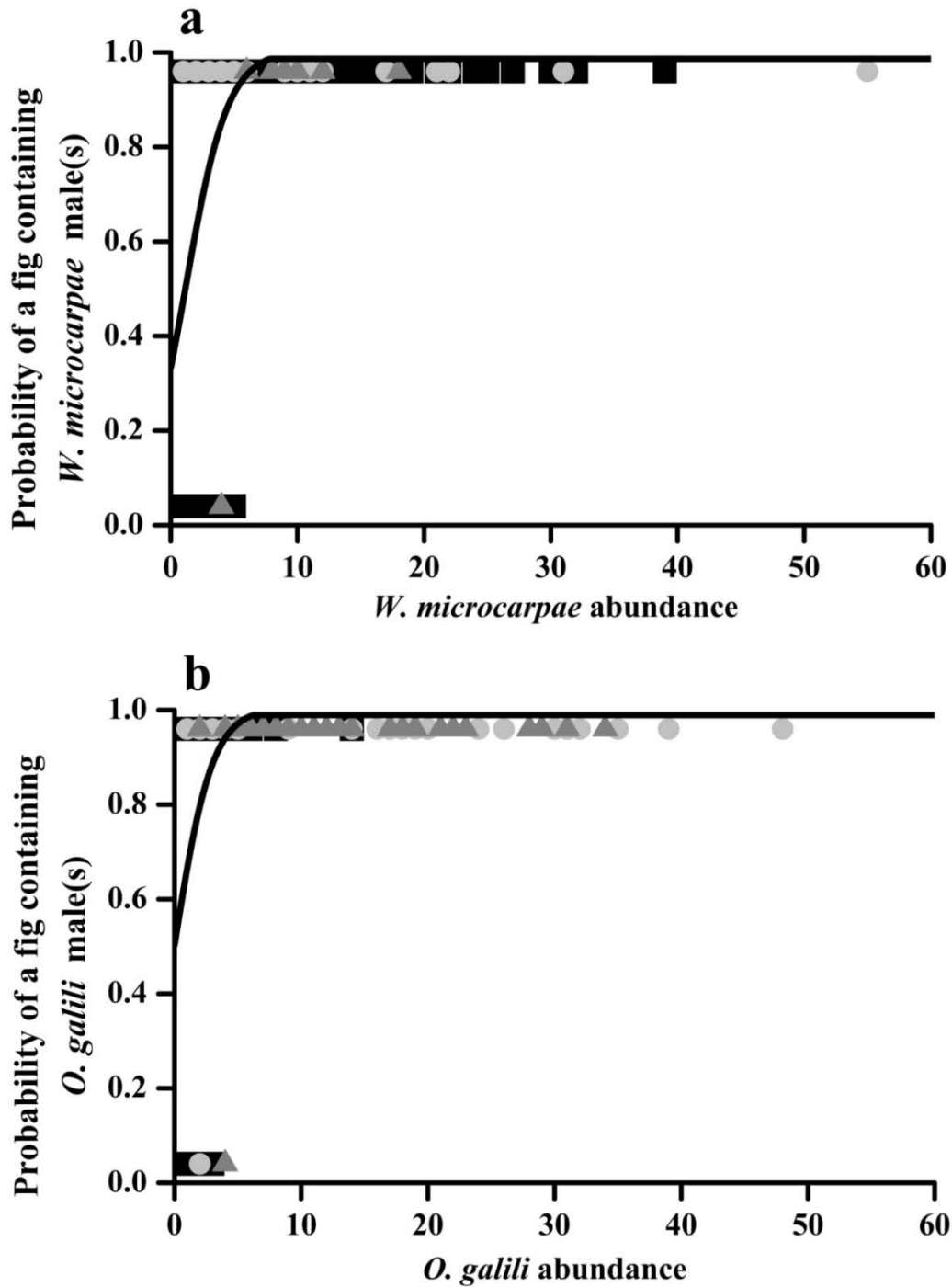


Fig. 3.2 Generalized linear functions reflecting relationships between the probability of a fig containing either *W. microcarpae* (a) or *O. galili* (b) males and the abundance of that species in *F. microcarpa* figs (data from all countries were combined).

Individual figs are represented by black squares (South African figs), grey triangles (Spanish figs) and light grey circles (Chinese figs).

3.4.5 Between-species facilitation

The proportion of figs that contained *W. microcarpae* females but no conspecific males was 21.8% (46 figs), which was very close to the proportion of male-free figs containing that wasp (20.4% (43 figs)) (GLMM: $p=0.666$; Table 3.4). Most figs with *O. galili* females but no conspecific males however contained *W. microcarpae* males, and just 7.4% (only 2 figs) of figs occupied by that species were entirely male-free (Table 3.4). Assuming that *O. galili* females never manage to escape unaided, then in South Africa, the females of this species in 22.2% of the figs they occupied had escaped from their natal figs thanks to the help provided by *W. microcarpae* males – a dramatic example of inter-specific facilitation.

3.5 Discussion

Fig wasps that become established on fig trees planted outside their natural range are utilizing the same host plants as before, but the biological communities inside the figs where their larvae develop are very different. Most obviously, these communities contain fewer species than in their natural range, some of which may be ‘keystone’ species (such as pollinator fig wasps), that have a major influence on the range of resources available within the figs. These differences in community richness and organization mean that colonizing fig wasps are operating under conditions unlike those where they evolved and that aspects of their reproductive strategies may be sub-optimal under these novel conditions.

Here we have focused on the fig wasp community associated with *F. microcarpa*, a fig tree that is of particular interest because it has become an invasive species in several areas of its introduced range. We compared fig wasp communities within/close to its native range with two contrasting areas where the same two NPFW species have also been introduced, one where the pollinator is also present (Spain), and one where the two NPFW are the only occupants of the figs (South Africa). In particular, we wished to determine whether individuals developing in less species-rich communities of non-indigenous fig wasps that are usually at low densities suffer increased mortalities during the period when young adults need to escape from their natal figs.

Density compensation (with species in less-rich communities present at higher densities within the figs they occupy) could potentially have reduced the risk of

developing in male-free *F. microcarpa* figs. Among figs colonized by *W. microcarpae* and *O. galili*, the densities of these individual species were not higher than in the Chinese figs, and given that few or no other fig wasp species were present, this resulted in fig wasps as a whole exploiting far fewer of the female flowers in the figs far from the native range. The lack of density compensation may reflect environmental factors such as unfavourable climatic conditions, e.g. much drier in South Africa and Spain than within their native range, which operate outside the figs to maintain the NPFW populations at low levels.

Inverse density dependence at low densities, where population growth rates decline as density declines, influences the dynamics of many plant and animal populations, especially if they are recent colonists (Lande 1998; Courchamp et al. 1999; Courchamp et al. 2008; Tobin et al. 2011). The situation in South Africa, where NPFW are utilising *F. microcarpa* figs in the absence of the tree's pollinators, provides an unusually clear cut example of this Allee effect, with comparatively low population densities in each fig resulting in a high proportion of figs lacking conspecific males, and finally creating many male-free figs where females failed to disperse, despite relatively large local populations. Loss of plenty progenitive females will definitely, in turn, reduce population growth rate and contribute to the limited population density. Nevertheless, unmated female fig wasps produce only male offspring, and the rarity of figs containing predominantly males of either species suggests that mating failure was uncommon in South Africa, which was consistent with that both NPFW species can mate outside figs, especially for *O. galili* that mates mainly or exclusively on the fig surface. In Spain, the additional presence of pollinator males in most figs meant that a smaller proportion of NPFW females were liable to be trapped in the figs than in South Africa due to the 'insurance males' strategy of the pollinator (Suleman et al. 2012).

It is surprising that fig wasp foundresses only lay eggs containing female offspring in a fig, because normally a foundress should have at least one male offspring (though the overall sex ratio can be extremely female-biased) to avoid strong location mating competition (Hamilton 1979; Herre 1985, 1987). Lack of male offspring has been reported in NPFW (Fellowes et al. 1999), but the underlying mechanisms are still unclear. The possible scenario is likely to reflect long-term interactions between NPFW and pollinators and the outbreeding behaviour of NPFW. In particular, many NPFW species are adapted for co-occurrence with the male pollinators that have been present throughout their co-evolutionary history. These pollinator males create exit

holes for females with which they have mated, but they also help other species. Further, the fully winged males in some NPFW and the actively-walking wingless males of others indicate that they can mate outside of figs, and thus their female do not need to share figs with conspecific males, so long as other males are present. The high frequencies of figs that lacked males in South Africa reflects patterns in NPFW oviposition behaviour and offspring sex ratios that are inappropriate to the ecological situation to which the insects are currently being exposed. Within the introduced and native ranges of the host plant where pollinators are present, pollinators colonise a majority of the figs and effective cooperation among their males help ensure that exit holes will usually be produced (Suleman et al. 2012). Even when pollinators are absent, additional species of NPFW will often be sharing the same figs in their native range, and some of these can contribute males to exit hole construction. The benefits that can be gained from sharing figs with other species were particularly clear in the South African figs, where males of the more common NPFW species routinely facilitated the escape of the other. This facilitation particularly benefited the rarer species (*O. galili*), because its females were much more likely to develop in figs containing *W. microcarpae* males than the reverse situation.

Sex ratios vary widely among wasps in general, as do the clutch sizes laid by females on individual patches (Smart & Mayhew 2009). Optimal oviposition strategies will be different in the introduced and native ranges of the two NPFW, because of the differences in the mix of species sharing the figs and their overall densities. Greater aggregation of offspring within figs will be favoured in the introduced range, because it reduces the chances of female offspring developing in figs that lack males. Some fig wasp parasitoids concentrate their attacks on figs that contain more hosts (Suleman et al. 2013), thereby favouring dispersion of potential host offspring across figs, but this is not a problem for the NPFW of *F. microcarpa* in Spain and South Africa, because parasitoids are absent. Other changes in the oviposition behavior of the two NPFW would also reduce the likelihood that their adult females would die without being able to escape their natal figs. These include less female-biased sex ratios and a preference for sharing figs with other species. Proximate mechanisms that could bring about such changes might include a reduction in antagonism among ovipositing females on the fig surface, a behavior that tends to reduce combined clutch sizes per fig (e.g. Suleman et al. 2012).

Intra-specific variation in traits associated with oviposition decisions are likely to have

an inherited component, and be potentially subject to selection (Charnov & Skinner 1984; Godfray 1994; Greeff 1997). Models (Kanarek & Webb 2010) suggest that adaptations mitigating against inverse density dependent effects among the small, sparse populations typical of founding populations occur at rates that are proportional to the genetic variation present in the populations. Both NPFW are likely to have arrived in South Africa after a series of stepping stones involving several countries, rather than arriving directly from SE Asia. If confirmed, this suggests that adaptations that would reduce Allee effects (Courchamp et al. 2008) by facilitating successful NPFW emergence from the figs of *F. microcarpa* may occur relatively slowly, despite the strong selection pressures that are being generated.

Introductions of insects often fail, even if they are part of an organised biological control programme, and Allee effects can be one of the contributory factors (Fauvergue et al. 2012). Along with the tree's pollinator, *W. microcarpae* and *O. galili* are globally the most widely introduced of the fig wasps associated with *F. microcarpa*. This is unlikely to reflect random selection of species from the twenty plus NPFW associated with its figs, and the ability of their males to allow escape from natal figs where pollinators are absent must have contributed to their success, even if Allee effects and a lack of density compensation mean that they exploit their host figs less effectively than might have been expected.

Chapter 4 Latitudinal variation in a Chinese fig wasp community: correlates and consequences

4.1 Abstract

Latitudinal gradients in species richness and diversity are an all-pervading feature of global biodiversity, with particularly strong gradients at higher trophic levels. Climatic features are generally major correlates of latitudinal gradients, but interactions within communities can make drivers difficult to detect. Here we focus on a community of fig wasps (Chalcidoidea) associated with a single plant resource (ovules of *Ficus microcarpa*) and record changes in faunal composition along a 1200 km transect in south-western China that extends beyond the tree's natural northern range limit. Fig wasp communities include at least two trophic levels, comprising exclusively phytophagous 'gallers' (which include a seed predator and the tree's pollinator) and their parasitoids. A total of 21 fig wasp species were recorded, 12 gallers and 9 parasitoids. The proportion of figs utilized by any fig wasps declined with increasing latitude, as did fig wasp species richness, diversity and abundance. Parasitoids declined more sharply with latitude than gallers, and winter low temperatures were much more strongly correlated with community attributes than summer highs. Lower winter temperatures are likely to act both directly (by excluding cold intolerant species) and indirectly via changes in the fruiting phenology of the host tree. Parasitoids are inherently more sensitive, because their distributions are further limited by declining host populations and the climatic tolerances of their hosts. The plant's poor reproductive success beyond its natural distribution provides an explanation for its northern range margin.

4.2 Introduction

The spatial distributions of species reflect the net effects of numerous historical, geographical, biotic and abiotic elements including speciation, migration, competition, resource availability and climatic tolerances (Gaston 2000; He et al. 2005). Latitudinal gradients are one of the most all-pervading features of species diversity patterns, with many different plant and animal groups containing fewer species at higher latitudes

(Gaston 2000; Willig et al. 2003; Witman et al. 2004; Buckley et al. 2010). Contemporary climatic gradients associated with latitude are generally assumed to be one of the main drivers of biodiversity gradients, because temperatures, precipitation and the extent of seasonal fluctuations in climate all change with latitude (Deutsch et al. 2008; Benton 2009; Feeley et al. 2012; Yasuhara et al. 2012), although phylo-geographic history cannot be ignored (Buckley et al. 2010; Romdal et al. 2013). The distributions of species at higher trophic levels, such as carnivores and parasitoids, often appear to be particularly sensitive to latitudinal effects (Hillebrand 2004; Freestone et al. 2011; Santos & Quicke 2011). Added ecological constraints provide one explanation for their greater sensitivity to latitude because suitable prey may be less abundant at high latitudes (Toscano & Griffen 2013), or entirely absent for parasites and parasitoids with highly specific host requirements (Condamine et al. 2012; Cruaud et al. 2012).

Interactions within complex communities, which may extend over large geographic areas, often make the drivers of latitudinal gradients in species richness difficult to distinguish. Relatively simple, spatially-defined communities associated with a single resource, provide a convenient alternative (Hawkins & Compton 1992; Bannerman et al. 2012). Here we focus on a community of parasitoid and plant-feeding fig wasps (Chalcidoidea) associated with a single plant resource, the figs produced by one species of fig tree in China. The obligate mutualism between fig trees (*Ficus* species, Moraceae) and their pollinating fig wasps (Chalcidoidea, Agaonidae) is highly taxon-specific, with most of the more than 750 species of fig tree species pollinated by one or a small number of fig wasps (Wiebes 1979; Chen et al. 2012; Herre et al. 2008). Related fig trees tend to be pollinated by related fig wasps, suggesting a long history of co-evolution, though some host switching between lineages has also taken place (Cook & Segar 2010; Cruaud et al. 2012). The mutualism is also of broader ecological significance because more species of vertebrates feed on ripe figs than any other fruits, resulting in fig trees and fig wasps often being keystone species, especially in tropical and subtropical forests (Shanahan et al. 2001; Herre et al. 2008). Most fig trees have tropical or sub-tropical distributions, and few species are exclusively temperate. Factors influencing their range margins may be linked to the trees themselves, or reflect limitations imposed by the environmental tolerances of their pollinators (Zhang et al. in press).

Figs are also utilised by other groups of fig wasps belonging to families of Chalcidoidea

other than Agaonidae. More than 30 non-pollinating fig wasps (NPFW) species have been recorded from a single *Ficus* species, but most support less than half this number (Compton & Hawkins 1992; Cook & Rasplus 2003). NPFW generally develop inside galled ovules, like the pollinators, though some species feed on the outer wall of their host figs. Some NPFW are capable of developing in unpollinated figs, which allows them to be independent of the pollinators. The host ranges of most NPFW are poorly known. Some lineages appear highly host plant specific, but others contain species that utilise more than one host plant (Cook & Segar 2010). Most NPFW lay their eggs from the outside of a fig, so they have no opportunity to pollinate, and their eggs are often concentrated in ovules located towards the periphery of the figs (Dunn et al. 2008a). As figs grow, the distance to the ovules from the outside increases, and NPFW that lay into older figs require longer ovipositors (Compton et al. 1994; Yu & Compton 2012). Detailed knowledge of the larval feeding behaviour of NPFW is only available for a tiny number of species, but it is becoming increasingly apparent that NPFW display a diverse range of feeding behaviours which includes seed predators, ovule and fig wall primary gallers, secondary gallers that enlarge the galls of primary gallers, primary parasitoids (most of which also feed on some plant tissue), and specialist hyper-parasitoids (Pereira et al. 2007; Compton et al. 2009; Segar & Cook 2012; Chen et al. 2013). The specific insect hosts attacked within figs by parasitoid NPFW are rarely documented (Cook & Segar 2010; Segar et al. 2013), but niche conservatism induced by morphological characters such as ovipositor length and body size generates some specific matching of parasitoids and gallers, and there is also some evidence for strict sense co-evolution between gall-formers and their specific parasitoids (Compton 1993b; West et al. 1996; Dunn et al. 2008a). Because of this limited knowledge, it is usually only possible to characterise the species within a particular fig wasp community as being either exclusively phytophagous ‘gallers’ (most or all of which are ovule gall-formers) and parasitoids in a broad sense, that kill larvae of other fig wasps and develop in galls that other species had initiated.

Fig wasp communities display convergence and relatively homogeneous structure across continents (Segar et al. 2013; R. Wang & S.G. Compton, unpublished), but also display between site variation in species richness. Latitudinal gradients in the species richness and composition of fig wasp faunas in Southern Africa have been investigated along a gradient extending from 6 degrees North to 34 degrees South (Compton & Hawkins 1992; Hawkins & Compton 1992). These two studies failed to detect

significant latitudinal trends in the species richness of galler NPFW, whereas species richness among parasitoid fig wasps was generally slightly smaller at lower latitudes, but for most species only a small number of crops were available for analysis. Here we focus on geographic variation in the community of fig wasps associated with the figs of a single species of Asian fig tree *Ficus microcarpa*. We recorded the fig wasp communities associated with *F. microcarpa* along a 1200 km roughly North-South transect that extended from within the plant's native range northwards into areas where it is widely planted, but does not become independently established. We recorded the composition of fig wasp fauna, and hypothesized that: (1) fig wasp community varied at different latitudes; (2) climatic factors especially winter low temperatures were correlated with community composition; (3) parasitoids were more sensitive to varying latitude and its associated climate factors than phytophages; and (4) changes in fig wasp community composition along latitudinal gradient may contribute to range boundary determination in *F. microcarpa*.

4.3 Materials and methods

4.3.1 *Ficus microcarpa* and its fig wasps

Ficus microcarpa L. is a monoecious fig tree with a natural distribution that extends from India and southern China to northern Australia (Berg & Corner 2005). As a result of its popularity as a street and ornamental tree, and the widespread introduction of its pollinators, *F. microcarpa* populations have become established in many tropical and subtropical areas, including the Mediterranean and Caribbean, mainland USA and Hawaii and Brazil, but not South Africa, where pollinators are absent (Nadel et al. 1992; Figueiredo et al. 1995; Kobbi et al. 1996; Beardsley 1998; Burrows & Burrows 2003; Starr et al. 2003; van Noort et al. 2013). In urban environments, small plants can cause damage to buildings, but the plant can also become a serious invader of natural habitats (Mckey 1989; Beardsley 1998; Starr et al. 2003; Corlett 2006; Caughlin et al. 2012). The plant's success in seasonal climates may be related to the ability of its pollinators' populations to rapidly recover from winter shortages of figs (Yang et al. 2013). *F. microcarpa* has tiny seeds that are mainly dispersed by frugivorous birds, and ants also serve as secondary dispersal agents (Kaufmann et al. 1991; Shanahan et al. 2001; Caughlin et al. 2012).

In China, *F. microcarpa* is indigenous to south Fujian, Guangdong, Guangxi, Hainan, south Yunnan Provinces and Taiwan. It is also one of the most widely-planted ornamental and street trees in southern China, both within its natural range and extending to around 1200 km north of its natural range margin (Y-Q Peng, unpublished). Within its native area, *F. microcarpa* is an uncommon component of natural forests. It is present at much higher densities in urban and we mainly focused on the fig wasp communities found on planted trees.

At least 30 species of fig wasps have larvae that develop inside the figs of *F. microcarpa* (Bouček 1988; Chen et al. 1999; Feng & Huang 2010; S.G. Compton & R. Wang, unpublished). Most of these fig wasps have *F. microcarpa* as their major or only host plant, but some are also associated with other related fig trees (Zhou et al. 2012; S.G. Compton & R. Wang, unpublished). *F. microcarpa* is pollinated by *Eupristina verticillata* Waterston (Agaonidae), a taxon that molecular data suggest may consist of a complex of morphologically similar species (Sun et al. 2011), but in SW China, only one species has been recorded (J.-Y. Rasplus & A. Cruaud, Pers. Comm.). In addition, *F. microcarpa* is one of the very few species of fig trees that supports an agaonid ‘cheater’ (*Eupristina* sp.) that fails to pollinate its host figs (J.-Y. Rasplus, Pers. Comm.). Adult females of the agaonids enter *F. microcarpa* figs in order to lay their eggs into the ovules via the styles of the flowers. Both agaonids have larvae that develop inside galled ovules and feed exclusively on plant tissue.

All the known fig wasps associated with *F. microcarpa* figs utilise its ovules for larval development. NPFW belonging to families of Chalcidoidea other than Agaonidae lay their eggs into these ovules via the outer wall of the fig, utilising their long or very long ovipositors (Galil & Copland 1981). As with the agaonids, a single larva develops inside each ovule. Parasitoid NPFW may or may not consume some plant tissue, but they always destroy the gall-causers. Some galls of *F. microcarpa* ovules can develop in figs that were not entered by pollinators. They have associated parasitoids that do not attack pollinator larvae (S.G. Compton & R. Wang, unpublished). Generally, species from subfamily Epichrysomallinae (family Pteromalidae) are the hosts of species from family Eurytomidae, and species from subfamily Sycoryctinae (family Pteromalidae) are parasitoids of agaonids and species from subfamily Otitesellinae (family Pteromalidae). *Philotrypesis taiwanensis* Chen (Sycoryctinae) is the only obligate seed predator, whose larvae consume seeds other than galler larvae (R. Wang & S.G. Compton, unpublished). In our analyses we grouped the species associated with *F.*

microcarpa into two trophic levels based on their feeding behavior, ‘gallers’ with larvae that feed exclusively on plant ovules (but including *P. taiwanensis*) - and ‘parasitoids’ with larvae that kill other species.

4.3.2 Study sites

F. microcarpa fig crops were sampled in Mianyang, Chengdu, Xichang and Panzhihua (Sichuan Province), and Kunming and Xishuangbanna (Yunnan Province). They formed a north-south transect across south-western China, covering about 1200 km and 9.5 degrees of latitude. Xishuangbanna is located on the border between subtropical and tropical China, with hot and humid summers and mild, dry winters and is the only study site believed to be within the native range of *F. microcarpa* (Table 4.1). At the other sites, *F. microcarpa* is not present in local natural forests, but has been widely planted in urban areas (Y-Q Peng, Pers. Comm.). Winter and summer temperatures at the sites generally decline with increasing latitude, but Kunming has a cooler climate than the other sites, because of its higher elevation (Table 4.1). Variation in annual precipitation among the study sites is slight, ranging from 850-1100 mm. The trees in Xishuangbanna were growing in a botanic garden. Elsewhere, they were planted along roadsides and in public amenity areas.

Table 4.1 Locations and collected dates of *Ficus microcarpa* fig samples and the proportion of figs that had been colonised by fig wasps.

Study sites are ordered from North to South. Meteorological data from the period 1971-2000 were obtained from the website the website of the China Meteorology Administration.

Study site	Location	Altitude (m)	Annual Precipitation (mm)	Mean monthly Minimum (°C)	Mean monthly Maximum (°C)	Sampling dates	N crops	Crops with fig wasps	N figs	Occupied figs (%) (mean± S.E.)
Mianyang	N 31° 28', E 104 ° 41'	460	927.5	12.9	20.8	July 2012	15	5	491	9.10 ± 4.77
Chengdu	N 30° 40',E 104 ° 06'	500	865.5	13.1	20.3	July 2012	6	3	77	24.37 ± 11.03
Xichang	N 27° 53',E 102 ° 17'	1533	1013.6	12.3	23.1	July 2012	14	12	277	60.62 ± 8.21
Panzhihua	N 26° 35',E 101 ° 43'	1150	849.4	15.5	27.6	July 2012	10	9	155	74.99 ± 9.81
Kunming	N 24° 53',E 102 ° 50'	1891	1011.2	10.3	20.8	Dec. 2010, July 2011	6	5	42	79.63 ± 16.33
Xishuangbanna	N 22° 00',E 100 ° 48'	553	1113.6	18.0	29.6	Dec. 2010, Jan. 2012	12	12	164	98.85 ± 0.82
Overall	--	--	--	--	--	--	63	46	1206	56.27 ± 5.20

4.3.3 Collecting methods

We haphazardly collected mature figs without fig wasp exit holes (late C/early D phase *sensu* (Galil & Eisikowitch 1968)) from at least six *F. microcarpa* trees at each study site, and stored them in 70% ethanol. Figs that are not colonised by fig wasps are retained on the trees for long periods before they abort. They continue to grow and could only be distinguished from figs entered by fig wasps after dissection. Numerous varieties and forms of *F. microcarpa* have been described within its extensive range (Berg & Corner 2005). The trees in SW China are uniform in appearance but could not be assigned to a particular variety. To record the contents of the figs, they were cut into quarters and soaked in water for at least 10 min to soften the galls before the figs were examined under a binocular dissecting microscope. Each flower was checked and recorded in one of the following categories: male flowers, seeds, unfertilized and ungalled female flowers, galls containing wasps and failed galls. Failed galls ('bladders') were hollow or contained the remains of dead fig wasp larvae. All the fig wasps were extracted from their galls and stored in 95% ethanol, and were identified as morphospecies mainly based on (Chen et al. 1999; Feng & Huang 2010). We used the classification of fig wasps according to (Rasplus et al. 1998; Campbell et al. 2000; Cruaud et al. 2010; Heraty et al. 2013) as shown in figweb (<http://www.figweb.org>).

4.3.4 Statistical analyses

All statistical analyses except species accumulation and estimated species richness curves were carried out using R version 2.14.2 (R Development Core Team 2012). Likelihood ratio tests were carried out to assess the significance of fixed effects, and data were transformed to natural logarithms where necessary. The effect of latitude on the presence or absence of fig wasps in the figs was analysed using a Generalized Linear Model (GLM) assuming a quasi-binomial distribution of residuals. Only figs that contained fig wasps were used in further analyses.

We tested whether we had detected most or all of fig wasp species in their regional species pools by delineating curves of accumulated species richness with increasing sample size using a first order jackknife algorithm (Burnham & Overton 1978; Heltshe & Forrester 1983), in SDR version 4.1.2 (Seaby & Henderson 2006).

The effect of latitude on fig wasp floral occupancy rates within figs (the proportion of

female flowers that supported fig wasp adult offspring) was evaluated by a GLM with a quasi-binomial distribution of residuals. The impacts of latitude on female flower number per fig, the numbers of total and female pollinator adult offspring (the plant's male reproductive function) and the numbers of seeds (the plant's female reproductive function) per fig were assessed using GLMs with quasi-Poisson distributions of residuals. The relationships between latitude and fig wasp abundance (total number of fig wasps), species richness and Shannon-Wiener index values per fig were analysed with Linear Models (LMs).

Meteorological data for the period 1971-2000 were obtained from the website of the China Meteorology Administration (Weather China.<http://www.weather.com.cn>). For each study site we collated nine climate factors: annual average temperature and precipitation, summer (three months from June to August) average and average high temperatures, summer average rainfall, winter (three months from December to February) average and average low temperatures, winter average rainfall and the difference between extreme high and low temperatures. We then ran a Principal Component Analysis (PCA) and selected the most important and reciprocally independent climate factors from the first and second components. These were found to be summer average high temperature and winter average low temperature (Tables 4.2 and 4.3 in supplementary materials). The effects of these two climate factors on occupancy rate, fig wasp abundance, species richness and Shannon-Wiener index per fig were tested using the same models as with latitude.

We ran separate GLMs with Quasi-Poisson distributions of residuals to evaluate the effects of latitude, summer high and winter low temperatures on fig wasp species richness (per fig) for gallers, parasitoids and the parasitoid: galler species ratio, and to compare trends of species richness at the two different trophic levels (interactions between trophic level and latitude, summer average high and winter average low temperature).

Table 4.2 The first and second principal components of climate factors using Principal Component Analysis (PCA).

	PC1	PC2
Standard deviation	2.070	1.874
Proportion of Variance	0.476	0.390
Cumulative Proportion	0.476	0.866
Average temperature	-0.453	0.173
Winter average temperature	-0.463	-0.003
Winter average low temperature	-0.480*	0.064
Summer average temperature	-0.218	0.441
Summer average high temperature	-0.259	0.449 *
Extreme temperature gap	0.364	0.341
Annual precipitation	-0.275	-0.402
Winter precipitation	-0.135	-0.342
Summer precipitation	-0.095	-0.419

* the climate factors contributing most to the first and second principal components.

Table 4.3 Pearson correlation coefficients of pair-wise comparisons of different climate factors.

	Winter average temperature	Winter average low temperature	Summer average temperature	Summer average high temperature	Extreme temperature gap	Annual precipitation	Winter precipitation	Summer precipitation (mm)
Average temperature (°C)	0.933**	0.970**	0.660	0.755	-0.515	0.249	-0.004	-0.050
Winter average temperature (°C)	--	0.951**	0.345	0.473	-0.761	0.460	0.139	0.240
Winter average low temperature (°C)	--	--	0.550	0.633	-0.671	0.474	0.195	0.107
Summer average temperature (°C)	--	--	--	0.979***	0.235	-0.283	-0.280	-0.615
Summer average low temperature (°C)	--	--	--	--	0.150	-0.267	-0.370	-0.514
Extreme temperature gap (°C)	--	--	--	--	--	-0.862*	-0.603	-0.629
Annual precipitation (mm)	--	--	--	--	--	--	0.755	0.681
Winter precipitation (mm)	--	--	--	--	--	--	--	0.175

*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$.

4.4 Results

4.4.1 Fig utilization by fig wasps

A total of 1206 mature/aborting figs were dissected from 63 crops, from which only 555 figs from 46 crops contained fig wasps (Table 4.1). More than 60% of the figs from the more southerly areas contained fig wasps, compared with less than 25% of the figs in the northerly Chengdu and Mianyang, where the crops that had no occupied figs were mainly located (Table 4.1). The proportion of figs lacking fig wasps increased significantly at higher latitudes (GLM: $df=1$, $LR=732.180$, $p<0.001$; Fig. 4.1).

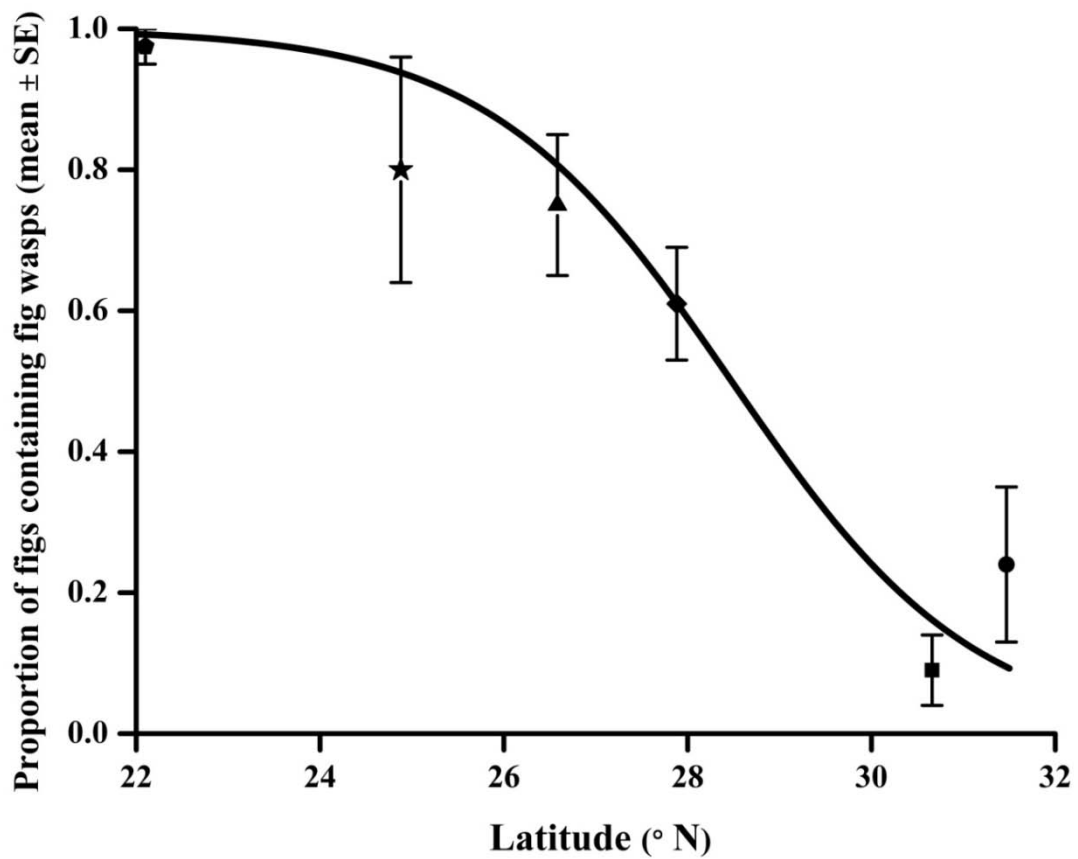


Fig. 4.1 Generalized linear relationship between the proportion of figs containing fig wasps in each crop and the latitudes of study sites.

A Quasi-binomial distribution of residuals was applied. Pentagons, stars, triangles, diamonds, squares and circles indicate Xishuangbanna, Kunming, Panzhihua, Xichang, Chengdu and Mianyang respectively, ordered from south to north.

4.4.2 The contents of figs

At most sites the figs contained around 160-210 female flowers that offered potential oviposition sites for fig wasps or could develop seeds. The figs from Mianyang, the most northerly site, were an exception and contained far fewer flowers of both sexes than figs elsewhere, because a large proportion of the figs there were colonised by *Meselatus bicolor*, a species that galls the figs early in their development and inhibits normal floral development, thereby making fewer flowers available to other species. When figs occupied by *M. bicolor* were excluded, female flower numbers per fig still declined with increasing latitude (LM: excluding figs occupied by *M. bicolor*: slope = -5.737 ± 0.773 , $df=1$, $R=55.15$, $p<0.001$).

Within the figs that had been colonised by fig wasps, occupancy was unusually high in Mianyang, because a majority of the figs containing fig wasps had been colonised by *M. bicolor* (Table 4.4). Elsewhere, floral occupancy was less than 25%, with the exception of Xishuangbanna, the most southerly site, where the figs contained far more fig wasps in total than elsewhere (Table 4.4). Total floral occupancy declined significantly with increasing latitude, a trend that was maintained even when figs that contained *M. bicolor* were excluded (Tables 4.4 and 4.5; Fig. 2 a & b).

Only 24.5% of the 555 figs with fig wasps contained any adult offspring of pollinating agaonids, with pollinator prevalence ranging from zero in Mianyang and Kunming to almost 70% in Xishuangbanna. The mean abundance of pollinator adult offspring in the figs where they were present also varied greatly, from about six at Chengdu to over 32 per fig at Xishuangbanna (Table 4.6). Pollinator sex ratios were female biased, resulting in about four female pollinator offspring per fig in Chengdu and 29 in Xishuangbanna (Table 4.6). The proportion of figs that contained seeds followed the same patterns as with pollinator offspring, with 30% or less of the figs with fig wasps containing seeds, except at Xishuangbanna (Table 4.6). Seed numbers in figs that contained seeds was also highly variable between sites, varying from only about two seeds per fig in Kunming, to around 29 seeds per fig at Xishuangbanna. Total and female *E. verticillata* adult offspring, and seed numbers declined with increasing latitude (GLM: pollinator: $\beta=-0.527 \pm 0.058$, $df=1$, $LR=6093.30$, $p<0.001$; female pollinator: $\beta=-0.563 \pm 0.061$, $df=1$, $LR=5759.10$, $p<0.001$; seeds: $\beta=-0.539 \pm 0.060$, $df=1$, $LR=5409.00$, $p<0.001$).

The cheater *Eupristina* species was found only in the figs from Kunming and Xishuangbanna, where it occupied 12.1% and 82.6% of the figs respectively (Table 4.6).

In a very small number of figs, it was recorded as an ‘accidental’ pollinator, with 50.0% (2 out of 4 figs in Kunming) and 19.6% (9 out of 46 figs in Xishuangbanna) of the figs that contained offspring of this species but not *E. verticillata*, also containing small numbers of seeds.

Table 4.4 The contents of *Ficus microcarpa* figs (Means \pm SE per fig).

Only figs containing fig wasps are included. Occupancy rates are the percentage of female flowers that were occupied by fig wasps. S (obs) = total number of species recorded at the site, S (est) is the estimated total number of species present at the site, based on a first order jack-knife algorithm. H is the Shannon-Wiener diversity index.

Study site	N crops	N figs	Male flowers	Female flowers	Occupancy rates (%)	Fig wasp numbers	Species richness per fig	S (obs)	S (est)	H
Mianyang	5	33	2.6 \pm 1.0	43.0 \pm 6.6	61.6 \pm 4.9	21.1 \pm 2.2	1.09 \pm 0.05	2	2	0.04 \pm 0.03
Chengdu	3	20	15.6 \pm 1.0	166.5 \pm 4.4	6.6 \pm 1.2	10.6 \pm 1.6	1.55 \pm 0.17	5	7	0.24 \pm 0.08
Xichang	12	181	16.7 \pm 0.4	173.1 \pm 3.5	12.7 \pm 1.1	16.7 \pm 0.9	2.02 \pm 0.06	9	9	0.49 \pm 0.03
Panzhihua	9	127	12.4 \pm 0.6	167.9 \pm 6.8	19.4 \pm 2.2	19.0 \pm 1.4	2.13 \pm 0.09	12	13	0.51 \pm 0.04
Kunming	5	33	17.0 \pm 2.0	181.9 \pm 11.5	17.4 \pm 2.8	26.6 \pm 3.6	2.55 \pm 0.16	10	12	0.63 \pm 0.07
Xishuangbanna	12	161	16.3 \pm 0.6	211.1 \pm 3.6	41.4 \pm 1.1	88.6 \pm 3.1	2.75 \pm 0.11	13	14	0.48 \pm 0.03
Overall	46	555	14.8 \pm 0.3	175.0 \pm 2.8	25.5 \pm 1.0	38.7 \pm 1.7	2.22 \pm 0.05	21	22	0.46 \pm 0.02

Table 4.5 Linear and generalized linear models examining aspects of fig wasp community composition in relation to latitude and climatic variables.

LR = likelihood ratio. Only figs that contained fig wasps are included. Response variables are: (1) occupancy rates (the proportion of female flowers that supported fig wasp adult offspring, calculated with or without inclusion of figs that contained *Meselatus bicolor* (MB)), (2) fig wasp abundance (the numbers of wasps present in figs occupied by fig wasps), (3) Shannon-Wiener diversity index (S-W index), (4) fig wasp species richness (SR), (5) galler fig wasp species richness (GSR), (6) parasitoid fig wasp species richness (PSR), (7) parasitoid species:galler species ratios (PPG), (8) the interaction between species richness per fig and trophic level (galler vs. parasitoid species) (SR × Trophic level).

Fixed effect	Response variables	Model	Residuals	Slope/ β (mean \pm SE)	df	LR
Latitude						
	Occupancy rate	GLM	Quasibinomial	-0.290 \pm 0.020	1	9939.10 ^{***}
	Occupancy rate (no MB)	GLM	Quasibinomial	-0.351 \pm 0.015	1	12838.00 ^{***}
	Fig wasp abundance	LM	Normal	-0.245 \pm 0.011	1	471.85 ^{***}
	SR	LM	Normal	-0.043 \pm 0.005	1	86.95 ^{***}
	S-W index	LM	Normal	-0.021 \pm 0.006	1	13.94 ^{***}
	GSR	GLM	QuasiPoisson	-0.051 \pm 0.006	1	21.05 ^{***}
	PSR	GLM	QuasiPoisson	-0.124 \pm 0.020	1	34.94 ^{***}
	PPG	GLM	QuasiPoisson	-0.075 \pm 0.022	1	8.38 ^{***}
	SR \times Trophic level	GLM	QuasiPoisson	--	1	9.50 ^{***}
Winter average low temperature						
	Occupancy rate	GLM	Quasibinomial	0.238 \pm 0.015	1	9657.20 ^{***}
	Occupancy rate (no MB)	GLM	Quasibinomial	0.274 \pm 0.013		11522.00 ^{***}
	Fig wasp abundance	LM	Normal	0.200 \pm 0.009	1	455.43 ^{***}
	SR	LM	Normal	0.029 \pm 0.004	1	48.92 ^{***}
	S-W index	LM	Normal	0.013 \pm 0.005	1	6.77 ^{**}
	GSR	GLM	QuasiPoisson	0.040 \pm 0.006	1	17.48 ^{***}
	PSR	GLM	QuasiPoisson	0.082 \pm 0.017	1	20.60 ^{***}
	PPG	GLM	QuasiPoisson	0.038 \pm 0.019	1	2.93 [*]
	SR \times Trophic level	GLM	QuasiPoisson	--	1	4.21 [*]
Summer average high temperature						
	Occupancy rate	GLM	Quasibinomial	0.256 \pm 0.026	1	3937.60 ^{***}
	Occupancy rate (no MB)	GLM	Quasibinomial	0.260 \pm 0.027		3823.20 ^{***}
	Fig wasp abundance	LM	Normal	0.167 \pm 0.019	1	80.38 ^{***}
	SR	LM	Normal	0.009 \pm 0.007	1	1.97 ^{NS}
	S-W index	LM	Normal	-0.012 \pm 0.008	1	2.31 ^{NS}
	GSR	GLM	QuasiPoisson	0.026 \pm 0.009	1	2.79 ^{**}
	PSR	GLM	QuasiPoisson	0.041 \pm 0.030	1	1.88 ^{NS}
	PPG	GLM	QuasiPoisson	-0.006 \pm 0.031	1	0.02 ^{NS}
	SR \times Trophic level	GLM	QuasiPoisson	--	1	0.19 ^{NS}

^{NS}: not significant, * : p<0.05; ** : p<0.01; *** : p<0.001.

Table 4.6 Prevalence and abundance of agaonids and seeds (only figs that contained fig wasps are considered).

Prevalence is the percentage of figs containing particular species or seeds. Males of pollinators could not be distinguished from those of a non-pollinating ‘cheater’ congener. Estimates assume equal sex ratios in figs where females of both *Eupristina* species were present.

Study site	Pollinator prevalence (%)	Pollinator abundance in figs it occupied (n figs)	Female pollinator abundance in figs it occupied (n figs)	Cheater prevalence (%)	Cheater abundance (n figs)	Seed prevalence (%)	N seeds (n figs)
Mianyang	0	--	--	--	--	0	--
Chengdu	30.0	6.3 ± 1.2 (6)	4.0 ± 0.7 (6)	--	--	30.0	8.2 ± 1.6 (6)
Xichang	1.7	10.0 ± 3.9 (3)	4.0 ± 1.5 (3)	--	--	2.8	6.4 ± 1.0 (5)
Panzhihua	12.6	30.6 ± 4.0 (16)	24.6 ± 5.6 (16)	--	--	11.8	23.9 ± 2.8 (15)
Kunming	0	--	--	12.1	11.3 ± 4.5 (4)	6.1	1.5 ± 0.7 (2)
Xishuangbanna	68.9	32.4 ± 2.9 (111)	29.2 ± 2.9 (111)	82.6	71.0 ± 4.1 (133)	72.7	28.6 ± 3.2 (117)
Overall	24.5	30.6 ± 2.7 (136)	27.0 ± 2.5 (136)	24.7	69.3 ± 4.1 (137)	26.1	26.1 ± 2.8 (145)

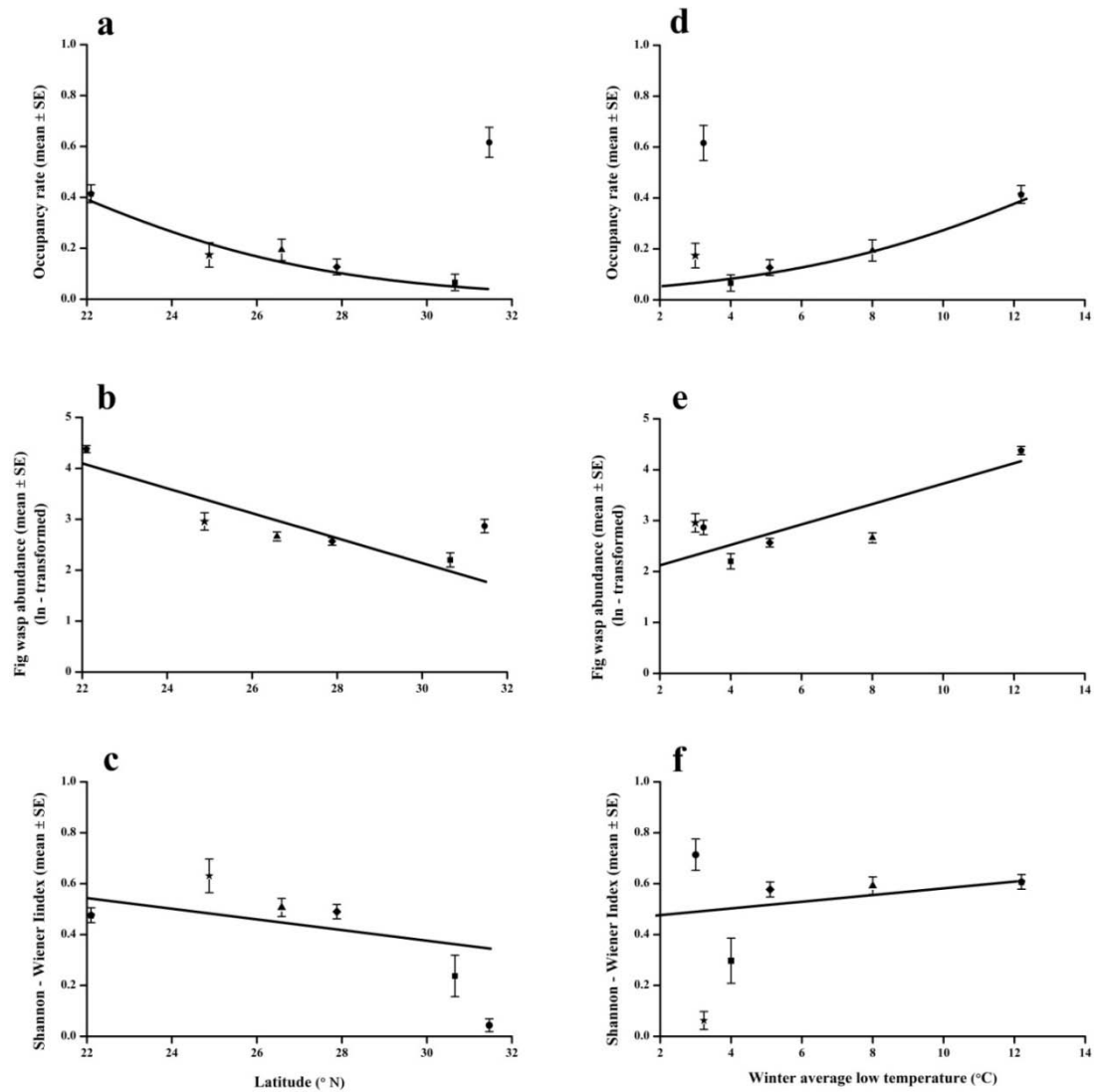


Fig. 4.2 The relationships of *Ficus microcarpa* fig wasp communities with latitude (a-c) and winter average low temperatures (d-f).

Only figs where fig wasps were present are considered. Floral occupancy rates are the proportion of female flowers that contained fig wasp adult offspring. They were analysed using GLM assuming a Quasi-binomial distribution of residuals. Fig wasp abundance is the number of fig wasp adult offspring in figs. Fig wasp abundance and Shannon-Wiener indices were analysed using LM. Pentagons, stars, triangles, diamonds, squares and circles indicate Xishuangbanna, Kunming, Panzhihua, Xichang, Chengdu and Mianyang respectively.

4.4.3 Latitudinal and climatic effects on the fig wasp fauna

Summer average high temperatures and winter average low temperatures at our study sites varied independently of latitude (Pearson's correlation: latitude vs. summer average high temperature: $t=0.105$, $df=4$, $p=0.922$; latitude vs. winter average low temperature: $t=-2.076$, $df=4$, $p=0.107$).

We recorded 21 fig wasp morphospecies from the figs of *F. microcarpa* in SW China (Table 4.7), but no more than 13 species were recorded from any individual site (Tables 4.4 and 4.7). Xishuangbanna had several species that were not recorded elsewhere, but there were also other species that were only recorded at other sites. Although several species of gallers and their associated parasitoids were only recorded at intermediate latitudes, no clearly northern species were present (Table 4.7). Both direct accumulation and first order jackknife methods suggest that regional species richness almost reached asymptotes within our range of sample sizes at every site (Fig. 4.3) and estimates of the size of the regional pools from which our samples were drawn suggest that we had recorded most but not all of the species predicted to be present at each site (Table 4.4).

Xishuangbanna supported the highest fig wasp species richness (Table 4.4). There was a latitudinal shift in the character of the fig wasp communities, with a northwards decline in the abundance of the agaonids and their associated parasitoids and an increasing preponderance of species that make larger galls such as *Meselatus*, *Odontofroggatia* and *Walkerella* species, together with their associated parasitoids (mainly *Sycophila maculafacies* and *Philotrypesis okinavensis*; Table 4.8). Mean species richness per fig declined significantly with latitude (Tables 4.4 and 4.5), but did not exceed three per fig, even at the southerly sites, where many more species were recorded overall.

Diversity, as measured by the Shannon-Wiener index, was highest at intermediate latitudes. In the two most northerly sites, Mianyang and Chengdu, this reflected the low species richness, while in the south, at Xishuangbanna, species richness was high, but many species were rare and offspring of the two *Eupristina* agaonids predominated, occupying over 97% of the figs and comprising over 91% of all the recorded fig wasp individuals (Tables 4.6 and 4.8). Despite this, there was a significant decline in the Shannon-Wiener index with increasing latitude (Table 4.5; Fig. 4.2 c).

Winter average minimum temperatures were positively correlated with ovule occupancy rate, fig wasp abundance, species richness and diversity (Shannon-Wiener index), suggesting it is an aspect of latitude with an important role in shaping the fig wasp fauna (Table 4.5; Fig. 2 d-f). In contrast, summer average maximum temperatures were not correlated with fig wasp community features, though there were positive relationships with occupancy rates and fig wasp abundance (Table 4.5).

Table 4.7 Distributions of fig wasp species in *Ficus microcarpa* figs.

Note that *Philotrypesis taiwanensis* is a seed predator that does not make morphologically distinct galls.

The species abbreviations are: *Ev*, *Eupristina verticillata* Waterston (Pollinator); *Es*, *Eupristina* sp. (cheater); *Aq*, *Acophila quinata* Zhang & Xiao; *Mb*, *Meselatus bicolor* Chen; *Oc*, *Odontofroggatia corneri* Wiebes; *Og*, *Odontofroggatia galili* Wiebes; *Oi*, *Odontofroggatia ishii* Wiebes; *Sbs*, *Sycobia* sp.; *Md*, *Micranisa degastris* Chen; *Wm*, *Walkerella microcarpae* Bouček; *Wn*, *Walkerella nigrabdomina* Ma & Yang; *Pt*, *Philotrypesis taiwanensis* Chen; *Bs*, *Bruchophagus sensoriae* Chen; *Sm*, *Sycophila maculafacies* Chen ('dark'); *Smp*, *Sycophila maculafacies* Chen ('pale'); *Sp*, *Sycophila petiolata* Chen; *Os*, *Ormyrus* sp.; *Pe*, *Philotrypesis emeryi* Grandi; *Po*, *Philotrypesis okinavensis* Ishii; *Srm*, *Sycoryctes moneres* Chen; *Scg*, *Sycoscapter gajimaru* Ishii.

Trophic level	Gallers and seed predator												Parasitoids									
Family (subfamily)	Agaonidae (Agaoninae)		Pteromalidae (Epichrysomallinae)						Pteromalidae (Otitesellinae)		Pteromalidae (Sycoryctinae)		Eurytomidae			Ormyridae		Pteromalidae (Sycoryctinae)				
Morphospecies	<i>Ev</i>	<i>Es</i>	<i>Aq</i>	<i>Mb</i>	<i>Oc</i>	<i>Og</i>	<i>Oi</i>	<i>Sbs</i>	<i>Md</i>	<i>Wm</i>	<i>Wn</i>	<i>Pt</i>	<i>Bs</i>	<i>Sm</i>	<i>Smp</i>	<i>Sp</i>	<i>Os</i>	<i>Pe</i>	<i>Po</i>	<i>Srm</i>	<i>Scg</i>	
Mianyang				√	√																	
Chengdu	√			√	√	√				√												
Xichang	√			√	√	√		√		√					√	√	√					
Panzhuhua	√			√	√	√		√		√		√	√	√	√	√				√		
Kunming		√	√	√	√				√	√			√	√			√				√	
Xishuangbanna	√	√	√		√		√		√	√	√	√		√					√		√	√

√ = present

Table 4.8 The most abundant fig wasp species at each study site. Only figs that contained fig wasps are considered. Sites are ordered from north-south.

Sample sizes are given in Table 4. Males of pollinators were estimated using the sex ratio for total agaonids in figs containing females of both *Eupristina* species.

	Study site	Predominant taxon	Prevalence (% figs occupied)	Abundance in occupied figs Mean \pm SE (n figs)	Relative abundance (% of all fig wasps at the site)
Gallers					
	Mianyang	<i>M. bicolor</i>	93.9	21.9 \pm 2.4 (31)	97.4
	Chengdu	<i>O. galili</i>	65.0	10.3 \pm 2.3 (13)	63.5
	Xichang	<i>O. corneri</i>	66.3	9.6 \pm 0.8 (120)	37.9
	Panzhihua	<i>W. microcarpae</i>	65.4	7.9 \pm 0.9 (83)	27.2
	Kunming	<i>O. corneri</i>	63.6	13.0 \pm 3.6 (21)	31.2
	Xishuangbanna	<i>Eupristina cheater</i>	82.6	71.0 \pm 4.1(133)	66.3
Parasitoids					
	Mianyang	None present			
	Chengdu	None present			
	Xichang	<i>S. maculafacies</i>	30.4	9.4 \pm 0.7 (55)	17.0
	Panzhihua	<i>P. okinavensis</i>	26.8	7.9 \pm 0.7 (34)	11.0
	Kunming	<i>P. okinavensis</i>	45.5	11.4 \pm 3.0 (15)	19.5
	Xishuangbanna	<i>S. gajimaru</i>	31.7	6.7 \pm 4.5 (51)	2.4

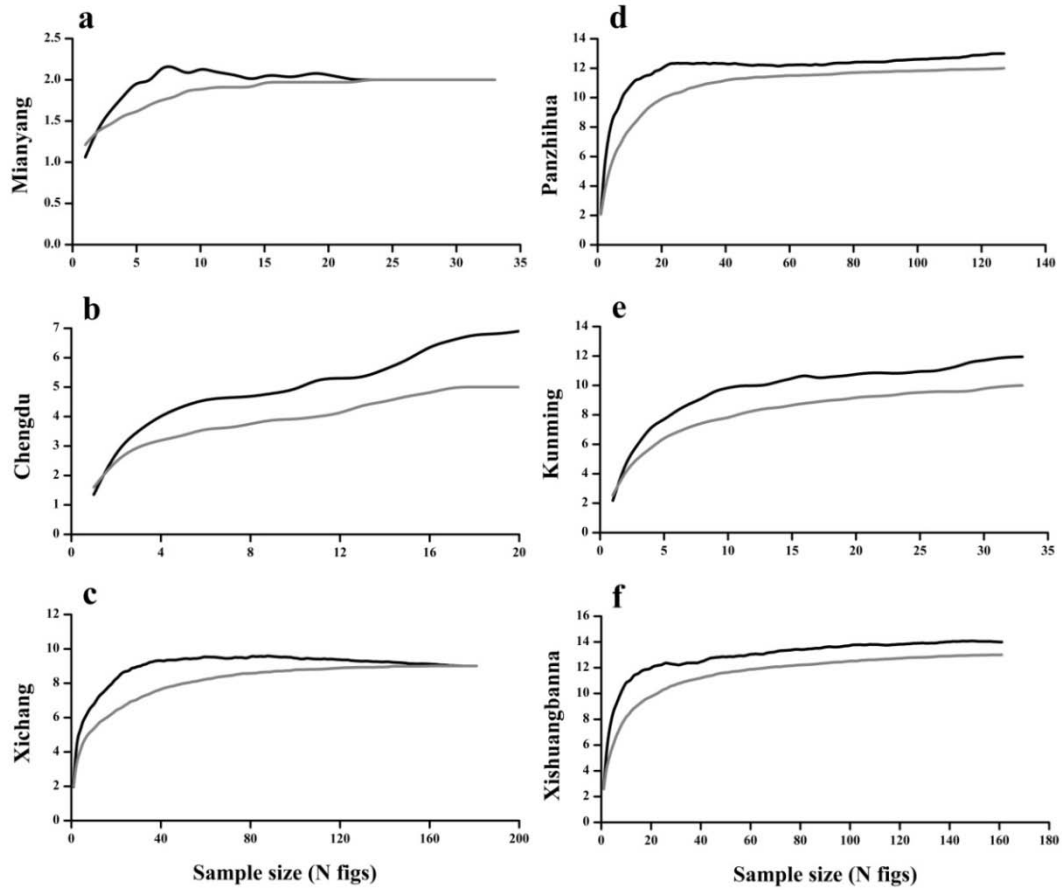


Fig. 4.3 Curves of estimated *Ficus microcarpa* fig wasp regional species richness in relation to sample size at six sites in SW China.

Black lines indicated estimated species accumulation using a first order jack-knife algorithm, while grey lines showed observed values. Note the variation in axis scales.

4.4.4 Comparisons between trophic levels

The overall fig wasp community from the combined six sites included 12 gallers and nine parasitoids (Table 4.7). Generally, offspring of galler species were far more abundant than those of parasitoid species, comprising 90.5% of the total number of fig wasp individuals in the figs (Table 4.9). There was no parasitoid present in the figs from the two most northerly sites.

Figs from higher latitude sites contained significantly fewer species at both trophic levels, but the ratio of parasitoid species to galler species per fig declined significantly with latitude, because parasitoid species declined significantly more rapidly with latitude than galler species (Tables 4.5 and 4.9; Fig. 4.4 a & b). A similar pattern was obtained with the gradient in winter average minimum temperatures (Table 4.5; Fig. 4.4 c & d). In contrast, summer average maximum temperatures only had a significant influence on species richness on galler species level (Table 4.5).

Table 4.9 The species richness and abundance of galler and parasitoid species in *F. microcarpa* figs (only figs that contained fig wasps are considered).

S (obs) = number of species recorded. Means \pm S.E (per fig) are presented. Parasitoid species: galler species ratios per site/per fig: PG per site/per fig. Abundance of gallers and parasitoids was based on all figs occupied by fig wasps, including those where parasitoids were absent.

	S (obs) gallers	S (obs) parasitoids	PG per site	Galler species richness	Parasitoid Species richness	Parasitoid species: galler species ratios	Gallers abundance	Parasitoids abundance
Mianyang	2	0	0	1.09 \pm 0.05	0	0	21.1 \pm 2.2	0
Chengdu	5	0	0	1.55 \pm 0.17	0	0	10.6 \pm 1.6	0
Xichang	6	3	0.50	1.61 \pm 0.05	0.41 \pm 0.04	0.32 \pm 0.03	12.9 \pm 0.8	3.9 \pm 0.5
Panzhihua	7	5	0.71	1.60 \pm 0.06	0.52 \pm 0.07	0.37 \pm 0.05	15.7 \pm 1.3	3.4 \pm 0.5
Kunming	6	4	0.67	1.67 \pm 0.13	0.88 \pm 0.12	0.60 \pm 0.09	17.3 \pm 3.5	7.8 \pm 1.8
Xishuangbanna	9	4	0.44	2.10 \pm 0.07	0.65 \pm 0.06	0.33 \pm 0.04	84.5 \pm 3.2	3.6 \pm 0.5
Overall	12	9	0.75	1.72 \pm 0.03	0.49 \pm 0.03	0.32 \pm 0.02	35.0 \pm 1.7	3.5 \pm 0.3

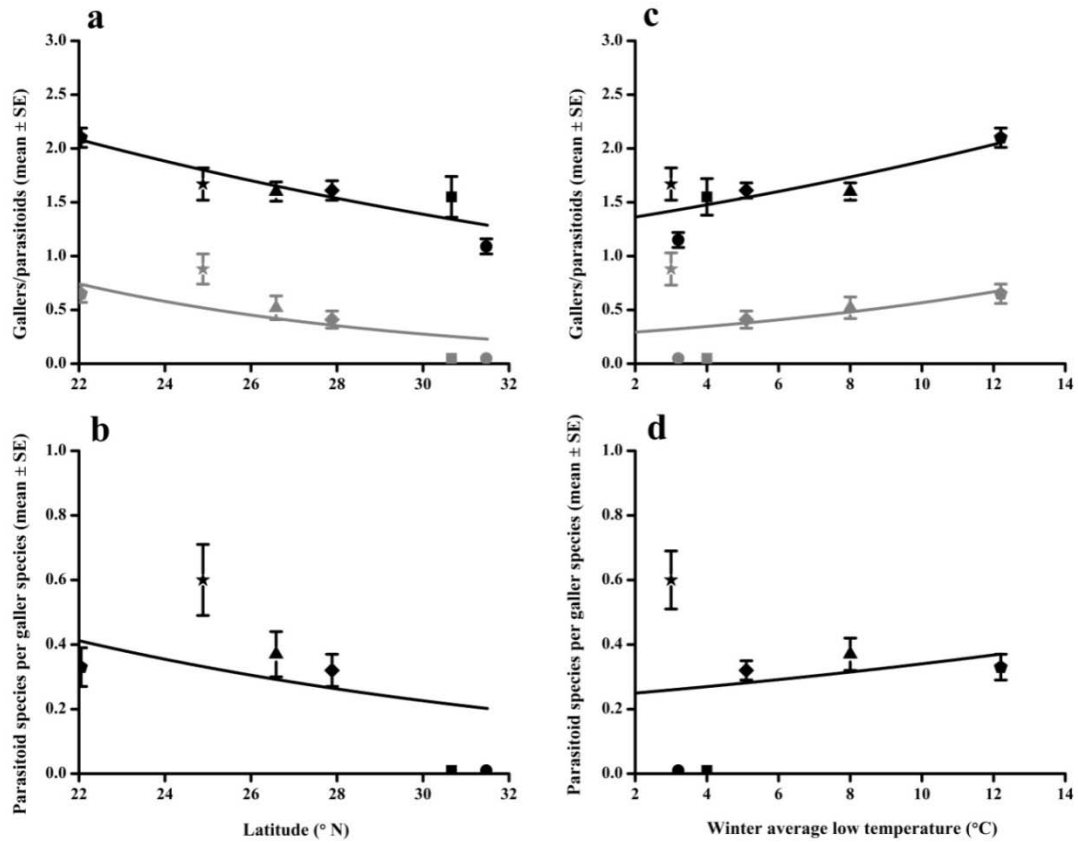


Fig. 4.4 The species numbers of gallers and parasitoids in fig wasp communities associated with *Ficus microcarpa* in relation to latitude (a-b) and winter average low temperatures (c-d).

GLMs assumed Quasi-Poisson distribution of residuals. In the upper figures the black lines are gallers, the grey lines are parasitoids. Exponential curves are indicated. Pentagons, stars, triangles, diamonds, squares and circles indicate Xishuangbanna, Kunming, Panzhihua, Xichang, Chengdu and Mianyang respectively, ordered from south to north.

4.5 Discussion

Most higher taxa of parasitoid hymenopterans display the all-pervasive decline in species richness towards the poles that is typical of insects in general (Hawkins & Compton 1992; Sime & Brower 1998), but this response is not universal and increased species richness among more gall wasp parasitoid assemblages has been reported from Canada (Bannerman et al. 2012). The fig wasp community associated with *F. microcarpa* in SW China changes in species richness and composition with latitude. There are no northern specialist fig wasps, but the extent to which each fig wasp species extends northwards beyond that natural range is variable. Some species also do not extend southwards into the less seasonal tropical forest environment of Xishuangbanna. The fall in species present further north was also reflected in less intense utilization of the floral resources offered by the fig tree. The speed of northwards decline in species richness differed between trophic levels, with parasitoids declining more rapidly than the exclusively phytophagous galls. From the tree's perspective, individuals growing further north increasingly produced figs that were of no reproductive value, because they were seldom or never colonized by pollinators.

Individual figs from *F. microcarpa* trees growing at different latitudes in China are likely to offer essentially identical resources to fig wasps, but the biotic and abiotic environments where the figs are offered are much more variable. Among the abiotic correlates that influence community composition, winter low temperatures, rather than summer high temperatures, appear to be particularly important. Physiological tolerances among the fig wasps are likely to have a significant role in determining the northerly limits of their distribution (Warren et al. 2010). Which stages of the insect's life cycles are particularly temperature sensitive are unclear, but low temperatures will influence larval development times, the ability of the adult offspring wasps to emerge from the figs and their ability to migrate between trees to look for oviposition sites (Yang et al. 2013). Under these circumstances, between-species variation in flexibility

of development rates may be critical. Potential hosts for parasitoids were present at lower densities at more northerly sites, both within individual figs and in terms of the proportion of the figs that contained any fig wasps. It appears that a shortage of hosts, rather than their complete absence, limits the distributions of these species.

Indirect effects via the response of the host tree to cold winter temperatures are also likely to be a major factor. The fruiting phenology of *F. microcarpa* is sensitive to temperature, with fewer figs produced during colder winter months and slower fig development rates, and initiation of new figs stops entirely during the winter at cooler sites (Peng et al. 2010; Yang et al. 2013; Zhang et al. in press). The number of fig tree species also declines regionally in response to latitude in China, so there are fewer opportunities at northerly locations for *F. microcarpa* to recruit fig wasps that also develop on other *Ficus* species (e.g. Zhang et al. in press).

Some postulated reasons for the global declines in animal species richness with latitude, such as a lack of alternative hosts at more northerly sites, can be rejected, because most fig wasps associated with *F. microcarpa* are probably host plant specific. Isolation by distance can influence species distributions (Bannerman et al. 2012), but the more abundant fig wasps associated with *F. microcarpa* are present across much of the extensive indigenous range of the plant (R. Wang & S.G. Compton, unpublished), indicating that limited dispersal is unlikely to restrict their distributions and fig wasps are well known for their extensive dispersal abilities (Ahmed et al. 2009). *F. microcarpa* trees may be present at higher densities in more southerly regions, making dispersal between trees easier, but agaonids and at least some NPFW are extremely effective at finding even isolated host trees (Ahmed et al. 2009), and densities of *F. microcarpa* fig trees in the most species-rich sample site, the botanic gardens at Xishuangbanna, were lower than those at most of the other sites, where rows of planted individuals were present (S.G. Compton, Pers. Comm.). The dispersal abilities of many fig wasps may also be contributing to the observed latitudinal gradient in species richness. If some species go extinct locally each winter at higher latitude sites then there

may be annual rescue effects from populations of these species further south. The direct and indirect effects of colder winter temperature nonetheless remain the most likely drivers of the declines in fig wasps we observed at higher latitudes.

Planted *F. microcarpa* are capable of surviving further north than its natural range limit, suggesting that there are germination and establishment issues that limit the tree's distribution. The extremely low prevalence or entire absence of the pollinator beyond the tree's natural range means that seed production is also severely limited. This results from an inability to sustain pollinator populations. The monoecious fig tree with a natural distribution that extends furthest north in China, *F. virens*, struggles to support its pollinator fig wasp populations through the winter at its northern range limit, where seed production nonetheless appears to be supported due to the migration of pollinators from further south (Zhang et al. in press). *F. virens* is pollinated by a species of *Platyscapa*, a genus where long distance pollinator dispersal has been reported (Harrison & Rasplus 2006). The pollinator of *F. microcarpa* appears to be less mobile.

Chapter 5 First record of an apparently rare fig wasp feeding strategy: obligate seed predation

5.1 Abstract

Fig trees require host specific agaonid fig wasps for pollination, but their figs also support numerous non-pollinating fig wasps (NPFW) that gall fig tissues or develop as parasitoids. *Ficus microcarpa* is widely naturalized outside its native range and the most invasive fig tree species. Seed predators are widely-used for the biological control of invasive plants, but no obligatory seed predatory (as opposed to ovule or fig wall-galling) NPFW have been recorded previously from any fig trees. *Philotrypesis* NPFW are usually regarded as parasitoids or ‘inquilines’ (parasitoids that also eat plant material) of pollinator fig wasps, but here we provide evidence that *Philotrypesis taiwanensis*, a NPFW associated with *F. microcarpa*, is an obligate seed predator: (1) adults emerge from seeds of typical appearance, with a surrounding elaiosome (2) it shows no preference for figs occupied by fig wasp species, other than the pollinator (3) it only develops in figs that contain pollinated ovules, avoiding figs occupied by an agaonid that fails to pollinate (4) larvae are distributed in layers where seeds are concentrated and (5) it has a negative impact on seed but not pollinator offspring numbers. *Philotrypesis* is a large genus, and further species are likely to be seed predators.

5.2 Introduction

Ficus is a largely pantropical genus comprising more than 800 species of fig trees. The genus is characterized by the structure of its inflorescences (figs) and its obligate mutualism with pollinating fig wasps (Hymenoptera, Agaonidae) (Wiebes 1979; Cook & Rasplus 2003; Harrison 2005). Each fig tree species is pollinated by one or a small number of host-specific agaonids (Cruaud et al. 2012). Adult female agaonids enter figs

in order to lay their eggs inside the ovules that line their inner surface. They also carry pollen into the figs and either actively or passively pollinate some of the ovules and gall the ovules where their eggs are laid. After several weeks, the next generation of females mate and then collect pollen before dispersing to receptive figs.

Fig trees are regarded as 'keystone' species in tropical forests because their figs are fed upon by more species of animals than any other tropical fruits (Shanahan et al. 2001; Herre et al. 2008), but after the introduction of their pollinators a small number of species have become naturalized outside their native ranges, where they can become invasive (McKey 1989). Factors seen as favouring rapid alien fig tree establishment and spread include their abundant seed production, good seed viability and extensive seed dispersal (Caughlin et al. 2012; Miao et al. 2012). The ability of fig trees to extend their distributions once pollinator populations become established should be favoured by a relative shortage of specialist fig seed-eating insects within the plants' introduced ranges, together with reduced predation of the pollinators by parasitoids - the 'enemy release hypothesis' (Keane & Crawley 2002; Müller-Schärer & Schaffner 2008; Pearson et al. 2011). Figs support a wide taxonomic range of natural enemies that feed on the figs and their pollinators, including ants, mites, beetles, flies and moths, some of which may have potential as biological control agents (Compton & Robertson 1988; Herre 1993; Miao et al. 2012), but the most prominent and most species-rich group of natural enemies are non-pollinating fig wasps (NPFW).

Fig trees can support up to 30 or more largely host tree-specific NPFW (Compton & Hawkins 1992). Like pollinator fig wasps, their larvae develop inside figs, with one larva developing per gall, but they reduce rather than enhance the reproductive success of their host plants. Most fig wasps utilize the ovules present inside figs for their larval development. Traditionally, the feeding biology of floral-feeding fig wasps (some also develop in the fig wall) has been placed in one of four categories: species that pollinate and gall the ovules, species that gall the ovules, parasitoids of other fig wasps and 'inquilines' that develop in ovules galled by other species, killing and eating their larvae,

but also feeding on plant tissue (Compton & van Noort 1992). Direct investigations of larvae ecology in figs are difficult (Tzeng et al. 2008), but it is increasingly apparent that this simplistic classification is inadequate and misleading and its use is often inconsistent with that used elsewhere (Chen et al. 2013). NPFW that feed in ovules galled by other species can also modify and expand the galls (secondary galls, Chen et al. 2013), and there are also specialist hyper-parasitoids that utilize ovules that had been galled by one species and were then attacked by primary parasitoids (Compton et al. 2009). Finally, there is also a species known to have larvae that are normally found in galled ovules, but small males can also complete their development inside fig seeds (Pereira et al. 2007).

Surprisingly, there are no recorded examples of NPFW species that are obligate seed eaters (feeding only on un-galled, fertilized ovules that would have developed into viable seeds if they had remained un-eaten). The seeds of the Asian fig tree *F. microcarpa* L. are unusual in that they are surrounded by a fleshy outer layer that functions as an elaiosome, attracting ants that help disperse the seeds (Kaufman et al. 1991). One of us (LB) observed that adults of the NPFW *Philotrypesis taiwanensis* Chen (Pteromalidae) were consistently emerging from what appeared to be typical seeds of this species, rather than galled ovules, suggesting that it was a potential seed predator. To test this we hypothesized that: (1) *P. taiwanensis* is independent of any other fig wasp species except the pollinator; (2) it impacts seed production but not pollinator numbers; (3) it is absent from figs that could not have contained seeds; and (4) *P. taiwanensis* larvae are developing in the more peripheral layers within figs, where most flowers develop as seeds.

5.3 Materials and methods

5.3.1 *Ficus microcarpa* and its fig wasps

F. microcarpa grows naturally as a strangler fig tree in tropical and subtropical forests

of SE Asia and Australasia, but is also widely planted in tropical and warm temperate urban areas as an ornamental tree, where it has often become naturalized (Beardsley 1998; Burrows & Burrows 2003; Caughlin et al. 2012). It produces crops of up to several thousand small figs in the leaf axils that reach about 10.1 ± 0.3 mm ($n = 20$ figs) in diameter when mature. As in other fig trees, its sexual reproduction depends on pollination by a host specific agaonid fig wasp. The pollinator is recorded as *Eupristina verticillata* (Agaonidae), but this taxon represents a complex of several closely related and morphologically similar species (Sun et al. 2011) including one species that is a 'cheater' (*Eupristina* sp.) that fails to pollinate the plant (J-Y Rasplus, Pers. Comm.). The cheater species is only recorded from Yunnan Province, China and has not been introduced elsewhere (R. Wang & S.G. Compton, unpublished), whereas pollinators of *F. microcarpa* have been introduced throughout most, but not all, of its introduced range (van Noort et al. 2013). Expansion of *F. microcarpa* populations is linked to extensive seed dispersal by birds (Shanahan et al. 2001). Seeds germinate on buildings and pavements in urban areas, where seedlings can cause architectural damage (Tan & Yeo 2009; Caughlin et al. 2012). Populations can also become established in natural areas and *F. microcarpa* has become invasive in Florida, Hawaii and Bermuda (Kaufmann et al. 1991; Nadel et al. 1992; Beardsley 1998; Starr et al. 2003).

Reflecting the plant's broad natural distribution and monoecious breeding system (Compton & Hawkins 1992), *F. microcarpa* figs are exploited by a large community of fig wasps comprising at least 30 NPFW species (Chen et al. 1999; Feng & Huang 2010; R. Wang & S.G. Compton, unpublished). Unlike agaonids, these NPFW do not enter the figs to lay their eggs. They have larvae that develop inside galled ovules, or are parasitoids of the gall-formers. They include approximately 14 species of putative gallers from subfamilies of Pteromalidae (Epichrysomallinae and Otitesellinae) and 16 species of putative parasitoids (Eurytomidae, Ormyridae and Pteromalidae (Sycoryctinae)) (R. Wang & S.G. Compton, unpublished).

Females of *Philotrypesis* species (Sycoryctinae) are distinguished by having the two

terminal segments of the gaster extended to partially enclose their long ovipositors (Bouček et al. 1981). On the basis of colour they can be separated into species that are all black, or have combinations of yellow or orange and black (Jiang et al. 2006). Males can be winged or flightless, and individuals often display dramatic variation within a single species (Murray 1987; Jousselein et al. 2004). Detailed early studies showed that the European *P. caricae* is a parasitoid of the tree's pollinator that also consumes plant material (Joseph 1957) and in the absence of further information this feeding behaviour has generally been assumed to be typical of the species-rich genus, as well as for sycorytines as a whole (Segar & Cook 2012; Zhou et al. 2012; Suleman et al. 2013). *P. taiwanensis* has black females and exclusively flightless males. It was initially described by Chen et al. (1999) from Taiwan, and has a wide distribution that covers most of the natural range of *F. microcarpa*, including mainland China, the Philippines and Thailand. It has also been introduced into Brazil, Florida, Puerto Rico and Hawaii (Beardsley 1998; Farache *et al.* 2009; R. Wang & S.G. Compton, unpublished). This species may also utilize another fig tree host, *Ficus benjamina* (Zhou et al. 2012). Little is recorded about its biology, other than that females were observed to oviposit into figs that had been pollinated weeks earlier, which suggested that it may be a parasitoid, because gall-forming species usually oviposit earlier (Compton 1993a; Chen et al. 1999).

5.3.2 Fig wasp faunal composition and impact of *P. taiwanensis*

From December 2010 to January 2013, mature *F. microcarpa* figs were collected from trees within the native range of the plant in Guangzhou (Guangdong Province, mainland China), Xishuangbanna (Yunnan Province, mainland China), Taipei (Taiwan) and Bangkok and Kanchanaburi (Thailand). Xishuangbanna, Bangkok and Kanchanaburi have seasonal climates, with distinct wet and dry seasons, whereas Guangzhou and Taipei have less seasonal but more humid subtropical climates.

At least five *F. microcarpa* crops were sampled at each location, with each sample

comprising at least 15 mature figs (late C or early D phases, *sensu* Galil & Eisikowitch (1968)). The total number of crops and figs sampled were 49 and 765 respectively (Table 5.1). The figs were initially stored in 70% ethanol. Each fig was sliced into quarters and soaked in water for more than 10 minutes to soften the galls before the contents of all the fig flowers were identified, using a binocular microscope. The flowers were allocated to the following categories: male flowers, seeds, unfertilized and undeveloped female flowers, galls containing wasps, and empty galls ('bladders'). Fig wasps were identified primarily using Chen et al. (1999) and Feng & Huang (2010), and scored as additional morpho-species where necessary. After fig dissection, figs with *P. taiwanensis* were selected to record the impacts of *P. taiwanensis* on the male and female reproductive successes of each fig. Note that only crops with at least three figs containing *P. taiwanensis* were chosen.

Table 5.1 The contents of *F. microcarpa* figs collected within the native range of the plant and the sub-set of figs with *Philotrypesis taiwanensis* used in the analyses.

E. verticillata was the only *Eupristina* species except at Xishuangbanna, where the ‘cheater’ *Eupristina* sp. was also present. Figs that did not contain agaonids had been colonized by galling NPFW that develop independently.

Study site	Total figs (% figs)				Selected figs (mean ± SE)							
	Crops	Figs	<i>Eupristina</i> species Prevalence (% figs)	<i>P. taiwanensis</i> prevalence	Crops	Figs	Female flowers	Seeds	<i>Eupristina</i> species	<i>P.</i> <i>taiwanensis</i>	Galler NPFW	Parasitoid NPFW
Guangzhou	12	169	75.1	35.5	5	52	136.3 ± 5.2	17.2 ± 2.1	28.6 ± 3.8	6.8 ± 0.8	2.7 ± 0.4	2.6 ± 0.5
Xishuangbanna	16	273	97.4	21.2	9	53	188.2 ± 7.0	28.1 ± 2.6	60.1 ± 6.8	7.2 ± 0.9	3.9 ± 0.7	3.1 ± 0.6
Taipei	11	220	45.5	20.9	3	34	150.1 ± 7.3	21.5 ± 2.3	21.2 ± 2.3	13.7 ± 2.6	3.7 ± 0.8	11.1 ± 2.0
Bangkok	4	40	45.0	17.5	2	7	169.4 ± 28.2	22.3 ± 3.9	30.7 ± 3.2	14.3 ± 6.0	1.3 ± 0.6	0
Kanchanaburi	6	63	68.3	34.9	1	21	203.6 ± 10.1	29.5 ± 3.6	43.6 ± 4.8	16.5 ± 2.4	2.0 ± 0.6	2.1 ± 0.9
Total	49	765	72.4	25.2	20	167	165.4 ± 3.9	23.3 ± 2.3	39.3 ± 5.1	9.8 ± 1.4	3.1 ± 0.6	4.3 ± 1.0

5.3.3 Relationships with agaonids and seeds

F. microcarpa is one of a small number of fig trees that support a non-pollinating ‘cheater’ agaonid (*Eupristina* sp.) in addition to a typical agaonid pollinator. Males of the two species cannot currently be distinguished on the basis of their morphology. Figs entered exclusively by the former hardly contain seeds, and are therefore unsuitable for seed eating species, but figs entered by both species contain galled ovules with agaonid larvae that are a potential resource for parasitoids or inquilines. *Eupristina* sp. was only present in the fig samples from Xishuangbanna, from where the sampled figs were divided according to which of the two agaonid species were present, or whether both species were present. The numbers of seeds, agaonids and *P. taiwanensis* that had developed in the three groups of figs were compared.

5.3.4 Spatial stratification of ovules containing seeds and *P. taiwanensis*

Ovule pedicel lengths provide an indication of where inside a fig that fig wasp larvae are developing, relative to the outer fig wall. Ovules with longer petioles are situated closer to the centre of a fig (Yu & Compton 2012). In many figs, ovules that develop into seeds are concentrated closer to the fig wall than those that support agaonids and their parasitoids, because agaonids preferentially oviposit into shorter-styled flowers, which have longer pedicels (Compton & Nefdt 1990; Dunn et al. 2008a). Figs from two *F. microcarpa* crops from Bangkok and Kanchanaburi contained high densities of *P. taiwanensis*. We recorded the pedicel lengths and contents of all female flowers that developed into either seeds or galls within a total of 26 figs from these two crops. The pedicel lengths were measured to the nearest 0.03 mm under a dissecting microscope using an eyepiece graticule. All the fig wasps inside the galls were then identified, and the female flowers were assigned into five categories: (1) seeds; (2) occupied by pollinators; (3) occupied by *P. taiwanensis*; (4) occupied by other NPFW and (5) empty galls where no larvae completed development (‘bladders’).

5.3.5 Statistical analyses

All statistical analyses were carried out using R 2.14.2 (R Development Core Team 2012). Response variables were square root or natural logarithm transformed where necessary. Likelihood ratio tests and multiple tests with Bonferroni correction were used to estimate the significance of fixed effects and pairwise comparisons respectively.

The effects of *P. taiwanensis* on seed and agaonid numbers (using all native range samples) were tested using LMM (linear mixed models) in R package nlme version 3.1 (Pinheiro et al. 2013), with crop identity as a random effect. This analysis was repeated after excluding data from Xishuangbanna, the only location where the non-pollinating agaonid *Eupristina* sp. was detected.

The effects of the presence/absence of seeds and agaonid identity (*Eupristina* sp. or *E. verticillata*) on the prevalence of *P. taiwanensis* (the proportion of figs where it was present) was tested using a generalized linear mixed model (GLMM) in R package lme4, version 1.0-5 (Bates et al. 2013). Differences in the abundance of *P. taiwanensis* in figs were examined using LMM. Crop identity was a random effect in both analyses. Differences in the pedicel lengths of flowers with different contents (other than bladders) were compared using LMM. We also used GLMM, assuming binomial distribution of residuals, to examine spatial stratification of flower categories by regressing the proportion of female flowers with different contents on pedicel lengths (to investigate the interaction between utilization type and pedicel length). Fig identity was set as a random effect in all analyses.

5.4 Results

5.4.1 Fig wasp community

At the five sites within the native range of *F. microcarpa*, *Eupristina* species were the most prevalent fig wasps (present in the highest proportion of figs) (Table 5.1). There

were twenty-eight NPFW species represented, including ten galler species that belong to subfamilies Epichrysomallinae and Otitesellinae, and eighteen putative parasitoid species in families Eurytomidae, Ormyridae and Pteromalidae, subfamilies Sycoryctinae and Pireninae. *P. taiwanensis* was also common and widespread, and was recorded from 25% of the figs.

A total of 167 figs containing *P. taiwanensis* were analysed from 20 crops where this species was present in three or more figs (Table 5.2). The average numbers of female flowers in the figs varied from less than 140 to over 200, depending on location (Table 5.1). *Eupristina* individuals were found in each fig and were the most numerous species of fig wasp, with *P. taiwanensis* the most abundant NPFW (57% of total NPFW) (Table 5.1). The assemblage of NPFW associated with *P. taiwanensis* was not consistent. *Odontofroggattia galili* (156 individuals, 5.4%) and *Sycoscapter gajimaru* (343 individuals, 11.9%) were the most common galler and parasitoid species but were only present in 35 (21.0% of the 167 figs) and 40 (24.0%) of the figs respectively. We also recorded six galler species including two *Walkerella*, one *Micranisa* and three *Odontofroggattia* species and eight putative parasitoid species comprising two *Philotrypesis*, two *Sycoryctes*, one *Sycoscapter* and three *Sycophila* species, but their prevalence and abundance were low (Table 5.2). Eleven additional NPFW species (one *Acophila*, one *Meselatus*, one *Walkerella*, one *Ormyrus*, four *Philotrypesis*, one *Sirovena* and two *Sycophila* species) were present in the same crops of figs, but absent from figs that contained *P. taiwanensis*.

Table 5.2. Prevalence (proportion of figs occupied) and total abundance of each fig wasp species in the 167 figs that contained *Philotrypesis taiwanensis*.

The full generic name was given only when it was first mentioned and abbreviation was used for other species in the same genus.

Study site	Crop	N figs	<i>Eupristina verticillata</i> complex		<i>Philotrypesis taiwanensis</i>		<i>Micranisa degastris</i>		<i>Odontofroggattia corneri</i>		<i>O. ishii</i>	
			N figs	Abundance	N figs	Abundance	N figs	Abundance	N figs	Abundance	N figs	Abundance
Guangzhou	1	6	6	109	6	27	--	--	--	--	3	5
Guangzhou	2	15	15	198	15	72	2	5	--	--	5	10
Guangzhou	3	4	4	84	4	12	--	--	--	--	2	5
Guangzhou	4	13	13	229	13	123	--	--	1	4	--	--
Guangzhou	5	14	14	859	14	117	--	--	--	--	--	--
Xishuangbanna	6	3	3	179	3	30	1	3	--	--	--	--
Xishuangbanna	7	7	7	367	7	53	--	--	--	--	2	7
Xishuangbanna	8	4	4	293	4	29	2	6	--	--	--	--
Xishuangbanna	9	3	3	369	3	8	--	--	--	--	--	--
Xishuangbanna	10	3	3	171	3	47	1	10	--	--	1	7
Xishuangbanna	11	5	5	829	5	26	--	--	--	--	1	7
Xishuangbanna	12	4	4	320	4	11	1	2	--	--	--	--
Xishuangbanna	13	20	20	622	20	166	--	--	7	29	--	--
Xishuangbanna	14	4	4	74	4	12	--	--	3	10	--	--
Taipei	15	13	13	313	13	289	5	17	1	1	--	--
Taipei	16	15	15	187	15	155	4	10	--	--	--	--
Taipei	17	6	6	221	6	21	1	1	--	--	2	6
Bangkok	18	3	3	88	3	60	1	2	--	--	1	1

Bangkok	19	4	4	127	4	40	--	--	--	--	2	5
Kanchanaburi	20	21	21	915	21	346	4	12	--	--	11	28
Overall	20	167	167	6554	167	1644	22	68	12	44	30	81

<i>O. galili</i>		<i>O. quinifuniculus</i>		<i>Walkerella microcarpae</i>		<i>W. nigrabdomina</i>		<i>Philotrypesis emeryi</i>		<i>P. okinavensis</i>		<i>Sycophila maculafacies</i>	
N figs	Abundance	N figs	Abundance	N figs	Abundance	N figs	Abundance	N figs	Abundance	N figs	Abundance	N figs	Abundance
--	--	--	--	3	7	--	--	--	--	6	18	--	--
3	4	--	--	3	12	--	--	--	--	4	17	--	--
1	2	--	--	--	--	--	--	--	--	1	1	1	1
10	41	--	--	--	--	--	--	--	--	4	8	1	3
8	28	--	--	3	12	--	--	--	--	1	1	--	--
--	--	--	--	--	--	--	--	--	--	--	--	--	--
--	--	--	--	--	--	--	--	1	6	--	--	--	--
--	--	--	--	--	--	--	--	3	15	--	--	--	--
--	--	--	--	--	--	--	--	1	1	--	--	--	--
--	--	--	--	--	--	--	--	--	--	--	--	--	--
--	--	--	--	--	--	1	2	2	11	--	--	--	--
--	--	--	--	--	--	1	2	3	8	--	--	--	--
--	--	--	--	17	84	--	--	3	4	4	10	1	3
--	--	--	--	4	35	--	--	--	--	2	2	--	--
3	9	1	1	--	--	--	--	--	--	--	--	--	--
5	51	--	--	--	--	--	--	1	3	--	--	--	--
5	21	--	--	--	--	--	--	2	4	--	--	5	8
--	--	--	--	--	--	--	--	--	--	--	--	--	--

--	--	--	--	1	1	--	--	--	--	--	--	--	--
--	--	--	--	--	--	--	--	--	--	--	--	2	3
35	156	1	1	31	151	2	4	16	52	22	57	10	18

<i>S. maculafacies</i> (pale)		<i>S. petiolata</i>		<i>Sycoryctes moneres</i>		<i>Sycoryctes</i> sp.2		<i>Sycoscapter gajimaru</i>		<i>Sycoscapter</i> sp.2	
N figs	Abundance	N figs	Abundance	N figs	Abundance	N figs	Abundance	N figs	Abundance	N figs	Abundance
--	--	--	--	3	10	--	--	--	--	--	--
--	--	2	2	4	6	--	--	6	21	--	--
--	--	1	2	--	--	--	--	3	9	--	--
--	--	--	--	4	16	--	--	4	10	--	--
--	--	--	--	3	8	--	--	--	--	--	--
--	--	--	--	--	--	--	--	2	9	--	--
--	--	--	--	--	--	1	9	2	15	--	--
--	--	--	--	--	--	1	4	--	--	--	--
--	--	--	--	--	--	--	--	--	--	--	--
--	--	--	--	--	--	--	--	2	7	--	--
--	--	--	--	--	--	--	--	1	1	--	--
--	--	--	--	--	--	--	--	1	6	--	--
--	--	--	--	5	13	--	--	--	--	--	--
--	--	--	--	2	3	--	--	--	--	--	--
--	--	--	--	2	2	--	--	--	--	--	--
3	9	--	--	7	37	--	--	14	236	3	12
--	--	4	10	1	29	--	--	4	26	--	--
--	--	--	--	--	--	--	--	--	--	--	--
--	--	--	--	--	--	--	--	--	--	--	--

--	--	--	--	7	39	--	--	1	3	--	--
3	9	7	14	38	163	2	13	40	343	3	12

5.4.2 Impacts of *P. taiwanensis*

Among the 167 figs containing *P. taiwanensis*, there was a significant negative relationship between the numbers of this species and the numbers of seeds, but no significant relationship between the numbers of *P. taiwanensis* and agaonids (LMM: seed numbers vs. *P. taiwanensis*: slope= -0.067 ± 0.013 , df=1, LR=26.61, $p < 0.001$; agaonids vs. *P. taiwanensis*: slope= 0.027 ± 0.014 , df=1, LR=3.59, $p = 0.058$; Figs. 5.1 a & b). Similar results were obtained when figs from Xishuangbanna (where cheater agaonids were present) were removed from the analysis (LMM: seed production vs. *P. taiwanensis*: slope= -0.079 ± 0.012 , df=1, LR=38.03, $p < 0.001$; *E. verticillata* vs. *P. taiwanensis*: slope= 0.023 ± 0.014 , df=1, LR=2.75, $p = 0.098$; Figs. 5.1 c & d).

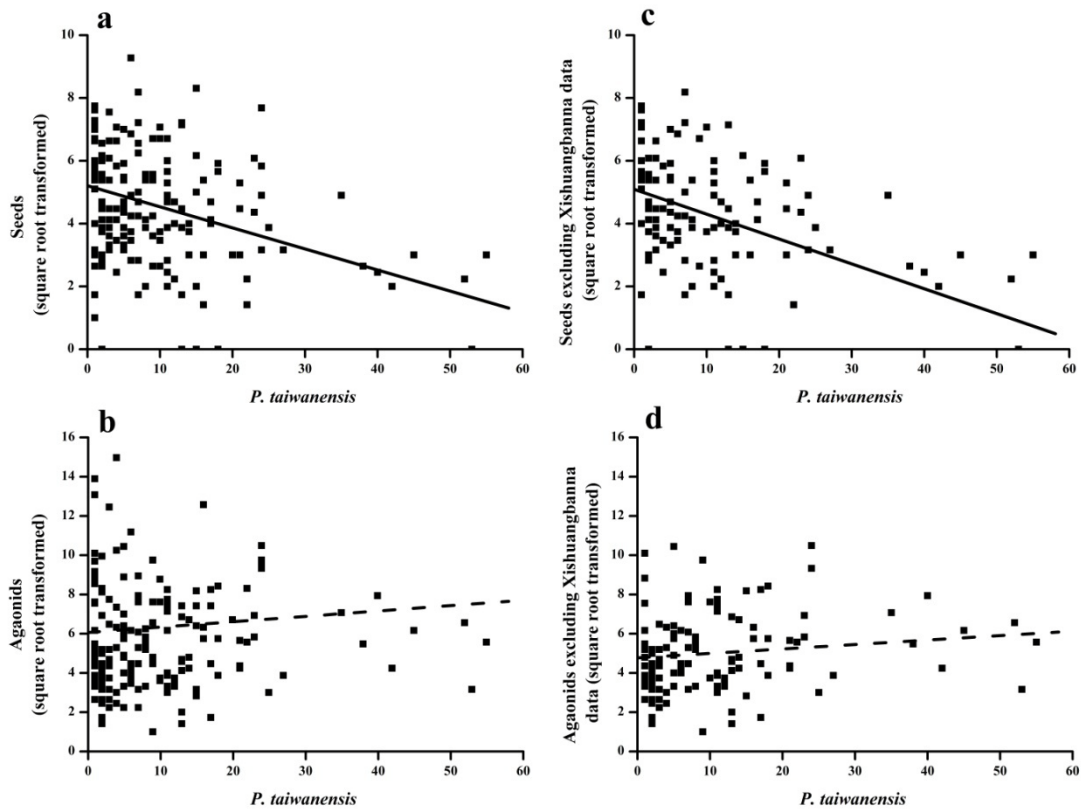


Fig. 5.1 The relationship between numbers of *P. taiwanensis* and seed and agaonid numbers in shared *F. microcarpa* figs. All figs (a & b) or after excluding figs from Xishuangbanna, where the cheater agaonid is present (c & d).

Note that the linear relationships between abundance of agaonids and *P. taiwanensis* (dashed lines) were not significant (LMMs).

5.4.3 Relationships with agaonids and seeds

Agaonid offspring were present in 266 of the figs collected from Xishuangbanna, of which 74, 86 and 106 contained offspring of only pollinators (*E. verticillata*), only cheaters (*Eupristina* sp.) or both species, respectively. Unexpectedly, seeds were recorded in 16 figs (18.6%) that contained no pollinator offspring, though at most only five seeds were present in any one fig – far fewer than in figs entered by the typical pollinator (Table 5.3). Seed numbers in the figs where offspring of both agaonid species were present also contained fewer seeds than figs where pollinator offspring were the only agaonids recorded. Total numbers of agaonid offspring were nonetheless higher in figs where both agaonids were present (Table 5.3).

P. taiwanensis emerged from four figs (6.5%) that lacked unattacked seeds, a prevalence that was significantly lower than that recorded from figs that also contained seeds (26.5%, $n=54$; GLMM: $df=1$, $LR=7.16$, $p=0.007$). Three out of the four figs that contained *P. taiwanensis* offspring but no seeds were occupied by cheater offspring, but no pollinator offspring and the other was occupied by both agaonid species. The abundance of *P. taiwanensis* offspring was also far lower in figs that contained only cheaters, compared with the other two groups of figs (Tables 5.3 and 5.4), but there was no significant difference in *P. taiwanensis* offspring abundance between figs containing only pollinator offspring and those that contained offspring of both agaonids (Tables 5.3 and 5.4).

Table 5.3 The presence of *Philotrypesis taiwanensis* in *F. microcarpa* figs from Xishuangbanna that had either been entered by the pollinator *E. verticillata*, by the ‘cheater’ *Eupristina* sp., or by both species.

Fig wasp abundance was calculated only in the figs where that particular species was present.

Contents	Total figs	<i>Eupristina</i>	Seeds	<i>Philotrypesis taiwanensis</i>	
		species (mean ± SE)	(mean ± SE)	Prevalence (% figs)	(mean ± SE)
Only pollinators	74	50.1 ± 3.7	43.6 ± 3.9	36.0	7.6 ± 1.0
Only cheaters	86	85.3 ± 4.2	0.5 ± 0.1	4.1	1.3 ± 0.3
Both species	106	83.8 ± 3.5	12.4 ± 1.3	22.6	6.5 ± 1.5
Total	266	73.3 ± 2.4	19.2 ± 1.7	21.8	6.8 ± 0.8

Table 5.4 The effects of agaonid species (*E. verticillata* pollinators, *Eupristina* sp. cheaters or a mix of both species) on the prevalence and abundance of *P. taiwanensis* in shared figs (LMMs).

Note that *P. taiwanensis* abundance was calculated only in the figs where it was recorded.

<i>P. taiwanensis</i>	Explanatory variable	Model	df	Likelihood ratio	Pairwise comparison	df	t/z value
Prevalence	Agaonid species	GLMM	1	14.40 ^{***}	Only pollinators vs. Mix	--	-1.437 ^{NS}
					Only pollinators vs. Only cheaters	--	-3.183 ^{**}
					Mix vs. Only cheaters	--	-2.588 [*]
Abundance	Agaonid species	LMM	1	6.15 [*]	Only pollinators vs. Mix	44	-1.59 ^{NS}
					Only pollinators vs. Only cheaters	44	-2.63 [*]
					Mix vs. Only cheaters	44	-2.00 ^{NS}

^{NS}: not significant, ^{*}: p<0.05, ^{**}: p<0.01 and ^{***}: p<0.001.

5.4.4 Spatial stratification of ovules containing seeds and *P. taiwanensis*

The lengths of the pedicels of 1863 female flowers were measured (n=26 figs). Galls containing offspring of *E. verticillata*, *P. taiwanensis*, *Odontofroggatia ishii* and *Sycosapter gajimaru* were present in these figs. Flowers containing pollinator offspring had pedicels 0.39 ± 0.01 mm in length (mean \pm SE, n=778) that were significantly longer than those of flowers that developed seeds (0.31 ± 0.01 mm, n=506). The pedicels of flowers containing *P. taiwanensis* (0.19 ± 0.01 mm, n=230) were significantly shorter than even those containing seeds, but not significantly shorter than those containing all other NPFW combined (0.26 ± 0.03 mm., n=74). The other NPFW occupied flowers with a wide range of pedicel lengths, reflecting between-species diversity, with some species preferring more central flowers and other species preferring more peripheral flowers (Table 5.5; Fig. 5.2 a).

With increasing pedicel lengths, an increasing proportion of female flowers were occupied by *E. verticillata* rather *P. taiwanensis*, other NPFW or seeds, leading to a significant interaction between pedicel lengths and utilization. Agaonid offspring were therefore concentrated in the centre of the figs, with seeds and *P. taiwanensis* concentrated towards the outer wall of the figs (Table 5.5; Fig. 5.2 b). The proportions of female flowers occupied by other NPFW and seeds was independent of pedicel lengths (GLMM: for other NPFW: $z=-1.85$, $p=0.065$; for seeds: $z=-0.61$, $p=0.542$).

Table 5.5 Comparisons of the pedicel lengths of flowers.

Linear mixed models (LMM) and generalized linear models (GLMM) were used to test the differences in pedicel lengths (PL) of female flowers among different contents (UT, i.e. other NPFW, pollinators, *P. taiwanensis* and seeds), and slopes of the proportions of female flowers being utilized (PFFU) by different utilization types (UT) with increasing pedicel lengths.

Region	Response variable	Explanatory variable	Model	df	Likelihood ratio	Pairwise comparison	df	t/z value
Thailand	PL	UT	LMM	3	180.37 ^{***}	other NPFW vs. pollinators	1559	-8.90 ^{***}
						other NPFW vs. <i>P. taiwanensis</i>	1559	-1.66 ^{NS}
						other NPFW vs. seeds	1559	-6.84 ^{***}
						pollinator vs. <i>P. taiwanensis</i>	1559	11.70 ^{***}
						pollinator vs. seeds	1559	4.04 ^{***}
						<i>P. taiwanensis</i> vs. seeds	1559	-7.98 ^{***}
Thailand	PFFU	PL×UT	GLMM	3	203.03 ^{***}	other NPFW vs. pollinators (along with PL)	--	-5.27 ^{***}
						other NPFW vs. <i>P. taiwanensis</i> (along with PL)	--	3.63 ^{**}
						other NPFW vs. seeds (along with PL)	--	-1.52 ^{NS}
						pollinator vs. <i>P. taiwanensis</i> (along with PL)	--	11.30 ^{***}
						pollinator vs. seeds (along with PL)	--	7.64 ^{***}
						<i>P. taiwanensis</i> vs. seeds (along with PL)	--	-6.94 ^{***}

^{NS}: not significant, * : p<0.05, ** : p<0.01 and *** : p<0.001.

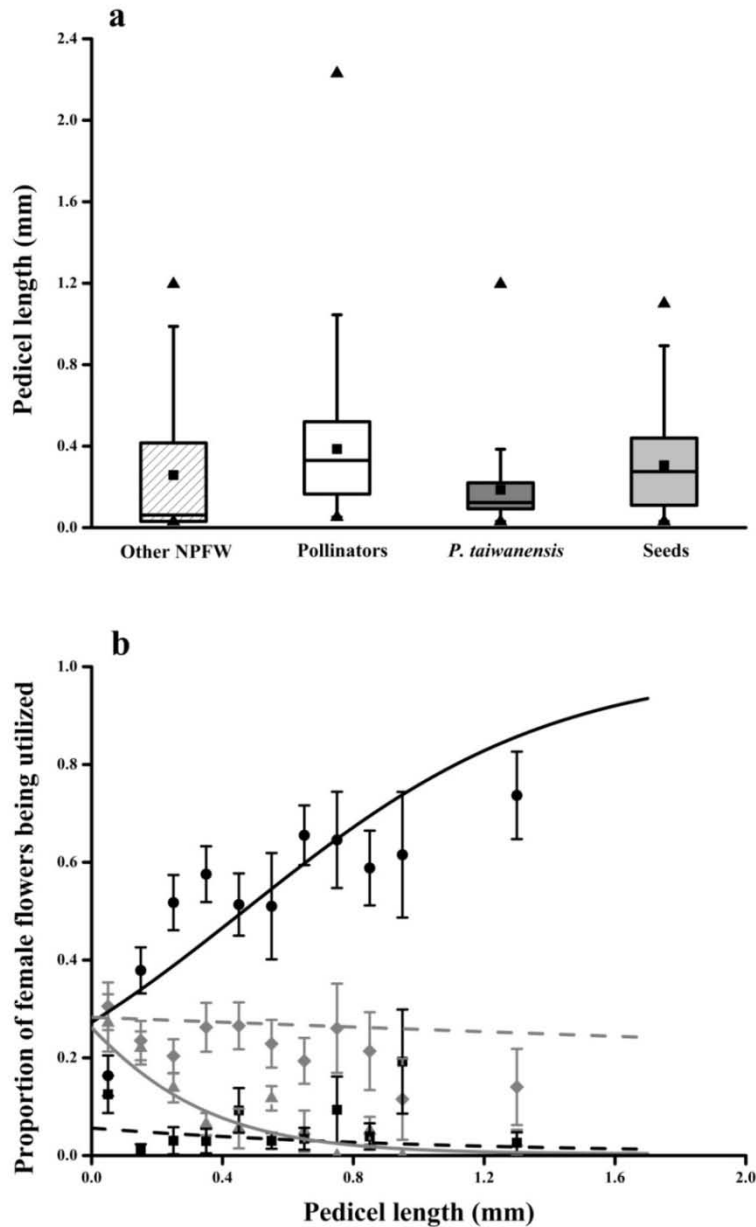


Fig. 5.2 (a) Box-plot for pedicel lengths of female flowers occupied by ‘other’ NPFW, pollinators, *P. taiwanensis* or seeds. (b) The relationship between ovule pedicel lengths and the proportions of the ovules containing other NPFW (black dashed line), agaonids (black line), *P. taiwanensis* (grey line) and seeds (grey dashed line).

(a) Line, box, whiskers, black squares and black triangles represent the median, the range from the first to third quartile, 1.5 times lower and upper quartiles, mean and minimum and maximum values of pedicel lengths in each utilization type. (b) Data (mean \pm SE) are shown for every 0.1 mm in pedicel length except the longest, which includes all data with pedicel lengths longer than 1.00 mm (mean pedicel length for this interval was *c.* 1.3 mm).

5.5 Discussion

Confirmation of the larval feeding behavior of fig wasps is difficult, but several forms of evidence consistently suggest that *P. taiwanensis* has larvae that feed on developing seeds. Adults emerge from ovules that are surrounded by an elaiosome, a feature absent from galled ovules. They come from figs that contain several different species of fig wasps, but display a preference only for figs that contain pollinators, suggesting that they are not parasitoids of NPFW, but are associated with agaonids. Furthermore, they avoided figs containing an agaonid species that fails to pollinate, suggesting that it is the presence of seeds, not agaonids *per se*, that is required. This does not rule out the possibility that *P. taiwanensis* is a highly specific parasitoid of *E. verticillata*, that avoids other *Eupristina* species, but *E. verticillata* galls do not develop an elaiosome around them. *P. taiwanensis* larvae were also distributed towards the periphery of the figs, where seeds (and other NPFW) were abundant, and not towards the centre of the figs, where *E. verticillata* galls are concentrated. Finally, *P. taiwanensis* had a negative impact on seed numbers, but not on pollinator offspring numbers.

A few figs that provided no evidence of pollinator entry nonetheless contained small numbers of seeds. It seems likely that foundresses of *Eupristina* sp., the cheater agaonid, occasionally carry a few pollen grains on their bodies when they enter figs, as was recorded with a cheater agaonid associated with a different species of fig tree (Compton et al. 1991). Alternatively, *E. verticillata* foundresses may also have entered the figs but failed to reproduce, or only unmated *E. verticillata* foundresses had entered them (unmated female foundresses are only able to produce sons, which we could not distinguish, West et al. 1997).

From the host tree's perspective, its female, but not male, reproductive output is reduced by *P. taiwanensis*, which is contrary to the more widespread situation, where NPFW reduce the male reproductive success of figs more than they reduce female reproductive success (Segar & Cook 2012; Suleman et al. 2013). The negative effects of *P. taiwanensis* on seed numbers were substantial, with figs containing 20 or more

offspring of *P. taiwanensis* containing less than half the number of seeds than figs from the same crops where it was absent (and pollinator offspring were present). Experimental addition of *P. taiwanensis* to figs is nevertheless required to accurately assess the impact of this species on seed production. Black *Philotrypesis* species do not form a monophyletic group (Jiang et al. 2006), hence there is reason to assume that other species with this colouration will have a similar biology.

The maximum abundance of *P. taiwanensis* in any one fig was noticeably higher than that achieved by the other *Philotrypesis* species (and other parasitoid NPFW), but similar to that achieved by the more common of the galling species. Host-specific seed predators have had notable success as bio-control agents against invasive plant species (Garren & Strauss 2009; Baraibar et al. 2011), if they can attain high densities (Knoche et al. 2010). *P. taiwanensis* may therefore have potential value as an agent for use against *F. microcarpa* outside its native range. However, we have not identified any parasitoid NPFW that target *P. taiwanensis* (possibly because seed tissue is likely to be chemically distinct from that present in galls, and most parasitoid NPFW also feed on some plant material). This suggests that *P. taiwanensis* will not benefit from an absence of its own natural enemies outside its native range (Keane & Crawley 2002; Müller-Schärer & Schaffner 2008; Pearson et al. 2011), and so is less likely to reach higher densities there. Consistent with this, *P. taiwanensis* has already been accidentally introduced with no notable impact into Brazil, Hawaii and Florida, where *F. microcarpa* has been planted (Beardsley 1998; Farache et al. 2009). Nonetheless, in combination with other NPFW such as *Sycosapter gajimaru* that reduce pollinator numbers and other NPFW that gall ovules, *P. taiwanensis* still has the potential to help reduce the rate of spread of its host plant.

An explanation for the rarity of typical seed predators among fig wasps is related to the constraints acting on fig trees as a result of their mutualistic association with pollinator fig wasps. The male component of fig tree reproduction (pollen donation) depends on the successful development of agaonid larvae inside ovules that the pollinators have

galled (and often pollinated). This limits the extent to which the plant can develop chemical defences to protect its ovules, because any increase in chemical defences is likely to harm the agaonid larvae, as well as other insects that are damaging the seeds. This constraint means that ovules inside figs are likely to be poorly defended, compared with those of other plants. After pollination, developing seeds are under no such constraints, and we hypothesise that levels of defensive compounds rise rapidly at this time, making them a less attractive resource than the tissues present in galled ovules. Constraints are absent throughout ovule development in about half of all fig tree species, because they have two functionally discrete sexes of trees, that specialize in either seed or pollinator production (Greeff & Compton 2002). Female (seed-producing) trees are not constrained by the need to avoid harming pollinator larvae and their ovules are utilized by very few species of NPFW, presumably because they are better defended than ovules in male figs.

Chapter 6 Mediterranean fig wasps and their impact on reproduction in *Ficus microcarpa*, an invasive fig tree

6.1 Abstract

Natural enemies that reduce the ability of plants to reproduce are often utilized for the biological control of invasive species. Reproduction in fig trees depends on host specific fig wasp pollinators that develop in galled ovules, but there are also many species of non-pollinating fig wasps (NPFW) that potentially reduce seed and pollinator numbers. In countries around the Mediterranean, we surveyed the fig wasps associated with a globally invasive fig tree, the Asian *Ficus microcarpa*. Seven NPFW species were recorded. We also compared the impact of the two most prevalent ovule-galling NPFW, the widely-introduced *Odontofroggattia galili* and *Meselatus bicolor*, a species not previously recorded outside its native range, on the tree's reproduction. Both gall-forming NPFW significantly reduced seed and pollinator production, but *M. bicolor* had a far greater impact, often entirely preventing seeds and pollinators from developing in the figs it occupied. *M. bicolor* has only been recorded from *F. microcarpa* and has the potential to be a valuable biological control agent in countries where *F. microcarpa* is invasive.

6.2 Introduction

Classical biological control, where natural enemies from the native range of an invasive species are released into its introduced range, is based on the assumption that a lack of specific natural enemies has allowed the weed or pest to become more abundant (the enemy release hypothesis: Keane & Crawley 2002; Müller-Schärer & Schaffner 2008; Pearson et al. 2011). Reflecting this, biological control programmes normally target species at higher trophic levels than the invasive organisms when selecting agents for

release (van Lenteren 2012). Phytophagous insects are frequently used to control invasive plant species, which represent one of the major threats to global biodiversity (Garren & Strauss 2009; Baraibar et al. 2011). Insects that feed on floral structures and seeds are especially useful where established plants have commercial value, but their reproduction and consequent establishment of their offspring is problematic (Zimmermann & Naser, 1999). This use also highlights that insects that reduce seed production are less likely to be effective against trees and other long-lived invasive species, and without complementary control activities will only have an impact on established plant populations over very long time intervals, or not at all. Conversely, agents that reduce plant sexual reproduction can provide effective control of short-lived plants that do not also reproduce asexually (Navntoft et al. 2009; Wilson et al. 2011), although they need to dramatically reduce seed production (Hill et al. 2000; Knochel et al. 2010).

Fig tree species (*Ficus*, Moraceae) are regarded as 'keystone' species in tropical forests because so many animals feed on their seeds (Shanahan et al. 2001; Herre et al. 2008). The genus contains more than 800 mainly tropical and sub-tropical species and is characterized by its unique inflorescences (figs) and a highly specific relationship with species of pollinating fig wasps (Hymenoptera, Agaonidae) (Wiebes 1979; Cook & Rasplus 2003; Harrison 2005). The majority of fig tree species are each pollinated by females of a single, host-specific species of agaonid (Cruaud et al. 2012). Monoecious fig trees have mutualistic relationships with their pollinators, where fig wasps that enter the figs both pollinate the flowers that line the inside and lay their eggs in some of them, which they also gall. Variation in style lengths results in more centrally located ovules tending to support the development of pollinator larvae, and more peripheral ovules being more likely to develop seeds.

Many fig trees are widely grown as ornamental species outside their native ranges, where they cannot reproduce sexually because they lack their specific pollinators, but a small number of pollinator species have now reached their hosts and potentially allow

the trees to become invasive (Mckey 1989; Caughlin et al. 2012; Miao et al. 2012). A wide variety of insects feed in or on figs and can have a negative impact on fig tree reproduction (Compton & Robertson 1988; Compton and Disney 1991; Herre 1993; Jauharlina et al., 2012; Miao et al. 2012). They include nematodes and mites, ants, beetles, moths and gall midges, but the most frequent non-mutualist occupants of figs are non-pollinating fig wasps (NPFW) belonging to several families of Chalcidoidea. The trophic relationships of NPFW are diverse, but poorly understood. They are traditionally classified as gallers, inquilines (kleptoparasites) and parasitoids (Kerdelhue et al. 2000; Compton et al. 2009; Cook & Segar 2010) but their biologies are proving to be more diverse than previously realized (Chen et al. 2013; van Noort et al. 2013). From their host plant's perspective, NPFW can be seen as reducing male reproductive success (by reducing numbers of pollen-carrying pollinator females), female reproductive success (seed production) or both. A negative impact of parasitoids on their pollinator hosts has been frequently reported, but their effects have also been seen as helping stabilize the mutualism, because they preferentially attack more peripheral galls, thereby favouring pollinators that lay their eggs more centrally and leave more peripheral ovules to develop into seeds (Dunn et al. 2008; Segar & Cook 2012; Yu & Compton 2012; Suleman et al. 2013). Obligate seed predatory NPFW appear to be very rare, but may be under-reported (Wang et al. in press a). Gall-forming NPFW can restrict both reproductive functions because they compete with pollinators for oviposition sites and occupy flowers that might have developed seeds (Kobbi et al. 1996). NPFW galls that develop quickly can also limit or prevent pollinator females from entering figs, and there may be competition for nutrients within figs containing galls of different species. NPFW can clearly reduce the reproductive success of fig trees and have the potential to act as biological control agents of invasive fig tree species. *Ficus microcarpa* L.f. is the most invasive species of fig tree. Here we describe which of its associated fig wasps have been introduced to the Mediterranean area and record the impact of two gall-forming NPFW on the tree's reproduction. *Odontofroggattia*

galili Wiebes is found almost everywhere that *F. microcarpa* is planted and was the first species reported from the Mediterranean (Galil and Copland 1991). It has been shown previously to reduce seed and pollinator numbers (Kobbi et al. 1996), but fails to prevent *F. microcarpa* from becoming invasive. *Meselatus bicolor* Chen appears to have only recently been introduced to the Mediterranean, and still has a limited distribution, but initial observations suggested that it may have a greater impact on the plant than *O. galili*. We first describe the distribution and abundance of the fig wasps associated with *F. microcarpa* in the Mediterranean area, and then hypothesized that *M. bicolor* had much greater effects on seed and pollinator production than *O. galili* because of its superior ability in nutrition acquisition.

6.3 Materials and methods

6.3.1 The tree and its associated fig wasps

F. microcarpa (the Malay banyan or Indian laurel) has been referred to previously as *F. nitida* and *F. retusa* (Berg and Corner 2005). It has a broad natural range in tropical and sub-tropical forests from India to Australia where it grows as a hemiepiphytic strangler of other trees, or directly from rocks. The figs (syconia) are small, produced in largely synchronized crops among the leaves and ripen to pink or black (Berg and Corner 2005). Development of the figs usually takes 4-8 weeks, depending on temperature (Yang et al. 2013). Frugivorous animals such as birds are mainly responsible for the primary dispersal of its seeds, with ants acting as secondary seed dispersal agents (Kaufmann et al. 1991; Shanahan et al. 2001). *F. microcarpa* has been widely planted outside its native range in streets, parks and gardens, in climates ranging from the humid tropics to strongly seasonal and semi-arid (Nadel et al. 1992; Figueiredo et al. 1995; Kobbi et al. 1996; Beardsley 1998; Starr et al. 2003; Berg & Corner 2005; van Noort et al. 2013). It is also salt tolerant and is widely-planted in coastal areas (Figueiredo et al. 1995; Kobbi et al. 1996; Beardsley 1998; van Noort et al. 2013). Where the tree's pollinator is also

introduced it has become invasive in Hawaii, Florida and Bermuda (Nadel et al. 1992; Beardsley 1998; Starr et al. 2003; Caughlin et al. 2012). The tree also sets seed around the Mediterranean, and some establishment in natural areas is reported, but it is mainly regarded as an urban nuisance because its seedlings damage walls and buildings.

Eupristina verticillata Waterston is the recorded pollinator of *F. microcarpa*, but this taxon may represent a complex of closely-related cryptic species (Sun et al. 2011). At least 42 NPFW species have also been reared from *F. microcarpa* figs (Chen et al. 1999; Feng & Huang 2010; Li et al. 2013, R. Wang & SG Compton, unpublished data). They include ovule gallers, parasitoids, and *Philotrypesis taiwanensis* Chen, a seed predator (Wang et al. in press a). The ovule galler *Odontofroggattia galili* Wiebes (Pteromalidae, Epichrysomallinae) is one of the two most widely introduced NPFW associated with *F. microcarpa* (the other is *Walkerella microcarpae* Bouček (Pteromalidae, Otitesellinae)). It has been introduced to most parts of the world where *F. microcarpa* is grown, including the Americas, Europe, Middle East and Pacific (Galil and Copland 1991; Bouček 1993; Compton, 1993; Beardsley 1998), and also South Africa, despite an absence of the tree's pollinator (van Noort et al. 2013). *F. microcarpa* is likely to be the only host plant of *O. galili* though there is an unconfirmed record from another *Ficus* species (Bouček 1988). *Sycophila* (Eurytomidae) are parasitoids of *Odontofroggattia* species in *F. microcarpa* figs. Asian *Sycophila* have been introduced to Florida and elsewhere (Beardsley 1998, R. Wang unpublished) and native African *Sycophila* species have also colonized *O. galili* in South Africa (van Noort et al. 2013). *F. microcarpa* was introduced around the Mediterranean over the course of the last two centuries (Mifsud et al. 2012). The pollinator of *F. microcarpa* has probably been in the Mediterranean area since at least the 1980s, allowing the plant to reproduce and colonise both urban and rural areas (Lo Verde et al. 1991; Kobbi et al. 1996; Doğanlar 2012; Mifsud et al. 2012). The first of its associated NPFW (*Odontofroggattia galili*) was recorded from Israel (Galil and Copland 1981) and then the Greek Isles (Compton 1989). Kobbi et al. 1996 subsequently recorded *O. galili*, a second ovule-galler

Walkerella microcarpae and the pollinator, from Tunisia. More recently, three further NPFW have been recorded from *F. microcarpa* figs in the Mediterranean area: *Odontofroggatia ishii* Wiebes, *Philotrypesis emeryi* Grandi and *Philotrypesis taiwanensis* Chen (Pteromalidae, Sycoryctinae) (Lo Verde & Porcelli 2010; Doğanlar 2012). *Odontofroggatia* species are ovule-gallers and subject to attack by *Sycophila* parasitoids. *P. emeryi* is a parasitoid, and *P. taiwanensis* is a seed predator (Wang et al. in press a). Mifsud et al. (2012) have also recorded the leaf-galling NPFW *J. microcarpae* from Malta.

Meselatus bicolor Chen (Epichrysomallinae) is a large gall-forming NPFW previously recorded only in figs of *F. microcarpa* from China (Chen et al. 1999). It is particularly abundant outside the tree's natural range in north Yunnan and Sichuan, to the north of the plant's native range, where *F. microcarpa* is widely planted, suggesting that it prefers seasonal climates (R. Wang & S.G. Compton, unpublished). *Bruchophagus sensoriae* Chen is its main parasitoid (R. Wang & S.G. Compton, unpublished), though it is also attacked by an *Ormyrus* species (Ormyridae) in the far north of its range (Y. Chen Pers. Comm.).

6.3.2 Study sites

Between 2011 and 2013, mature figs were collected from *F. microcarpa* trees planted in the following Mediterranean locations: Rhodes and Symi (Greece), Sicily (Italy), Tripoli (Libya), Malta (Malta), Majorca (Spain) and Marmaris (Turkey) and from trees in Tenerife (Spain), the largest of the Canary Islands, situated in the Atlantic off the coast of North Africa. These areas have typical Mediterranean climates with mild, rainy winters and hot, dry summers. Their annual precipitation ranges from about 330 mm in semi-arid Tripoli to 1100 mm in Marmaris. Tenerife has a warmer climate with mild winters and low annual rainfall of about 410 mm.

6.3.3 Sampling methods

At least ten mature figs at late C/early D phase *sensu* Galil & Eisikowitch (1968) were collected haphazardly from crops on different trees at each site and stored in 70% ethanol (Table 6.1). Figs at this time contain all the adult offspring of fig wasps that had oviposited into the figs. Each fig was cut into quarters and soaked in water for approximately 10 minutes to soften the galls before dissection. Each flower was checked under a dissecting microscope and was assigned into one of five categories: male flowers, unfertilized and un-galled female flowers, galls containing wasp offspring, seeds and empty galls ('bladders') where fig wasps had not completed their development. Fig wasps extracted from the galls were identified using mainly Chen et al. (1999) and Feng & Huang (2010), and then stored in 95% ethanol.

Table 6.1 The sample sizes (only figs containing fig wasps) and fig wasp species breeding in figs of *F. microcarpa* in the Mediterranean and Tenerife.

The number of crops where each fig wasp species emerged are shown. Abbreviations: Eupristina verticillata: EV, Meselatus bicolor: MB, Micranisa degastris: MD, Odontofroggatia galili: OG, Odontofroggatia ishii: OI, Walkerella microcarpae: WM, Philotrypesis emeryi: PE and Sycophila maculafacies: SM.

Country	Site	N figs	N crops	Years	Fig wasp species richness	NPFW species richness	Galler (n crops)						Parasitoid (n crops)	
							EV	MB	MD	OG	OI	WM	PE	SM
Greece	Rhodes & Symi	331	31	2011-2012	7	6	13	27	0	21	1	3	3	8
Italy	Sicily	99	10	2012	4	3	10	0	0	9	0	1	1	0
Libya	Tripoli	96	7	2012	2	1	7	0	0	7	0	0	0	0
Malta	Malta	130	9	2011	2	1	9	0	0	9	0	0	0	0
Spain	Majorca	101	6	2012	4	3	6	0	2	4	0	4	0	0
Spain	Tenerife	30	1	2013	2	1	1	0	1	0	0	0	0	0
Turkey	Marmaris	10	1	2012	1	0	1	0	0	0	0	0	0	0
Overall		797	65	2011-2013	8	7	47	27	3	50	1	8	4	8

6.3.4 Sizes of figs and galls

A total of 409 dissected figs (from 35 crops) had their lengths and widths measured to the nearest 0.02 cm using a dissecting microscope with an eyepiece graticule. We also randomly selected 138 figs from 9 crops from which the lengths and widths of 3745 galls containing *M. bicolor*, *O. galili* and pollinating agaonids were measured to the nearest 0.04 mm. The volumes of the figs and galls were then estimated using the formula for an ellipsoid, their approximate shape.

6.3.5 Statistical analyses

Figs containing any fig wasps other than *E. verticillata*, *M. bicolor* and *O. galili* were excluded from all analyses. All statistical analyses were carried out using R 2.14.2 (R Development Core Team, 2012). Response variables in linear mixed models (LMMs) were square-root or natural logarithm transformed if necessary. Likelihood ratio tests were used to assess the significance of fixed effects in LMMs and Generalized Linear Mixed Models (GLMMs), and multiple tests with Bonferroni correction were applied in pairwise comparisons. Crop identity was set as the random effect in all analyses except the one relevant to gall size.

We quantified the effects of *M. bicolor* and *O. galili* on the other contents of the figs they occupied. All figs were sorted into three types: (1) figs containing only *E. verticillata*, (2) figs containing *M. bicolor*, and (3) figs containing *O. galili*. Differences in male and female flower numbers, total and female pollinator offspring abundance, and seed production among different fig types were tested using LMMs in R package nlme version 3.1 (Pinheiro et al. 2013). The impacts of densities of the two NPFW on the plant's male (female pollinator abundance) and female (seed production) reproductive functions of figs were also analysed using GLMMs in R package lme4 version 1.0-5 (Bates et al. 2013) assuming Poisson error distributions.

All three fig wasps gall the ovules of their host and in figs shared by two or more

species their galls are potentially competing locally to acquire nutrients, but unfortunately we could not identify the original contents of failed galls in figs containing mixtures of species. To examine competitive effects we compared the sizes of successful mature galls, gall failure rates and the sizes of mature figs. Variation in gall size among species was tested using LMMs with fig identity as the random effect. Figs containing a single species of fig wasp were used to test whether gall failure rates (the proportion of galls that failed to generate adult offspring) and fig size varied among species, and whether the relationships between gall failure rate, fig size and total number of galls (per fig) differed among species using GLMMs (gall failure rates) assuming binomial error distribution and LMMs (fig size). The combination of *E. verticillata* and *O. galili* were only included in the analyses comparing gall failure rates, and *M. bicolor* did not share figs with other species.

6.4 Results

6.4.1 Fig wasps associated with Mediterranean *F. microcarpa*

We recorded the contents of 797 mature figs (from 65 crops) in the Mediterranean area and Tenerife (Table 6.1). Additional figs that lacked fig wasp offspring are not considered here. In addition to the pollinator (*E. verticillata*), a total of seven NPFW species were recorded, including five gall-forming species (*M. bicolor*, *Micranisa degastris* Chen, *O. galili*, *O. ishii* and *W. microcarpae* and two parasitoids, *P. emeryi* and *Sycophila maculafacies* Chen. The former utilize pollinator larvae as hosts, the latter is a parasitoid of *Odontofroggatia* species (including *O. galili* and *O. ishii*). These are the first records of *M. bicolor* outside its native range and the first records of *M. degastris* and *S. maculafacies* in the Mediterranean area. *E. verticillata* was found in all seven study sites. The most widespread NPFW species was *O. galili* which was recorded everywhere except Marmaris and Tenerife (Table 6.1). The most diverse fig wasp communities were present in figs from the Greek islands of Rhodes and Symi,

where six NPFW species were recorded (five from Rhodes and four from Syri), with three NPFW species recorded from the figs in Sicily and Majorca and just one or no NPFW species recorded from the other areas (Table 6.1).

The pollinating fig wasp, *E. verticillata*, was the most abundant species in most collections, emerging from 50.8% (405) of the figs and comprising 54.3% of all the recorded fig wasp adult offspring (33715 individuals). It was noticeably less frequent in collections from the islands of Rhodes and Syri, where only 23.0% of the figs contained this species (Table 6.2). *M. bicolor* and *O. galili*, were the most abundant NPFW overall, recorded from 25.2% (201) and 47.8% (381) of the figs and comprising 18.4% and 23.5% of the total fig wasp offspring respectively (Table 6.3). *M. bicolor* was present at high densities in the figs it occupied, where it excluded all other fig wasp species, but its distribution was limited to Rhodes and Syri (Table 6.3; Fig. 6.1 a). *O. galili* was the most prevalent NPFW overall (occupying the most figs), but its offspring were at relatively low densities in the figs where it occupied (Table 6.3; Fig. 6.1 b). The other five NPFW species were always rare, in total emerging from just 7.5% (60) of the figs and comprising only 1.3% of the fig wasp offspring. Note that we failed to identify some of the NPFW offspring (2.5% of the total fig wasps) because they had already emerged from their natal figs.

Table 6.2 The contents of *F. microcarpa* figs (means \pm SE).

Contents are calculated for all figs at each site, not just the figs where a particular species was present. Occupancy rate is the proportion of female flowers whose ovules contained adult fig wasps, and failed gall rate is the proportion of galls that failed to support fig wasps to adulthood.

Site	Male flowers	Female flowers	Fig wasp abundance	Occupancy rate (%)	Pollinator abundance	NPFW abundance	Gall failure rate (%)	Female pollinators	Seed production
Rhodes & Symi	7.1 \pm 0.5	91.4 \pm 4.1	39.2 \pm 1.8	54.4 \pm 1.5	13.7 \pm 1.8	25.4 \pm 1.3	7.3 \pm 1.9	11.5 \pm 1.6	6.9 \pm 1.1
Sicily	20.8 \pm 0.8	237.8 \pm 4.6	64.3 \pm 3.5	26.5 \pm 1.3	44.5 \pm 3.8	19.8 \pm 2.2	21.7 \pm 1.8	38.8 \pm 3.3	27.5 \pm 2.9
Tripoli	19.8 \pm 0.6	190.6 \pm 3.1	51.9 \pm 3.2	27.6 \pm 1.7	39.4 \pm 3.9	12.5 \pm 1.3	11.4 \pm 1.3	29.8 \pm 3.3	25.0 \pm 2.3
Malta	18.4 \pm 0.5	190.8 \pm 3.6	42.6 \pm 2.3	22.1 \pm 1.1	18.8 \pm 2.5	23.8 \pm 1.4	4.9 \pm 0.8	14.2 \pm 2.3	10.3 \pm 1.6
Majorca	19.1 \pm 0.6	199.3 \pm 4.0	16.7 \pm 1.1	8.6 \pm 0.5	9.7 \pm 1.0	7.1 \pm 0.9	18.1 \pm 1.9	7.2 \pm 0.8	7.7 \pm 1.2
Tenerife	15.7 \pm 1.0	168.6 \pm 6.1	41.6 \pm 2.9	25.5 \pm 1.9	40.3 \pm 3.0	1.3 \pm 0.4	19.1 \pm 1.8	27.2 \pm 1.9	31.1 \pm 2.3
Marmaris	26.3 \pm 2.2	263.0 \pm 6.1	93.1 \pm 10.8	35.7 \pm 4.3	93.1 \pm 10.8	0	1.6 \pm 0.8	85.0 \pm 9.5	113.3 \pm 8.9
Overall	14.2 \pm 0.3	156.5 \pm 2.8	42.3 \pm 1.2	35.3 \pm 0.9	23.0 \pm 1.2	19.3 \pm 0.7	10.9 \pm 0.9	18.5 \pm 1.1	14.5 \pm 0.9

Table 6.3 The prevalence and abundance of the three most common fig wasp species, *Eupristina verticillata*, *Meselatus bicolor* and *Odontofroggattia galili* in *F. microcarpa* figs.

Note that both abundance and relative abundance are based only on the figs where each particular species was present.

Country	<i>E. verticillata</i>			<i>M. bicolor</i>			<i>O. galili</i>		
	Prevalence (%)	Abundance (N occupied figs)	Relative abundance (%)	Prevalence (%)	Abundance (N occupied figs)	Relative abundance (%)	Prevalence (%)	Abundance (N occupied figs)	Relative abundance (%)
Rhodes & Symi	23.0	59.8 ± 4.3 (76)	82.9 ± 2.8	60.7	30.9 ± 1.7 (n=201)	100 ± 0	25.1	13.8 ± 0.9 (n=83)	48.6 ± 4.3
Sicily	87.9	50.6 ± 4.0 (87)	70.3 ± 3.6	0	NA	NA	62.6	31.3 ± 2.2 (n=62)	59.4 ± 4.2
Tripoli	78.1	50.5 ± 4.4 (75)	79.9 ± 2.9	0	NA	NA	76.0	16.4 ± 1.5 (n=73)	49.4 ± 4.5
Malta	43.8	42.9 ± 3.8 (57)	65.8 ± 3.4	0	NA	NA	94.6	25.1 ± 1.4 (n=123)	75.2 ± 3.0
Majorca	69.3	13.9 ± 3.3 (70)	83.6 ± 3.2	0	NA	NA	39.6	14.1 ± 1.5 (n=40)	92.0 ± 2.5
Tenerife	100	40.3 ± 3.0 (30)	96.1 ± 1.2	0	NA	NA	0	NA	NA
Marmaris	100	93.1 ± 10.8 (10)	100 ± 0	0	NA	NA	0	NA	NA
Overall	50.8	45.2 ± 1.8 (405)	78.7 ± 1.4	25.2	30.9 ± 1.7 (n=201)	100 ± 0	47.8	20.8 ± 0.8 (n=381)	63.6 ± 1.9

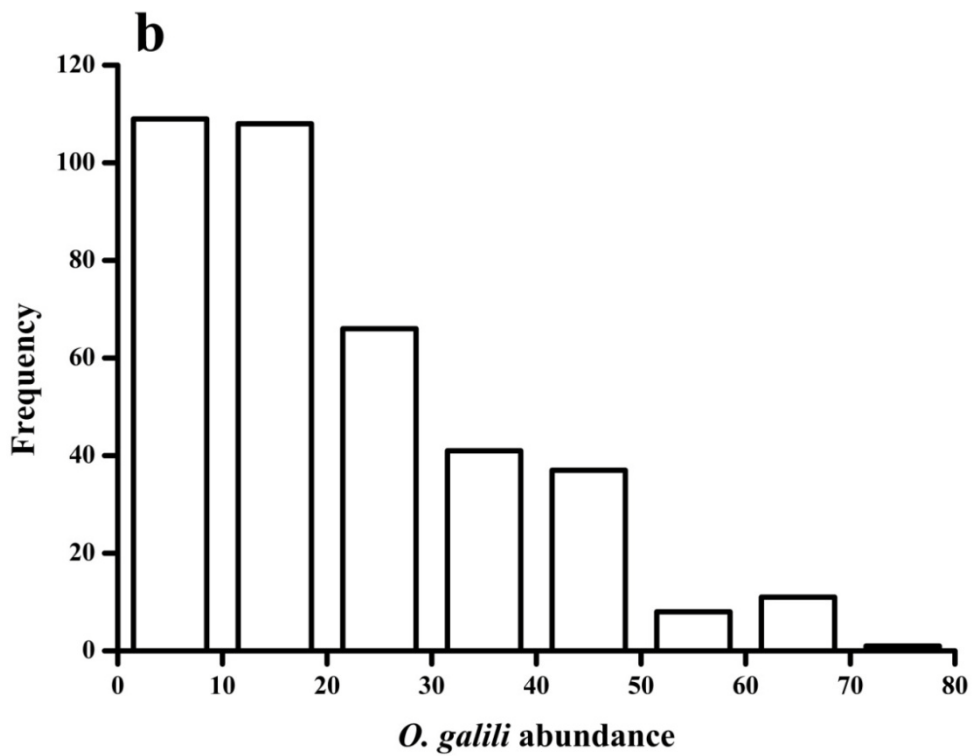
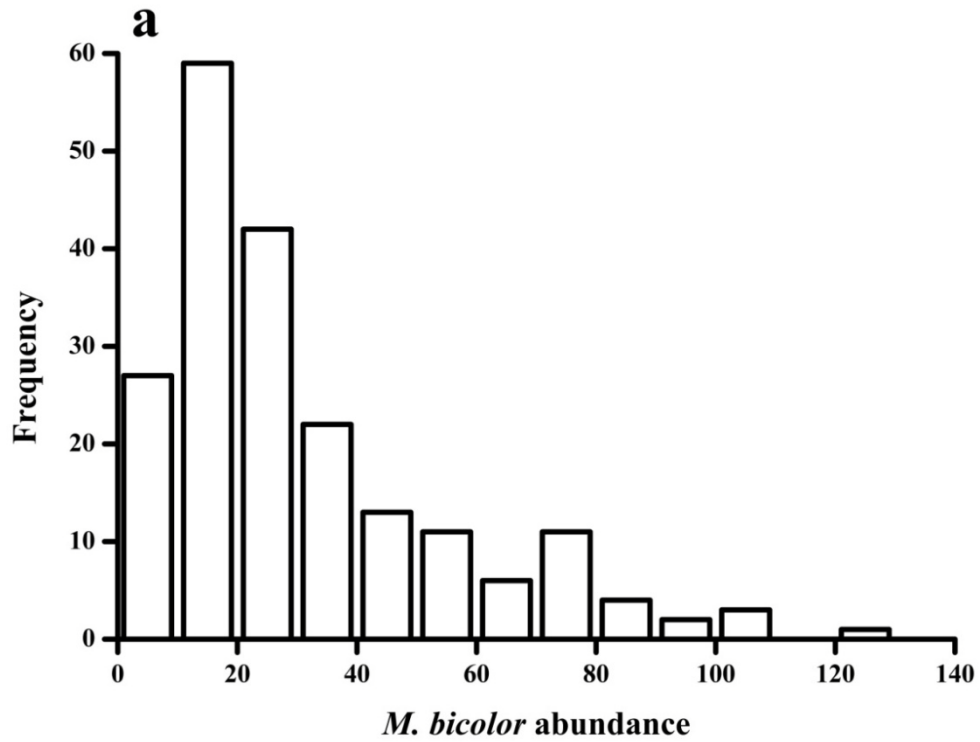


Fig. 6.1 The abundance of *M. bicolor* (a) and *O. galili* (b) adult offspring in the figs where they were present.

Note the different X axis scales.

6.4.2 Effects of *M. bicolor* and *O. galili* on fig contents

We included 737 figs that did not contain any fig wasps other than either *E. verticillata*, *M. bicolor* or *O. galili* in the following analyses. The numbers of male flowers in figs containing *M. bicolor* adult offspring were reduced by more than 95% compared with those occupied by the other species (Tables 6.4 and 6.5; Fig. 6.2). Similarly, female flower numbers in the figs occupied by *M. bicolor* were just 22.6% and 20.9% of those recorded in figs occupied by *O. galili* or *E. verticillata*, respectively (Tables 6.4 and 6.5; Fig. 6.2). Small but significant differences in male and female flower numbers were also detected between figs containing *O. galili* or *E. verticillata* (Tables 6.4 and 6.5; Fig. 6.2). None of the figs containing *M. bicolor* offspring contained any pollinator offspring or seeds, even when as few as six *M. bicolor* were present.

O. galili had less dramatic, but still significant, effects on host plant reproduction. Pollinator offspring, female pollinator offspring and seeds were reduced by 61.3%, 61.9% and 73.6%) in figs containing *O. galili*, relative to figs containing only *E. verticillata* (Tables 6.4 and 6.5; Fig. 6.2). Both male (measured as female pollinator abundance) and female (seed production) reproductive successes of figs were negatively related to *O. galili* abundance (GLMM: female pollinator abundance: $\beta = -0.030 \pm 0.001$ (mean \pm SE), $df=1$, Likelihood ratio (LR)=537.66, $p < 0.001$; seed production: $\beta = -0.028 \pm 0.002$, $df=1$, LR=304.39, $p < 0.001$; Fig. 6.3 a & b).

Table 6.4 The contents (means \pm SE) of figs containing only *E. verticillata*, *M. bicolor* or *O. galili*.

Fig wasp	N crops	N figs	Male flowers	Female flowers	Pollinators	Female pollinators	Seeds
<i>E. verticillata</i>	37	179	20.3 \pm 0.5	206.4 \pm 4.0	54.0 \pm 3.2	44.4 \pm 2.9	39.4 \pm 2.5
<i>M. bicolor</i>	27	201	0.9 \pm 0.1	43.2 \pm 1.8	0	0	0
<i>O. galili</i>	48	357	18.4 \pm 0.3	191.5 \pm 2.6	20.9 \pm 1.6	16.9 \pm 1.4	10.4 \pm 0.9

Table 6.5 LMM comparisons of the numbers of male and female flowers, total and female pollinator adult offspring and seeds in figs containing only *E. verticillata*, only *M. bicolor* or only *O. galili*.

Response variable	Fixed effect	df	Likelihood ratio	Pairwise comparisons	df	t value
Male flowers	Fig wasp	2	454.38 ***	<i>E. verticillata</i> vs. <i>M. bicolor</i>	671	25.91 ***
				<i>E. verticillata</i> vs. <i>O. galili</i>	671	3.92 ***
				<i>M. bicolor</i> vs. <i>O. galili</i>	671	-25.73 ***
Female flowers	Fig wasp	2	379.32 ***	<i>E. verticillata</i> vs. <i>M. bicolor</i>	671	22.27 ***
				<i>E. verticillata</i> vs. <i>O. galili</i>	671	3.18 **
				<i>M. bicolor</i> vs. <i>O. galili</i>	671	-22.20 ***
Pollinators	Fig wasp	2	286.68 ***	<i>E. verticillata</i> vs. <i>M. bicolor</i>	671	18.31 ***
				<i>E. verticillata</i> vs. <i>O. galili</i>	671	13.59 ***
				<i>M. bicolor</i> vs. <i>O. galili</i>	671	-9.22 ***
Female pollinators	Fig wasp	2	253.44 ***	<i>E. verticillata</i> vs. <i>M. bicolor</i>	631	17.15 ***
				<i>E. verticillata</i> vs. <i>O. galili</i>	631	12.43 ***
				<i>M. bicolor</i> vs. <i>O. galili</i>	631	-8.77 ***
Seed production	Fig wasp	2	286.34 ***	<i>E. verticillata</i> vs. <i>M. bicolor</i>	671	18.22 ***
				<i>E. verticillata</i> vs. <i>O. galili</i>	671	14.62 ***
				<i>M. bicolor</i> vs. <i>O. galili</i>	671	-8.23 ***

** : p<0.01, *** : p<0.001.

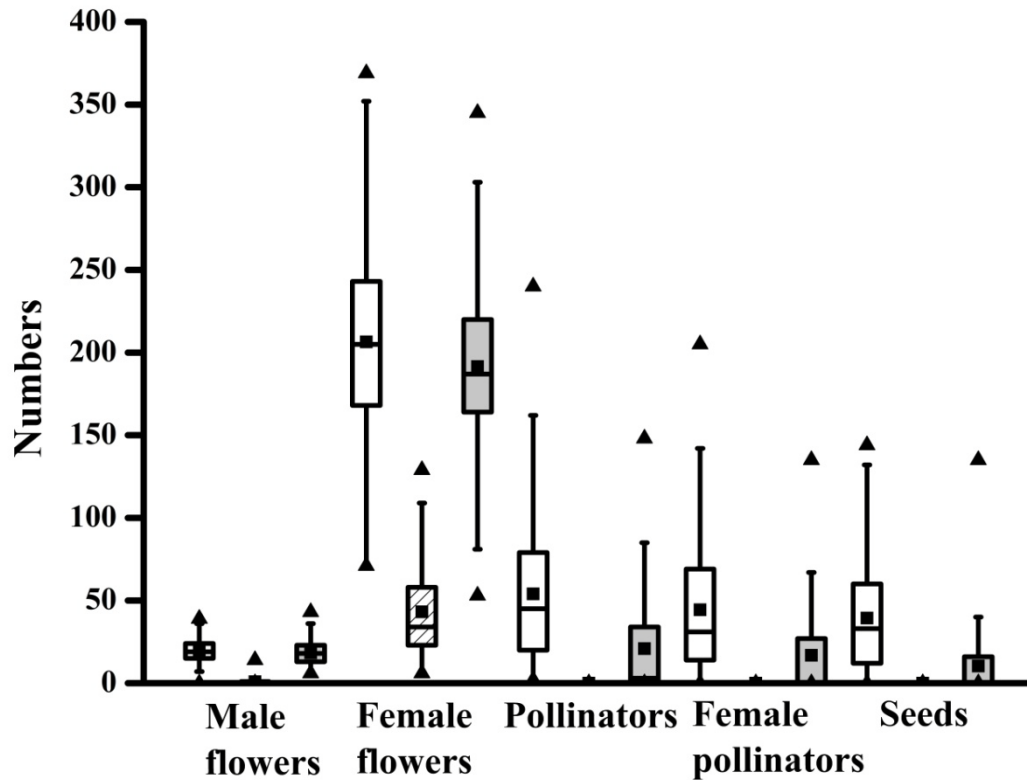


Fig. 6.2 Numbers of male and female flowers, total and female pollinator adult offspring and seeds in figs containing *M. bicolor* (hatched bars), *O. galili* (grey bars) and only *E. verticillata* (open bars).

In the box-plot, lines, boxes, whiskers, black squares and black triangles represent the median, the range from the first to third quartiles, 1.5 times lower and upper quartiles, mean, minimum and maximum values respectively.

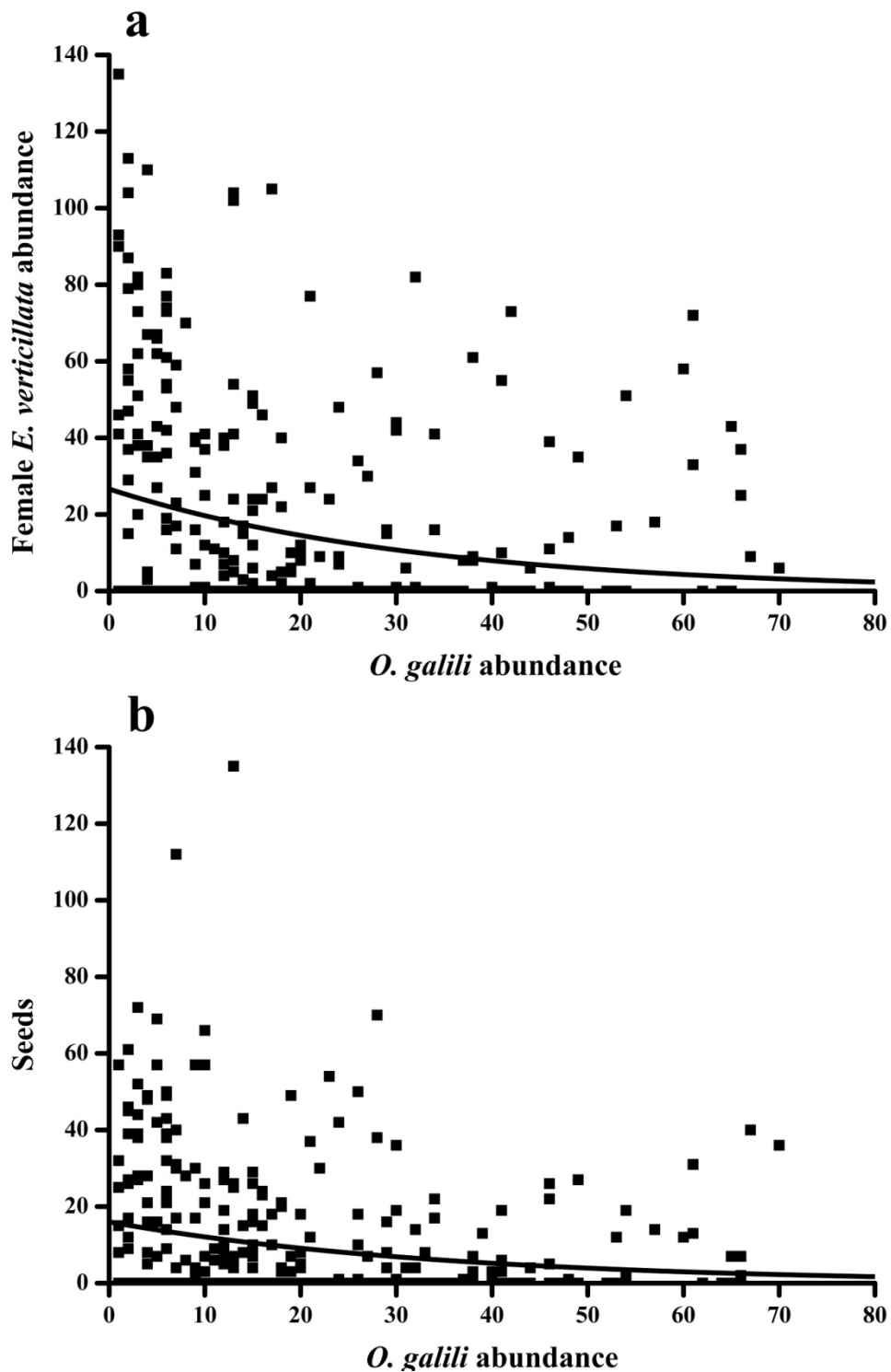


Fig. 6.3 The effects of *O. galili* on male (female pollinator abundance, a) and female (seed production, b) reproductive success of *F. microcarpa* figs using GLMMs setting Poisson distribution in residuals.

6.4.3 Gall sizes

M. bicolor produced the largest galls (gall size= $2.951 \pm 0.022 \text{ mm}^3$, N galls=1051, N figs=26). They were 9.4 times the volume of *E. verticillata* galls ($0.312 \pm 0.004 \text{ mm}^3$, N galls=1184, N figs=50) and 3.0 times the volume of *O. galili* galls ($0.974 \pm 0.006 \text{ mm}^3$, N galls=1510, N figs=96). The galls of the three species differed significantly from each other (LMM: fixed effect: species: df=2, LR=3130.46, $p < 0.001$; pairwise comparisons: *E. verticillata* vs. *M. bicolor*: df=3605, $t = -96.66$, $p < 0.001$; *E. verticillata* vs. *O. galili*: df=3605, $t = -78.57$, $p < 0.001$; *M. bicolor* vs. *O. galili*: df=3605, $t = 50.05$, $p < 0.001$; Fig. 6.4).

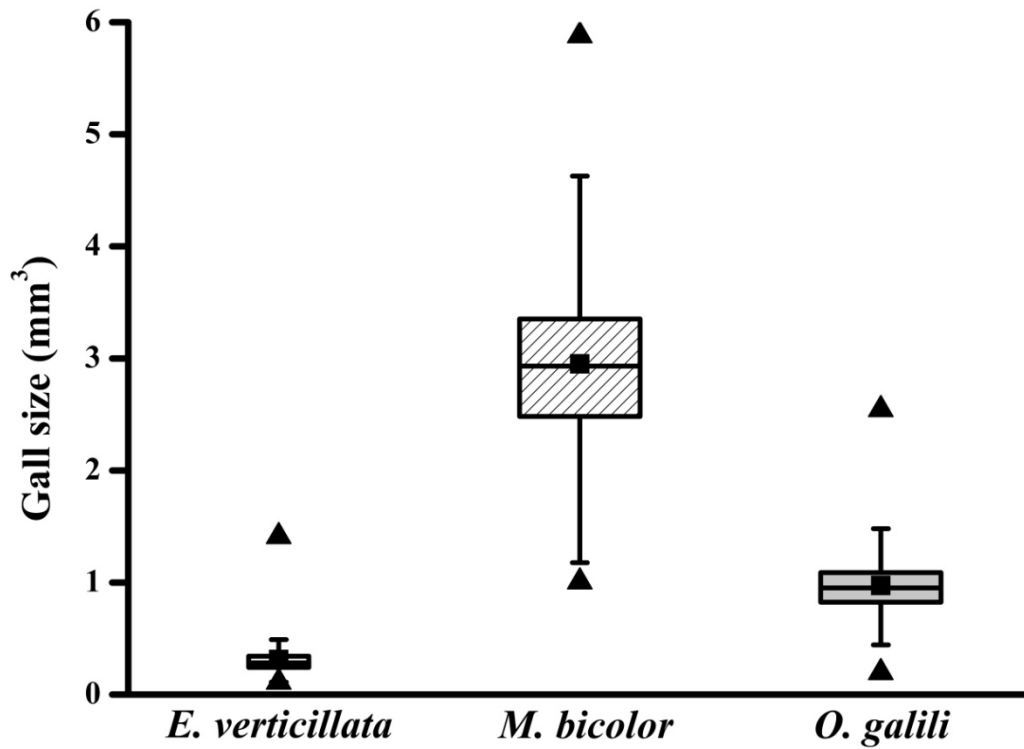


Fig. 6.4 The estimated volumes of galls that contained adult offspring of *E. verticillata*, *M. bicolor* and *O. galili* (grey bar).

Lines, boxes, whiskers, black squares and black triangles represent median, range from the first to third quartile, 1.5 times lower and upper quartiles, mean, minimum and maximum values respectively.

6.4.4 Gall failure rates

A total of 552 figs contained offspring of a single species and a combination of *E. verticillata* and *O. galili* were detected in 185 figs. Figs containing only *E. verticillata* or a combination of *E. verticillata* and *O. galili* had far higher gall failure rates, averaging 15.6% and 17.5%, which were at least 4.0 and 3.5 times as high as in the figs that contained only *M. bicolor* and only *O. galili*, respectively (Tables 6.6 and 6.7; Fig. 6.5 a). The two NPFW had similar gall failure rates, whereas there was a slight but significant difference in gall failure rates between figs containing only *E. verticillata* and those with a combination of *E. verticillata* and *O. galili* (Tables 6.6 and 6.7; Fig. 6.5 a) probably because 27.4% (48 figs) of figs containing only pollinators but only 13.0% (24 figs) of figs containing a combination of *E. verticillata* and *O. galili* did not produce any failed galls.

For all three species, gall failure rates were independent of total number of galls in a fig, and there was also no variation in the strength of this relationship between species (GLMMs: figs containing only *E. verticillata*: $\beta = -0.002 \pm 0.001$, $z = -1.62$, $p = 0.106$; figs containing only *M. bicolor*: $\beta = 0.004 \pm 0.003$, $z = 1.40$, $p = 0.163$; figs containing only *O. galili*: $\beta = -0.003 \pm 0.006$, $z = -0.57$, $p = 0.571$; Table 6.7). We could not confidently identify which fig wasps had generated the empty galls in figs that contained combinations of species, but most were small, suggesting that they were failed pollinator galls.

Table 6.6 Gall failures rate and fig volumes (cm³) (means ± SE) in figs containing only *E. verticillata*, only *M. bicolor* or only *O. galili*.

Gall failure rates are the proportion of galls that failed to support fig wasps to adulthood, and this rate was also calculated in figs with a combination of *E. verticillata* and *O. galili*.

Fig type	Gall failure rate (N crops, N figs)	Fig size (N crops, N figs)
Only <i>E. verticillata</i>	0.156 ± 0.013 (37, 179)	0.482 ± 0.028 (16, 60)
Only <i>M. bicolor</i>	0.039 ± 0.006 (27, 201)	0.928 ± 0.063 (15, 112)
Only <i>O. galili</i>	0.045 ± 0.007 (37, 172)	0.420 ± 0.016 (23, 130)
<i>E. verticillata</i> and <i>O. galili</i>	0.175 ± 0.011 (36, 185)	--

Table 6.7 Effects of fig wasp presence (figs containing only *E. verticillata*, only *M. bicolor* or only *O. galili*) and total number of galls on gall failure rates and fig sizes.

GLMMs assumed binomial distributions of residuals and LMMs were used, and figs containing a combination of *E. verticillata* and *O. galili* were included in the analyses comparing gall failure rates in different fig wasp presence as another type of fig wasp presence.

Response variable	Fixed effect(s)	Model	df	Likelihood ratio	Pairwise comparisons	df	z/t value				
Gall failure rate	Fig wasp presence	GLMM	2	379.05 ^{***}	Only <i>E. verticillata</i> vs. Only <i>M. bicolor</i>	--	9.83 ^{***}				
					Only <i>E. verticillata</i> vs. Only <i>O. galili</i>	--	10.46 ^{***}				
					Only <i>E. verticillata</i> vs. <i>E. verticillata</i> and <i>O. galili</i>	--	-6.68 ^{***}				
					Only <i>M. bicolor</i> vs. Only <i>O. galili</i>	--	-1.14 ^{NS}				
					Only <i>M. bicolor</i> vs. <i>E. verticillata</i> and <i>O. galili</i>	--	-12.57 ^{***}				
					Only <i>O. galili</i> vs. <i>E. verticillata</i> and <i>O. galili</i>	--	-15.34 ^{***}				
					Fig wasp presence × total number of galls	GLMM	2	3.40 ^{NS}	Only <i>E. verticillata</i> vs. Only <i>M. bicolor</i>	--	-1.84 ^{NS}
Fig size	Fig wasp presence	LMM	2	29.96 ^{***}	Only <i>E. verticillata</i> vs. Only <i>M. bicolor</i>	265	-4.44 ^{***}				
					Only <i>E. verticillata</i> vs. Only <i>O. galili</i>	265	1.65 ^{NS}				
					Only <i>M. bicolor</i> vs. Only <i>O. galili</i>	265	7.30 ^{***}				
					Fig wasp presence × total number of galls	LMM	2	62.56 ^{***}	Only <i>E. verticillata</i> vs. Only <i>M. bicolor</i>	262	-8.53 ^{***}
					Only <i>E. verticillata</i> vs. Only <i>O. galili</i>	262	-2.80 [*]				
					Only <i>M. bicolor</i> vs. Only <i>O. galili</i>	262	3.99 ^{***}				

^{NS}: not significant, ^{*}: p<0.05, ^{***}: p<0.001.

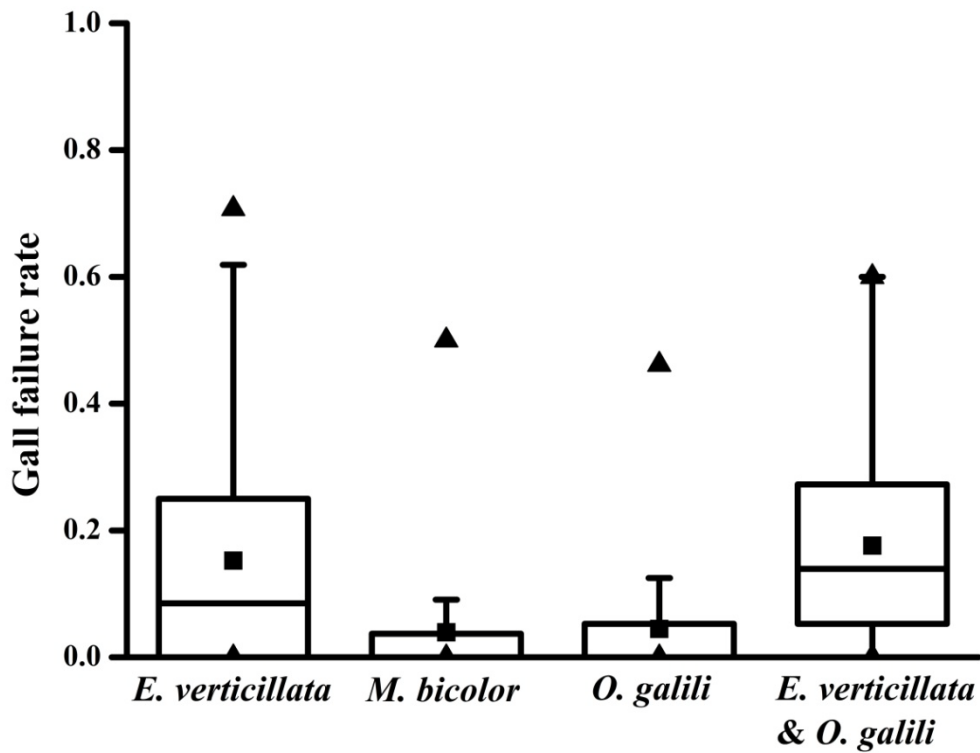


Fig. 6.5 Gall failure rates among figs containing only *E. verticillata*, only *M. bicolor*, only *O. galili* and combination of *E. verticillata* and *O. galili* adult offspring.

Lines, boxes, whiskers, black squares and black triangles represent median, range from the first to third quartile, 1.5 times lower and upper quartiles, mean and minimum and maximum values respectively.

6.4.5 Fig sizes

302 of the 409 figs whose volumes were estimated contained only one of the three fig wasp species. Figs containing only *M. bicolor* were 1.9 and 2.2 times as those containing only *E. verticillata* or *O. galili* offspring. Figs containing only *E. verticillata* were similar to those containing only *O. galili* (Tables 6.6 and 6.7; Fig. 6.6 a). Fig size increased with increasing numbers of fig wasp galls in figs containing only *M. bicolor* (LMM: slope= 0.022 ± 0.002 , df=262, t=12.12, p<0.001) and only *O. galili* (LMM: slope= 0.009 ± 0.003 , df=262, t=3.62, p<0.001) with a significantly stronger slope in the former, but the sizes of figs where only *E. verticillata* was present (and where seeds also contributed to their volume) were independent of total number of galls (LMM: slope= 0.001 ± 0.002 , df=262, t=0.61, p=0.541; Table 6.7; Fig. 6.6 b).

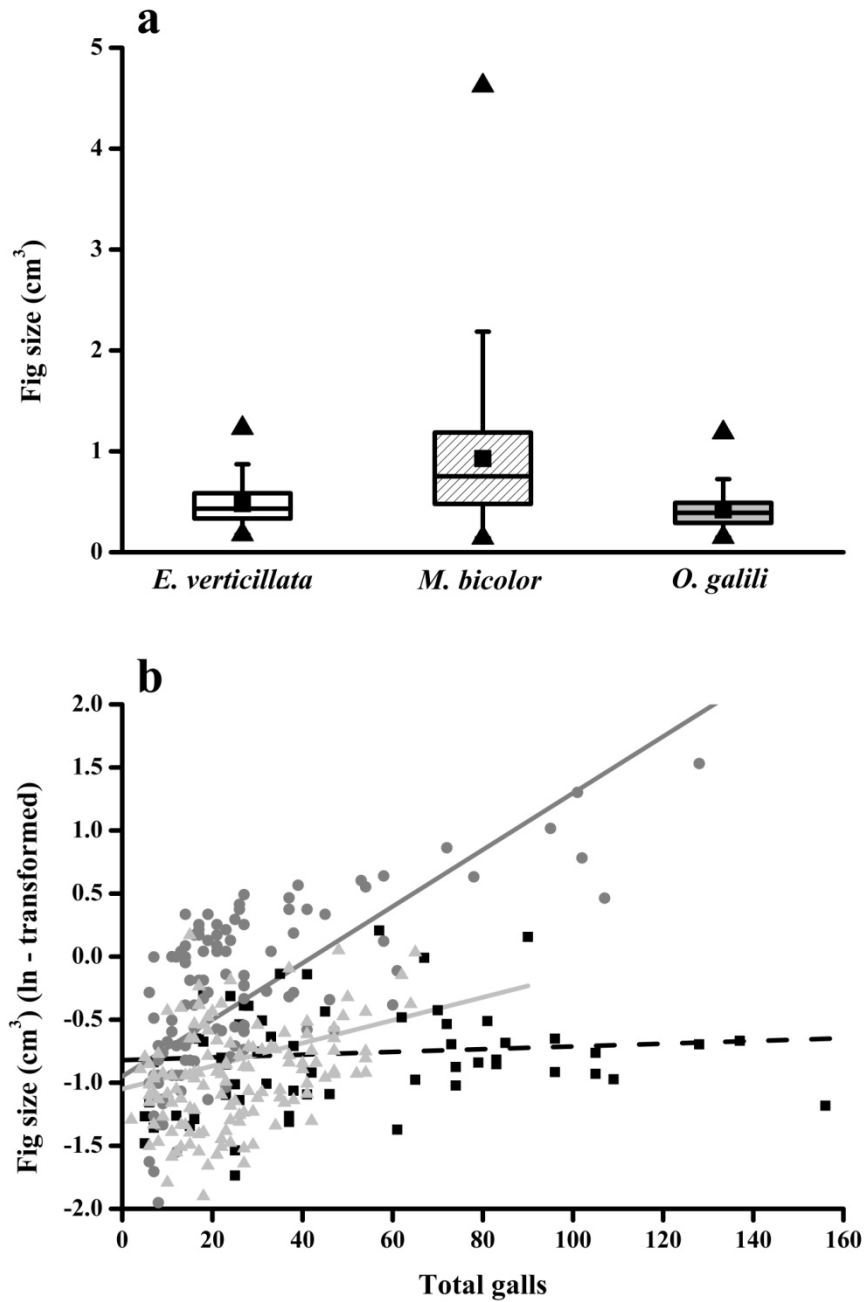


Fig. 6.6 Effects of fig wasp species on fig volume.

(a) The estimated volumes of figs in figs containing only *E. verticillata*, only *M. bicolor* or only *O. galili*. Lines, boxes, whiskers, black squares and black triangles represent median, range from the first to third quartile, 1.5 times lower and upper quartiles, mean and minimum and maximum values respectively. (b) Differences in the linear relationship between fig size and total number of galls in figs containing only *E. verticillata* (black dashed line (non-significant) and squares), only *M. bicolor* (grey line and circles) and only *O. galili* (light grey line and triangles) using LMMs.

6.5 Discussion

We recorded the presence in the Mediterranean area of the pollinator of *F. microcarpa* together with seven species of NPFW. Three species were recorded from the area for the first time (*M. bicolor*, *M. degastris* and *S. maculafacies*), but we failed to detect an eighth species, *P. taiwanensis*, which was reported recently from Hatay, Turkey (Doğanlar 2012). The fig wasp fauna associated with *F. microcarpa* in the Mediterranean currently includes over one third of the NPFW recorded routinely in *F. microcarpa* figs in its native range (R. Wang and S.G. Compton, unpublished). An early survey in the Greek Isles detected only *O. galili* (Compton 1989), since when an additional six species appear to have arrived, including the tree's pollinator. The rapid expansion in the fauna is presumably as a result of increasing international trade (Lo Verde et al. 1991; Doğanlar 2012; Mifsud et al. 2012). Secondary spread around the Mediterranean, either by natural dispersal or human activities, is likely to result in further local enrichment of communities as new species arrive from elsewhere in the area and may lead to eventual homogenization in community composition across the Mediterranean as a whole.

O. galili was the first fig wasp reported from the Mediterranean, whereas *M. bicolor* has apparently arrived recently and may be restricted to the Greek Isles. Both species reduce the reproductive success of *F. microcarpa*, but *M. bicolor* has a much greater impact, totally inhibiting pollinator and seed production in the figs it occupies, even when present in small numbers. Both NPFW can develop independently of the pollinator, but only *M. bicolor* prevents pollinator females from developing in figs it has galled. *M. bicolor* females oviposit before the fig developmental stage when pollinators enter, and the rapid development of their large galls often prevents pollinator females from entering the figs. For any pollinators that do manage to enter, the inside of the figs is clearly not conducive to oviposition and larval development. Figs containing *M. bicolor* become noticeably larger than normal, and may also be having a less noticeable impact on larval development in other figs, if there is

competition for nutritional resources between adjacent figs.

Rates of flower occupancy were consistently low throughout the Mediterranean, suggesting that the figs could have supported more fig wasp larvae and that the impact of *O. galili* on pollinator offspring numbers was not a result of competition for oviposition sites (Dunn et al. 2008; Segar & Cook 2012). Perhaps surprisingly, given that it has a mutualistic association with its host plant, *E. verticillata* galls were far more likely to fail than those of either *M. bicolor* and *O. galili*, despite being smaller and supporting the development of smaller fig wasps. In one fig tree species, at least, failed galls had been oviposited in as well as galled (Ghana et al. 2012), which suggests that failures in larval development are a major mortality factor for *E. verticillata*. The gall failure rate of the pollinator was much lower in the tree's native range, indicating a poor adaptation of *F. microcarpa*, a common phenomenon in exotic species (Carroll 2011). Both *M. bicolor* and *O. galili* are able to generate larger galls, with lower failure rates, suggesting that they are more efficient than the pollinator at directing nutrients to their galls. Besides, even though the identity of the failed galls is unknown, high gall failure rates in figs with a combination of *E. verticillata* and *O. galili* could also reflect much lower survival rate of pollinator offspring because *O. galili* galls are less likely to become failed galls. The mechanisms that allow some galls to be stronger assimilate sinks than others are poorly understood (Dorchin et al. 2006), but the effects of competition between gall inhabitants are well documented (Burstein et al. 1994; McGeoch & Chown 1997; Hartley 1998).

Despite its ubiquity and demonstrably negative impact on seed and pollinator offspring numbers, *O. galili* has failed to prevent *F. microcarpa* from becoming invasive in areas such as Florida and Hawaii, and the tree is now also becoming established in parts of the Mediterranean (www.maltawildplants.com). *M. bicolor* clearly offers better prospects for reducing the damage to buildings caused by *F. microcarpa* seedlings in urban environments, and also for slowing down the spread of this species in natural areas, though seed predation alone offers no guarantee of successful control (Garren &

Strauss 2009). The arrival of *M. bicolor* in the Mediterranean provides an opportunity to study how its impact on *F. microcarpa* and other fig wasps changes over time. It is rare or absent from many sites in the native range of *F. microcarpa* in China (R. Wang et al. unpublished data), where it seems to prefer areas with more seasonal climates and avoid more tropical areas. The Mediterranean is extra-tropical, like many other areas where *F. microcarpa* is introduced, and *M. bicolor* is likely to do well there.

Chapter 7 Food web structure in a complex fig wasp community: compartments are linked to gall size

7.1 Abstract

The trophic interactions summarized in food webs and are central to the structure of ecological communities. Fig trees (*Ficus*) are a conspicuous pan-tropical plant group pollinated by ovule-galling fig wasps (Agaonidae). Their fruits (figs) also support up to 30 or more non-pollinating fig wasp species. Untangling the trophic relationships within the more diverse fig wasp communities has proved difficult. *Ficus microcarpa* is widely-grown outside its native range, where it can become invasive. Sub-sets of its diverse (>20 species) fig wasp community are also widely introduced. Using a standardized sampling method throughout much of its introduced and native range, we recorded which species are found together. Together with gall sizes, spatial distributions within figs and how their numbers co-vary this allowed us to construct the first food web for a complex fig wasp community. All the phytophagous fig wasps utilized female flowers for larval development, and they were far more common than parasitoids. Most are ovule galls, but there was one seed predator. Sycoryctines (Pteromalidae) were parasitoids of agaonids, and eurytomids were parasitoids of epichrysomallines. No parasitoids of the seed-eating species were recorded. Parasitoid host specificity was variable, but each galler genus (or subfamily) had characteristic parasitoids, suggesting strong evolutionary constraints and niche conservatism within the community. Parasitoid host ranges were generally linked to the size of the galls generated by hosts. Frequent host density-dependent aggregation among the parasitoids suggests they contribute to the stability of fig wasp communities.

7.2 Introduction

Natural ecosystems contain communities of interacting plants and animals. Species located at different trophic levels form food webs comprising those species that are eaten and those that do the eating. Food web structure is integral of ecosystems (Pace et al. 1999; Yamaguchi et al. 2011). Bottom-up and top-down regulation via food chains contribute to the sustainability of biodiversity and ecosystem function (Finke & Denno 2004; O'Corner et al. 2009; Estes et al. 2011). Species at higher trophic levels prey on those at lower levels, providing top-down regulation that can control host density and reduce intra-guild competition, and in turn help protect species at even lower positions in each food chain (Estes et al. 2011). Density-dependent foraging behaviour of predators can also generate stability (West et al. 1996; Skelhorn et al. 2011; Horning & Mellish 2012). Bottom-up regulation of populations involves factors such as induced secondary metabolites of plants produced in response to feeding by herbivores (Enge et al. 2012), host-shifts of phytophages for escaping from their natural enemies (Leppänen et al. 2013) and long-distance movements between resource patches (Bartel et al. 2011). Destruction of food web structure by human activities can cause the collapse of local communities (Kruess & Tscharrntke 1994; Estes et al. 2011) and consequently facilitate biological invasions (Tylianakis et al. 2007; Gurevitch et al. 2011).

Recent studies have revealed that the stability of food web tends to be positively linked with their complexity, and that the existence of generalists that generate increased connectances may generate more stability than specialists (Pillar et al. 2011; Rooney & McCann 2012). Most of those studies are however based on large scale open ecosystems. This contrasts with many insect communities on plants that are mainly composed of specialists that co-exist at much smaller spatial scales. Such communities are often characterized by high local species richness and contribute significantly to global biodiversity.

The genus *Ficus* (fig trees) is widely recognised as contributing to the sustainability of pantropic and pansubtropic forest ecosystems because it not only contains at least 800

species, but also supports numerous invertebrate and vertebrate species (Shanahan et al. 2001; Harrison 2005; Herre et al. 2008; Compton et al. 2010). All fig tree species produce figs (syconia), complex inflorescences inside which male and female flowers develop in a largely isolated environment. Sexual reproduction of the plants relies on pollinating fig wasps (Agaonidae). Often there is a one to one relationship with each fig tree species only having one pollinator species and vice versa, but there are exceptions, usually with one tree pollinated by several agaonids (Cook & Rasplus 2003; Harrison 2005). Fig trees can be sub-divided on the basis of their breeding systems into monoecious and functionally dioecious species (Cook & Rasplus 2003; Herre et al. 2008). In monoecious figs, female flowers in all figs have various style lengths and female pollinators generally pollinate female flowers close to the fig wall, but oviposit into and gall mainly ovules in the center of a fig. In functionally dioecious figs, those on female trees only produce seeds because the styles of their female flowers are too long for oviposition (and the stigmas make ovipositor penetration difficult), whereas flowers in functionally male figs have short styles and only raise pollinator offspring. The larvae of pollinators feed on the galled ovules, and when become mature, adult males mate with females before helping to release them from their natal figs. They then fly away to search for receptive figs elsewhere.

Besides pollinating agaonids, figs are also exploited by a large number of non-pollinating fig wasps (NPFW) belonging to families of Chalcidoidea. These include species of Agaonidae (a small number of which do not pollinate), Eurytomidae, Ormyridae, Pteromalidae and Torymidae (Bouček 1988; Cook & Segar 2010). Like pollinating fig wasps, most NPFW are believed to have one or only a few host fig tree species, but they primarily lay eggs from outside figs rather than entering the figs first (Cook & Segar 2010; Li et al. 2010; Zhou et al. 2012). NPFW can be allocated into two trophic levels. Phytophages only feed on plant tissues and do not kill other fig wasps. Some form galls in the fig wall, or are seed predators (Periera et al. 2007), but most develop inside ovules that they have galled. Parasitoids have larvae that develop at the

expense of other species. They include secondary gallers that modify galls of other species (Chen et al. 2013), parasitoids that may or may not also feed on plant tissues (Segar & Cook 2012), and specialist hyper-parasitoids (Compton et al. 2009). Generally, NPFW are thought to kill or compete for oviposition sites with pollinators (Kerdelhué & Rasplus 1996; Segar & Cook 2012) though some NPFW are parasitoids of other parasitoids (Compton 1993).

The detailed feeding biology and host relationships of most NPFW has not been confirmed, especially in the case of putative parasitoids. Species in Pteromalidae subfamilies Epichrysomallinae and Otitesellinae are believed to all be gall formers (Bouček 1988), and some species of Eurytomidae (*Sycophila*) are the obligate parasitoids of epichrysomallines (Compton 1993). Species of Sycoryctinae (Pteromalidae) are generally regarded as parasitoids of agaonids (Segar & Cook 2012; Suleman et al. 2013), but one species is a seed predator (Wang et al. in press a).

Related fig trees are often pollinated by related agaonids, suggesting that they often share long co-evolutionary histories (Cruaud et al. 2012). Some gall-forming NPFW show similar patterns (Jousselin et al. 2008) but parasitoid NPFW may be more likely to display host or niche shifts probably because of their wide host range (Segar & Cook 2010), which may play an important role in shaping community structure (Segar et al. 2013). Nevertheless, niche conservatism induced by morphological characters such as ovipositor length and body size still strongly contribute to some matches between parasitoids and gallers at genus/subfamily level, therefore reflecting the role of evolutionary constraints (Dunn et al. 2008; Segar & Cook 2012; Yu & Compton 2012; Segar et al. 2013).

There is some evidence of similar fig wasp community structure across continents (Segar et al. 2013), but very little is known about the dynamics and stability of fig wasps food webs. The mutualism may have been established as long ago as 75 million years after which several groups of Chalcidoidea have independently colonized the resources it provides. The oldest known NPFW are found in Dominican Amber dated at

about 20 myr BP (S.G. Compton, unpublished) and belong to a modern genus, so there has been at least some stability in community composition for this period. Parasitoid NPFW may also play an important role in stabilizing the mutualism in some monoecious figs, providing selection pressures on agaonids to avoid longer styled flowers and forcing them to oviposit in the ovules located towards the central area of a fig (Dunn et al. 2008; Segar & Cook 2012). Parasitoid NPFW may also have influenced the galling behavior of agaonids in dioecious figs (Yu & Compton. 2012). We surveyed the fig wasp fauna associated with figs of *Ficus microcarpa*, a fig tree that has been widely dispersed outside its native range, with the aim of revealing major links in its fig wasp food web. The widespread introduction of *F. microcarpa* allowed us to take advantage of sample sites where only a small subset of the species in the native range were present. Several forms of evidence were collected. Putative parasitoids of each phytophage were expected to occur at the same sites and in the same figs as their hosts, and be more likely to occur in figs where their hosts were present. They were also expected to have a negative impact on host numbers, to develop in galls of similar size and to develop in galls located within similar locations as their hosts within the figs.

7.3 Methods and materials

7.3.1 *F. microcarpa* and its fig wasps

F. microcarpa is a monoecious fig tree with a natural distribution in tropical and subtropical forests of SE Asia and Australasia, where it grows as a strangler or from bare rocks (Berg & Corner, 2005). During the last 200 years it has also been transplanted widely as an ornamental and shade tree in tropical and warm temperate urban areas including Brazil, the Caribbean, East Asia, the Mediterranean, South Africa and southern USA (Lo Verde et al. 1991; Nadel et al. 1992; Figueiredo et al. 1995; Kobbi et al. 1996; Beardsley 1998; Burrows & Burrows 2003; Lo Verde & Porcelli 2010; Caughlin et al. 2012). It has also been planted outside its natural range in

temperate areas of China and Australia.

As in other fig trees, sexual reproduction of *F. microcarpa* depends on a host specific pollinating agaonid fig wasp, *Eupristina verticillata* Waterston (Agaonidae). Recent studies have revealed that this taxon represents a complex of several closely-related and morphologically similar species (Sun et al. 2011), including one species (*Eupristina* sp. 'cheater') that no longer pollinates the plant (J-Y Rasplus, Pers. Comm.). The non-pollinating agaonid is only recorded from Yunnan Province, China. Only one species of pollinator has been introduced outside the plants introduced range, and is found almost everywhere the tree is planted (van Noort et al. 2013; A. Cruaud and J-Y. Rasplus, Pers. Comm.).

A crop of *F. microcarpa* can consist of up to several thousand small figs located in the leaf axils. They reach about 10.1 ± 0.3 (mean \pm SE; n=20) mm in diameter when mature and can contain several tens of seeds. Crop sizes are influenced by season and temperature (Yang et al. 2013). Mature figs of *F. microcarpa* are eaten by a wide range of bird species that contribute to the rapid expansion of *F. microcarpa* populations in some areas, where the plant has the potential to threaten both native plants and animals (Kaufmann et al. 1991; Nadel et al. 1992; Shanahan et al. 2001; Corlett 2006). Seeds routinely germinate on buildings, walls and pavements in urban areas, where seedlings cause damage (Tan & Yeo 2009; Caughlin et al. 2012). *F. microcarpa* is therefore regarded as invasive in Bermuda, Florida, and Hawaii and a nuisance species elsewhere (Nadel et al. 1992; Beardsley 1998; Starr et al. 2003).

Reflecting the broad natural distribution and monoecious breeding system of *F. microcarpa* (Compton & Hawkins 1992), its figs are exploited by a large community of NPFW comprising at least 21 species. They utilise the plant's ovules for larval development but do not pollinate (Chen et al. 1999; Feng & Huang 2010; Li et al. 2013). Except for the non-pollinating agaonid, all the known NPFW belong to other families of Chalcidoidea, which lay their eggs into these ovules via the outer wall of the fig, utilising their long or very long ovipositors (Galil and Copland 1981). Like the

agaonids, a single larva of other NPFW develops inside each ovule. Species from subfamilies Epichrysomallinae and Otitesellinae are considered to be gallers, whereas putative parasitoids comprise fig wasps from the families Eurytomidae and Ormyridae and Pteromalidae, subfamily Sycoryctinae. *Philotrypesis taiwanensis* (Sycoryctinae) is an exception as it is an obligate seed predator, with larvae that consume seeds rather than hosts in galled ovules (Wang et al. in press a).

In our analyses we assigned the fig wasps associated with *F. microcarpa* into two trophic levels, those with larvae that feed exclusively on plant tissue: ‘phytophages’ (that are mainly ovule-gallers but including the obligate seed-feeder, *P. taiwanensis* and agaonids) and ‘parasitoids’ with larvae that develop at the expense of gall forming species. We have not detected any obligate hyper-parasitoids, but intra-guild predation among parasitoids is possible.

The extent of host specificity among NPFW associated with each fig tree species is largely unknown, but preliminary observations based largely on the sizes of the NPFWs suggested several putative host-parasitoid associations in the figs. These included (host: parasitoid) *Meselatus bicolor* Chen (Epichrysomallinae): *Bruchophagus sensoriae* Chen (Eurytomidae); *Odontofroggattia* spp. (Epichrysomallinae): *Sycophila* spp. (Eurytomidae); *Eupristina* (Agaonidae), *Walkerella* and *Micranisa* spp. (both Otitesellinae): *Philotrypesis* spp. (except *P. taiwanensis*, Sycoryctinae), and *Eupristina* spp.: *Sycoryctes* and *Sycoscapter* spp. (both Sycoryctinae).

7.3.2 Sample sites

Fig crops were sampled in both the introduced and native ranges of *F. microcarpa* (Table 7.1). We sampled figs at seven sites located in East and Southeast Asia including southern mainland China, Taiwan, Thailand and the Philippines. Figs were also collected from 20 sites in the plant's introduced range in southeast and southwest China close to the plant's native range and also Australia, Brazil, Caribbean, Florida (USA), countries around the Mediterranean and South Africa. The plant's native range is

largely tropical and characterized by relatively high humidities, at least in some seasons, and generally hotter climates than the introduced range. The plant has mainly been introduced into subtropical and warm temperate regions, but they have a wide range of climates.

Table 7.1 Locations of sample sites and sampling dates.

Area	Site (abbreviation)	Location	Year(s)
Native range			
China mainland	Guangzhou (GZ)	N 23°11', E 113°22'	2011-2012
China mainland	Xishuangbanna (XS)	N 22°00', E 100°48'	2010-2013
Taiwan	Taibei (TB)	N 25°01', E 121°33'	2012
Thailand	Bangkok (BK)	N 13°44', E 100°33'	2012-2013
Thailand	Chiang Mai (CM)	N 18°46', E 98°59'	2012
Thailand	Kanchanaburi (KC)	N 14°04', E 99°32'	2012-2013
The Philippines	Manila (MN)	N 14°40', E 121°04'	2012
Introduced range			
Australia (southern)	Brisbane (BR)	S 27°29', E 153°06'	2012
Brazil	Rio de Janeiro (RJ)	S 22°53', W 43°34'	2012
Canary Islands	Tenerife (TN)	N 28°29', W 16°19'	2013
China mainland	Chengdu (CD)	N 30°40', E 104°06'	2012
China mainland	Kunming (KM)	N 24°53', E 102°50'	2010-2011
China mainland	Mianyang (MY)	N 31°28', E 104°41'	2012
China mainland	Panzhihua (PZ)	N 26°35', E 101°43'	2012
China mainland	Sanming (SM)	N 26°16', E 117°38'	2013
China mainland	Xichang (XC)	N 27°53', E 102°17'	2012
Florida (USA)	Davie (DV)	N 26°04', W 80°14'	2012
Greece	Rhodes (RD)	N 36°10', E 27°58'	2011-2012
Greece	Symi (SY)	N 36°35', E 27°50'	2012
Italy	Sicily (SC)	N 38°07', E 13°22'	2012
Libya	Tripoli (TP)	N 32°51', E 13°12'	2011-2012
Malta	Malta (MT)	N 35°56', E 14°23'	2011
Puerto Rico	Puerto Rico (PR)	N 18°23', W 66°04'	2013
South Africa	Grahamstown (GH)	S 33°19', E 26°31'	2011
South Africa	Port Elizabeth (PE)	S 33°58', E 25°37'	2011
Spain	Majorca (MJ)	N 39°35', E 2°40'	2012
Turkey	Marmaris (MM)	N 36°51', E 28°15'	2012

7.3.3 Fig wasp faunas

From December 2010 to July 2013, several *F. microcarpa* crops were sampled at each site, with each sample comprising at least 10 haphazardly-selected mature figs (late C or early D phases, *sensu* Galil & Eisikowitch 1968). The figs were stored in 70% ethanol prior to dissection. Each fig was sliced into quarters, and the galls inside a fig was softened by being soaked in water for more than 10 minutes before the contents of all the fig flowers were identified under a binocular microscope. All the flowers were sorted into the following categories: male flowers, seeds, unfertilized and undeveloped female flowers, galls containing wasps, and empty galls ('bladders'). Only figs that had been utilized by fig wasps were included in the analyses.

The fig wasps were identified morphologically using primarily Chen et al. (1999) and Feng & Huang (2010), or scored as new morpho-species where necessary. The higher taxonomy of the fig wasps was based on Rasplus et al. (1998), Campbell et al. (2000), Cruaud et al. (2010) and Heraty et al. (2013) as shown in figweb (<http://www.figweb.org>). *Philotrypesis okinavensis* and *P. emeryi* are closely related species, with females and smaller males that are hard to separate. We have segregated these species on the basis of the presence of one, or a line of hairs on the cheeks (present on the head of male *P. okinavensis*, absent in *P. emeryi* males). The names are applied *sensu* Chen et al. (1999), but not Bouček (1993). We did not attempt to distinguish between species of pollinators within the *E. verticillata* complex. Male pollinators could also not be distinguished from those of the non-pollinating 'cheater' congener. In figs where females of both *Eupristina* were present, abundance of male pollinators and cheaters was estimated assuming they had equal sex ratios.

7.3.4 The sizes of fig wasp galls

Body size differences among fig wasps reflect the size of their galls. Gall size contributes to the specificity of galler-parasitoid relationships inside figs because most parasitoids can't enlarge the galls they occupied (Segar et al. 2013). We randomly

selected 68 Asian figs collected from Chengdu, Mianyang, Panzhihua, Xichang and Xishuangbanna (all from mainland China), Taipei (Taiwan) and Manila (the Philippines). At least five galls with adult fig wasp offspring were haphazardly sub-sampled in each fig and their lengths and widths were measured to the nearest 0.04 mm under a dissecting microscope using an eyepiece graticule. Fig wasps inside the measured galls were then identified. The volumes of the galls were calculated assuming their shape to be ellipsoid.

7.3.5 Spatial stratification of fig wasps

Pedicels grow after being galled and their lengths can be used to delineate the spatial distribution of the galls relative to the outer wall of the figs (Yu and Compton 2012). Ovules with longer petioles are located closer to the centre of a fig. The pedicel lengths and contents of all ovules that developed into either seeds or galls (including both failed galls and galls supporting fig wasp adult offspring) were recorded in 20 figs that were randomly chosen from seven Asian *F. microcarpa* crops located in Panzhihua, Xichang, Xishuangbanna (China mainland), Bangkok and Kanchanaburi (Thailand). The pedicel lengths were measured to the nearest 0.02 mm under a dissecting microscope using an eyepiece graticule, and the fig wasp adult offspring inside the galls were then identified. Note that in we did not measure the pedicel lengths of galls containing adult male agaonids (34 galls) in seven figs from Xishuangbanna, where both *Eupristina* taxa were present, because we could not distinguish between their males.

7.3.6 Statistical analyses

All statistical analyses were carried out using R 3.0.0 (R Development Core Team, 2013). Response variables were square root or natural logarithm transformed where necessary. Likelihood ratio tests and multiple tests with Bonferroni corrections were used to estimate the significance of fixed effects and pairwise comparisons respectively.

We tested the differences in fig wasp prevalence, abundance and species richness between gallers and parasitoids using Generalized Linear Models (GLMs) assuming either quasi-binomial (for prevalence) or quasi-Poisson (for abundance and species richness) distributions of residuals.

Parasitoids are expected to (1) negatively impact the abundance of their hosts; (2) emerge in figs with more hosts (density-dependent response) and (3) emerge in galls with similar sizes and pedicel lengths to those containing their hosts (niche overlap).

Potential interactions among species were analysed after the following selection process (1) rare fig wasp species that were present in less than 20 figs was excluded (2) each of the remaining parasitoid species was paired with the remaining species of both phytophages (composed of both *P. taiwanensis* and galler NPFW) and other parasitoids (in case parasitoids were using other parasitoids as hosts). Only the pairs that co-occurred in at least 20 figs were assessed. The relationships between the abundance of the pairs of species were tested using Linear Mixed Models (LMMs) in R package nlme version 3.1 (Pinheiro et al. 2013), setting crop identity as a random effect.

The fig wasps were placed in nine groups including four groups of phytophages: *Eupristina* spp., *Meselatus* spp., *Odontofroggatia* spp. and Otitesellinae spp.; and five groups of parasitoids: *Bruchophagus* spp., *Philotrypesis* spp. (comprising all *Philotrypesis* species except *P. taiwanensis*), *Sycophila* spp., *Sycoryctes* spp. and *Sycoscapter* spp. We did not assign the only seed predator, *P. taiwanensis* into any species group because no parasitoids were found to negatively influence its abundance (see results below). We combined *Walkerella* and *Micranisa* spp. (Pteromalidae, Otitesellinae) because they are closely related and were relatively rare in the figs containing parasitoids. They form similar-sized galls that are intermediate in size between those of *Eupristina* spp. and epichrysomallines. We then assessed the effects of the abundance of parasitoid groups on that of their putative host groups, namely *Eupristina* spp. vs. *Philotrypesis* spp., *Sycoryctes* spp. and *Sycoscapter* spp.; *Meselatus* spp. vs. *Bruchophagus* spp.; *Odontofroggatia* spp. vs. *Sycophila* spp.; and Otitesellinae

spp. vs. *Philotrypesis* spp. using Generalized Linear Mixed Models (GLMM) in R package lme4 version 1.0-5 (Bates et al. 2013), assuming a Poisson distribution of residuals and setting crop identity as a random effect. Only the figs that contained representatives of at least one parasitoid group were included in these analyses.

Response of a parasitoid species/group (absence/presence) to the density of its tentative host species/group was tested using GLMMs assuming binomial distribution in residuals with crop identity as the random effect. The density of host species/group (per fig) was estimated by either the sum of its density and the density of its putative parasitoid species/group or only its density per fig. The independence between the prevalence of parasitoids and the density of their putative hosts may be influenced if their own density is added, but given the low prevalence and density of parasitoids (see results below), such effect could be ignorable. Only parasitoid-host pairs where there were significant negative impacts of parasitoids on their host phytophages were examined based only on figs from those sample sites where both the parasitoids and hosts were present (see Table 7.2 for details).

We tested whether there was niche differentiation among phytophage groups and parasitoid groups with different putative hosts, and among the species allocated to the same species groups, by comparing the sizes and pedicel lengths of their galls using LMMs. Niche overlap in gall size and pedicle length between galls containing adult offspring of each phytophage species/group and those of its putative parasitoid species/group(s) was examined using LMMs. In addition, niche overlap in spatial distributions of galls inside the figs were analysed further by testing the homogeneity of the spatial stratification between galls supporting adult offspring of each phytophage group and those containing its putative parasitoid group(s) (i.e. the interaction between species groups and pedicel length on the proportion of galls containing adult fig wasp offspring, in relation to all the measured ovules) using GLMMs assuming a binomial distribution of residuals. We were not able to describe the spatial stratification of galls at the single species level due to limited sample sizes in most species (each species

emerged from <5% of the measured ovules). Only the pollinating and non-pollinating
agaonids were more abundant. Fig identity was set as a random effect in all analyses.

Table 7.2 Global distributions and prevalence of fig wasp species associated with *F. microcarpa* figs.

Order and abbreviations of sample sites are as in Table 7.1; Order and abbreviations of species are as in Table 7.4. **S_g**: species richness of putative gallers; **S_p**: species richness of putative parasitoids; **S_t**: total fig wasp species richness. Note that the seed predator, *Philotrypesis taiwanensis*, is included with the putative phytophages. Prevalence (proportion of figs occupied) of a species was calculated based on all figs containing fig wasps at each site (figs that contained no fig wasps are excluded).

Site	Putative phytophages														Putative parasitoids														S _t									
	Ev	Es	Aq	Mb	Oc	Og	Oi	Oq	Sbs	Md	Wm	Wn	Ws	Pt	S _g	Bs	Sc	Sm	Smp	Sp	Ss	Ol	Os	Sic	Pe	Po	Ps1	Ps2		Ps3	Srm	Srs	Scg	Scs	S _p			
Native range																																						
GZ	75				2	28	12	6		2	24			36	8			20		8		1			4	28					20		16		7	15		
XS	70	65	0.4		11	0.4	6	4		9	14	5		21	11		1	3							15	6				4	9	19		7	18			
TB	45				2	72	9	1		10	3			21	8			39	7	58	2	2			3	3				10		22	2	10	18			
BK	46			21	21		10	8		3	5		33	18	9										3	13	3	10						4	13			
CM	100				2							9		2	4															11		13		2	6			
KC	68			3	24		17			10	14			35	7			6	10	8					8	2	2	2	6	16		3	10	17				
MN	100													10	2														80					1	3			
Introduced range																																						
BR	95					43		20						3			33	15																	2	5		
RJ	78						19				38			25	4										1	44									2	6		
TN	100									37					2																				0	2		
CD	30			1	1	65					5			5																					0	5		
KM		38	2	8	52	31			10	33				7	6		21						2			35								4	11			
MY				94	15									2																					0	2		

PZ	12	20	29	27	7	61	1	7	14	7	4	4	25	5	12	
SM	100		6	4	4	1	6	6					1	1	7	
XC	2	2	66	62	3	27		6		30	6	6		3	9	
DV	75			84		91	19	4		3			5	48	3	7
RD	23	60		26		1		4		9			2		2	6
SY	24	65		16	3			4					8		1	5
SC	88			63		1		3					1		1	4
TP	78			76				2							0	2
MT	44			95				2							0	2
PR	94				17	49	11	17	5				6	2	2	7
GH				18			93		2						0	2
PE				2			100		2						0	2
MJ	69			40		5	10		4						0	4
MM	100								1						0	1

7.4 Results

7.4.1 Fig wasp communities

We recorded the contents of 2681 figs that contained fig wasps, obtained from 192 crops. A total of 99038 fig wasps were present (Table 7.3). The mean numbers of male and female flowers in the figs were about 15 and 160 respectively (Table 7.3). Around 27% of the female flowers were galled by fig wasps, and about 8% developed seeds (Table 7.3). The remaining female flowers remained undeveloped.

We identified a total of 32 fig wasp morpho-species, comprising 14 phytophages and 18 parasitoids (Table 7.4). Eleven of these species were not previously described from *F. microcarpa* figs, including 10 new morpho-species belonging to the genera *Eupristina* (1 species, the ‘cheater’), *Sycobia* (1 species), *Walkerella* (1 species), *Ormyrus* (1 species), *Philotrypesis* (3 species), *Sycophila* (1 species), *Sycoryctes* (1 species) and *Sycoscapter* (1 species). One further species, *Walkerella nigrabdomina* Ma & Yang, has been also recorded previously from *Ficus pisocarpa* (Table 7.4). Each of these newly-recorded species were only present at one or two sites, and usually at extremely low prevalence (present in a small proportion of the figs) (Table 7.4). They may be species that mainly utilize figs of other fig tree species, but occasionally colonise *F. microcarpa*. In addition, small numbers of several native African fig wasp species have colonized *F. microcarpa* figs in South Africa, where pollinators of this species are absent (van Noort et al. 2013).

Although the pollinating agaonid was entirely absent from four sites (in South Africa and outside the native range in China – sites GH, KM, MY and PE), it was the dominant fig wasp species in both the introduced and native ranges of the plant (Tables 7.3 and 7.4). Generally, phytophagous NPFW were far more common than parasitoids, with higher prevalence rates and abundance per fig (GLM: prevalence (binomial): phytophage NPFW vs. parasitoids: $df=1$, $LR=1435.600$, $p<0.001$; abundance (quasi-Poisson): phytophage NPFW vs. parasitoids: $df=1$, $LR=30970.000$, $p<0.001$;

Table 7.5).

Fig wasp species richness varied from 1 to 18 across the sites with an average total species richness per fig of 1.962 ± 0.023 (mean \pm SE), comprising 0.410 ± 0.014 parasitoid species per fig and 1.552 ± 0.015 phytophages (GLM: phytophage vs. parasitoid species richness (quasi-Poisson): $df=1$, $LR=1900.9$, $p<0.001$; Fig. 7.1 a-f). Parasitoids were entirely absent from nine sites in the plant's introduced range. Species of *Eupristina*, *Meselatus*, *Odontofroggata*, *Micranisa*, *Walkerella*, *Bruchophagus*, *Sycophila*, *Philotrypesis*, *Sycoryctes* and *Sycosapter* were widespread, usually with high prevalence within the sites where they occurred, whereas *Acophila*, *Sycobia*, *Ormyrus* and *Sirovena* species were rare and are excluded from the following analyses (Table 7.4).

Table 7.3 Sample sizes and contents of *F. microcarpa* figs (means \pm SE).

We only included figs that had been colonized by fig wasps when calculating sample sizes and all calculations are based only on figs containing fig wasps. Occupancy rate is the proportion of ovules containing fig wasp adult offspring.

Area	Site (abbreviation)	N crops	N figs	Male flowers	Female flowers	Prevalence pollinators (%)	Pollinator abundance	Prevalence NPFW (%)	NPFW abundance	Total wasp abundance	Occupancy rate (%)	Seeds
Native range												
China mainland	Guangzhou (GZ)	12	169	14.1 \pm 0.4	134.4 \pm 2.8	75.1	24.2 \pm 2.1	89.3	11.7 \pm 0.8	35.9 \pm 2.1	26.2 \pm 1.3	13.6 \pm 1.2
China mainland	Xishuangbanna (XS)	17	279	16.0 \pm 0.4	192.9 \pm 2.7	70.3	23.7 \pm 1.8	83.2	54.4 \pm 2.7	78.1 \pm 2.3	40.4 \pm 1.0	20.2 \pm 1.7
Taiwan	Taibei (TB)	11	220	13.9 \pm 0.4	142.8 \pm 2.8	45.5	11.8 \pm 1.3	94.5	17.7 \pm 0.9	29.5 \pm 1.6	21.1 \pm 1.0	10.4 \pm 1.1
Thailand	Bangkok (BK)	4	39	12.7 \pm 1.2	130.9 \pm 9.1	46.2	12.5 \pm 2.6	89.7	19.1 \pm 3.3	31.6 \pm 3.4	32.7 \pm 5.1	9.9 \pm 2.1
Thailand	Chiang Mai (CM)	4	47	22.8 \pm 1.1	202.8 \pm 3.8	100.0	67.6 \pm 5.8	31.9	0.9 \pm 0.3	68.6 \pm 5.8	33.5 \pm 2.7	35.9 \pm 3.1
Thailand	Kanchanaburi (KC)	6	63	14.0 \pm 0.8	154.8 \pm 7.9	68.3	20.8 \pm 2.9	76.2	15.6 \pm 1.8	36.3 \pm 3.6	24.7 \pm 2.1	15.8 \pm 2.1
The Philippines	Manila (MN)	2	40	20.8 \pm 1.0	197.3 \pm 4.0	100.0	35.0 \pm 5.2	80.0	11.8 \pm 2.0	46.8 \pm 4.9	23.8 \pm 2.4	33.1 \pm 2.4
Overall	--	56	857	15.4 \pm 0.2	163.6 \pm 1.7	66.6	23.0 \pm 1.0	84.1	27.2 \pm 1.2	50.1 \pm 1.3	30.0 \pm 0.6	17.1 \pm 0.8
Introduced range												
Australia	Brisbane (BR)	2	40	18.1 \pm 0.9	160.5 \pm 5.5	95.0	26.2 \pm 2.6	62.5	6.6 \pm 1.3	32.8 \pm 2.4	22.1 \pm 1.8	16.8 \pm 1.8
Brazil	Rio de Janeiro (RJ)	6	130	13.5 \pm 0.5	106.7 \pm 3.9	77.7	15.5 \pm 1.9	79.2	3.8 \pm 0.4	19.3 \pm 1.9	15.6 \pm 1.2	22.4 \pm 2.3
Canary Islands	Tenerife (TN)	1	30	15.7 \pm 1.0	168.6 \pm 6.1	100.0	40.3 \pm 3.0	36.7	1.3 \pm 0.4	41.6 \pm 2.9	25.5 \pm 1.9	31.1 \pm 2.3
China mainland	Chengdu (CD)	3	20	15.6 \pm 1.0	165.5 \pm 4.4	30.0	1.9 \pm 0.7	85.0	8.7 \pm 1.8	10.6 \pm 1.6	6.6 \pm 1.2	2.5 \pm 0.9
China mainland	Kunming (KM)	6	48	16.9 \pm 1.4	200.1 \pm 9.2	0	0	100.0	32.0 \pm 3.0	32.0 \pm 3.0	17.7 \pm 2.0	0.2 \pm 0.1
China mainland	Mianyang (MY)	5	33	2.6 \pm 1.0	43.0 \pm 6.6	0	0	100.0	21.1 \pm 2.2	21.1 \pm 2.2	61.6 \pm 4.9	0

China mainland	Panzhuhua (PZ)	10	136	11.6 ± 0.7	159.1 ± 7.0	11.8	3.6 ± 1.2	98.5	16.1 ± 1.0	19.7 ± 1.3	23.7 ± 2.5	2.6 ± 0.8
China mainland	Sanming (SM)	4	71	15.0 ± 0.6	178.7 ± 5.0	100.0	42.7 ± 3.3	16.9	2.5 ± 0.8	45.2 ± 3.1	26.2 ± 1.6	31.4 ± 1.5
China mainland	Xichang (XC)	12	181	16.7 ± 0.4	173.1 ± 3.5	1.7	0.2 ± 0.1	99.4	16.6 ± 0.9	16.7 ± 0.9	12.7 ± 1.1	0.2 ± 0.1
Florida (USA)	Davie (DV)	4	100	17.2 ± 0.6	172.8 ± 4.1	75.0	13.0 ± 2.0	99.0	22.7 ± 0.9	35.7 ± 2.1	21.9 ± 1.4	3.7 ± 0.5
Greece	Rhodes (RD)	27	294	7.3 ± 0.5	93.3 ± 4.4	22.8	14.3 ± 2.0	89.1	25.7 ± 1.4	40.0 ± 2.0	53.7 ± 1.6	7.4 ± 1.2
Greece	Symi (SY)	4	37	5.5 ± 1.3	76.0 ± 10.3	24.3	9.1 ± 4.1	89.2	22.9 ± 2.5	32.0 ± 3.7	59.7 ± 4.7	2.9 ± 1.1
Italy	Sicily (SC)	10	99	20.8 ± 0.8	237.8 ± 4.6	87.9	44.5 ± 3.8	64.6	19.8 ± 2.2	64.3 ± 3.5	26.5 ± 1.3	27.5 ± 2.9
Libya	Tripoli (TP)	7	96	19.8 ± 0.6	190.6 ± 3.1	78.1	39.4 ± 3.9	76.0	12.5 ± 1.3	51.9 ± 3.2	27.6 ± 1.7	25.0 ± 2.3
Malta	Malta (MT)	9	130	18.4 ± 0.5	190.8 ± 3.6	43.8	18.8 ± 2.5	94.6	23.8 ± 1.4	42.6 ± 2.3	22.1 ± 1.1	10.3 ± 1.6
Puerto Rico	Puerto Rico (PR)	7	47	14.1 ± 0.9	173.0 ± 6.8	93.6	42.0 ± 6.9	63.8	4.5 ± 0.8	46.4 ± 6.5	23.6 ± 2.8	25.2 ± 4.4
South Africa	Grahamstown (GH)	7	140	20.9 ± 0.5	168.4 ± 4.5	0	0	100.0	7.0 ± 0.6	7.0 ± 0.6	4.4 ± 0.4	0
South Africa	Port Elizabeth (PE)	5	81	23.5 ± 0.8	194.6 ± 2.8	0	0	100.0	5.6 ± 0.6	5.6 ± 0.6	2.9 ± 0.3	0
Spain	Majorca (MJ)	6	101	19.1 ± 0.6	199.3 ± 4.0	69.3	9.7 ± 1.0	53.5	7.1 ± 0.9	16.7 ± 1.1	8.6 ± 0.5	7.7 ± 1.2
Turkey	Marmaris (MM)	1	10	26.3 ± 2.2	263.0 ± 6.1	100.0	93.1 ± 10.8	0	0	93.1 ± 10.8	35.7 ± 4.3	113.3 ± 8.9
Overall	--	136	1824	15.1 ± 0.2	158.6 ± 1.6	41.6	15.5 ± 0.7	83.4	15.3 ± 0.4	30.7 ± 0.7	25.5 ± 0.6	10.6 ± 0.5
All figs												
Overall	--	192	2681	15.2 ± 0.2	160.3 ± 1.2	49.6	17.9 ± 0.6	83.7	19.1 ± 0.5	36.9 ± 0.6	26.9 ± 0.4	12.7 ± 0.4

Table 7.4 Summary of fig wasp taxa associated with *F. microcarpa* (with abbreviations).

The seed predator, *Philotrypesis taiwanensis* is included with gallers in the putative phytophages. Calculations of prevalence (proportion of figs occupied) were based on all figs with fig wasps in the native or introduced ranges of the plant.

Trophic level	Wasp taxon	Native range		Introduced range	
		N sites present	Prevalence (%)	N sites present	Prevalence (%)
Putative phytophages					
	Agaonidae, Agaoninae				
<i>Ev</i>	<i>Eupristina verticillata</i> Waterston	7	66.6	16	41.6
<i>Es</i>	<i>Eupristina</i> sp. ('Cheater')	1	21.0	1	1.0
	Pteromalidae, Epichrysomallinae				
<i>Aq</i>	<i>Acophila quinata</i> Zhang & Xiao	1	0.1	1	0.1
<i>Mb</i>	<i>Meselatus bicolor</i> Chen	2	1.2	7	14.6
<i>Oc</i>	<i>Odontofroggatia corneri</i> Wiebes	6	10.2	6	11.2
<i>Og</i>	<i>Odontofroggatia galili</i> Wiebes	3	24.0	15	37.8
<i>Oi</i>	<i>Odontofroggatia ishii</i> Wiebes	5	8.4	4	2.0
<i>Oq</i>	<i>Odontofroggatia quinifuniculus</i> Feng & Huang	4	2.9	1	0.4
<i>Sbs</i>	<i>Sycobia</i> sp.	0	0	2	0.8
	Pteromalidae, Otitesellinae				
<i>Md</i>	<i>Micranisa degastris</i> Chen	5	6.8	6	7.5
<i>Wm</i>	<i>Walkerella microcarpae</i> Bouček	5	11.6	12	23.7
<i>Wn</i>	<i>Walkerella nigrabdomina</i> Ma & Yang	2	2.0	0	0
<i>Ws</i>	<i>Walkerella</i> sp.	1	1.5	0	0
	Pteromalidae, Sycoryctinae				
<i>Pt</i>	<i>Philotrypesis taiwanensis</i> Chen	7	23.1	4	3.3

Putative parasitoids

Eurytomidae

<i>Bs</i>	<i>Bruchophagus sensoriae</i> Chen	0	0	2	1.2
<i>Sc</i>	<i>Sycophila curta</i> Chen	1	0.2	0	0
<i>Sm</i>	<i>Sycophila maculafacies</i> Chen	4	15.4	6	6.3
<i>Smp</i>	<i>Sycophila maculafacies</i> ('pale')	2	2.5	3	1.3
<i>Sp</i>	<i>Sycophila petiolata</i> Chen	3	17.0	2	0.9
<i>Ss</i>	<i>Sycophila</i> sp.	1	0.6	0	0

Ormyridae

<i>Ol</i>	<i>Ormyrus lini</i> Chen	2	0.8	0	0
<i>Os</i>	<i>Ormyrus</i> sp.	0	0	1	0.1

Pteromalidae, Pireninae

<i>Sic</i>	<i>Sirovena costallifera</i> Li, Xiao & Huang	1	0.1	0	0
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Pteromalidae, Sycoryctinae

<i>Pe</i>	<i>Philotrypesis emeryi</i> Grandi	5	7.7	5	0.7
<i>Po</i>	<i>Philotrypesis okinavensis</i> Ishii	5	8.3	6	6.8
<i>Ps1</i>	<i>Philotrypesis</i> sp.1	2	0.6	0	0
<i>Ps2</i>	<i>Philotrypesis</i> sp.2	1	0.1	0	0
<i>Ps3</i>	<i>Philotrypesis</i> sp.3	1	0.5	0	0
<i>Srm</i>	<i>Sycoryctes moneres</i> Chen	6	13.1	0	0
<i>Srs</i>	<i>Sycoryctes</i> sp.	1	2.8	0	0
<i>Scg</i>	<i>Sycoscapter gajimaru</i> Ishii	5	15.9	1	2.6
<i>Scs</i>	<i>Sycoscapter</i> sp.	1	0.5	0	0

Table 7.5 Prevalence and abundance of phytophagous NPFW (including the seed predator, *Philotrypesis taiwanensis*) and their parasitoids (means \pm SE).

Site order and abbreviations of sample sites are as in Table 7.1. Calculations were based on all figs containing fig wasps.

Site	Phytophage NPFW		Parasitoid	
	Prevalence (%)	Abundance	Prevalence (%)	Abundance
Native range				
GZ	81.1	7.2 \pm 0.6	57.4	4.4 \pm 0.5
XS	81.7	51.3 \pm 2.7	39.1	2.8 \pm 0.3
TB	85.9	9.4 \pm 0.7	78.6	8.2 \pm 0.5
BK	76.9	17.1 \pm 3.4	25.6	1.5 \pm 0.6
CM	10.6	0.3 \pm 0.2	21.3	0.5 \pm 0.2
KC	73.0	11.9 \pm 1.5	42.9	3.4 \pm 0.9
MN	10.0	0.1 \pm 0.1	80.0	11.7 \pm 1.9
Overall	75.0	22.2 \pm 1.2	53.4	4.8 \pm 0.3
Introduced range				
BR	62.5	4.5 \pm 0.9	32.5	2.2 \pm 0.6
RJ	64.6	2.3 \pm 0.3	44.6	1.5 \pm 0.3
TN	36.7	1.3 \pm 0.4	0	0
CD	85.0	8.7 \pm 1.8	0	0
KM	100.0	26.0 \pm 3.1	52.1	5.9 \pm 1.4
MY	100.0	21.1 \pm 2.2	0	0
PZ	98.5	12.5 \pm 0.8	45.4	3.6 \pm 0.5
SM	16.9	2.4 \pm 0.8	1.4	0.1 \pm 0.1
XC	99.4	12.7 \pm 0.8	39.2	3.9 \pm 0.5
DV	99.0	19.2 \pm 0.8	53.0	3.5 \pm 0.5
RD	86.7	22.4 \pm 1.4	9.9	0.5 \pm 0.2
SY	83.8	20.9 \pm 2.6	8.1	1.4 \pm 0.9
SC	63.6	19.7 \pm 2.2	1.0	0.02 \pm 0.02
TP	76.0	12.5 \pm 1.3	0	0
MT	94.6	23.8 \pm 1.4	0	0
PR	55.3	3.8 \pm 0.8	27.7	0.7 \pm 0.2
GH	100.0	7.0 \pm 0.6	0	0
PE	100.0	5.6 \pm 0.6	0	0
MJ	53.5	7.1 \pm 0.9	0	0
MM	0	0	0	0
Overall	81.6	13.5 \pm 0.4	18.0	1.3 \pm 0.1
All figs				
Overall	79.5	16.3 \pm 0.5	29.3	2.4 \pm 0.1

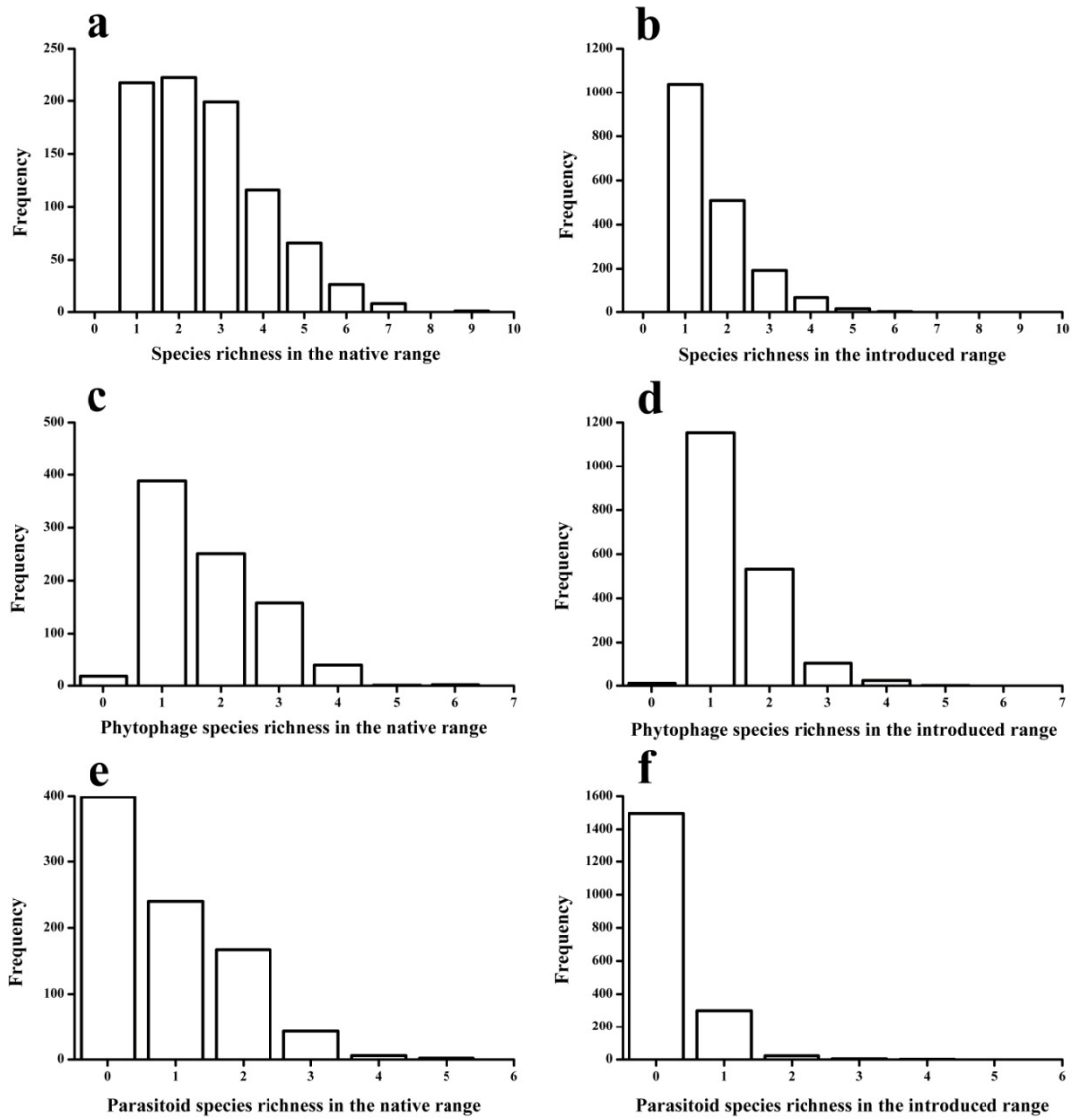


Fig. 7.1 Frequency distributions of species richness per fig for all species (a & b), phytophages (c & d) and parasitoids (e & f) in the native and introduced ranges of *F. microcarpa*.

Only figs that contained at least one species of fig wasp are included.

7.4.2 Species interactions

A total of 34 fig wasp species pairs co-occurred in more than 20 figs (Table 7.6). These pairs involved 18 (9 phytophage and 9 parasitoid) species that comprised 98.4% (97476 individuals) of the total fig wasps recorded overall. *E. verticillata* was negatively correlated with *Philotrypesis emeryi* Grandi, *Philotrypesis okinavensis* Ishii, *Sycoryctes moneres* Chen and *Sycoscapter gajimaru* Ishii. The ‘cheater’ *Eupristina* sp., which has much more limited distribution, was negatively correlated with *Sycoryctes* sp. and *S. gajimaru* (Table 7.6; Fig. 7.2). *Odontofroggattia corneri* Wiebes and *Odontofroggattia galili* Wiebes were the likely hosts of three *Sycophila* species (*Sycophila maculafacies* Chen, *Sycophila maculafacies* ‘pale’ and *Sycophila petiolata* Chen), but abundance of *Odontofroggattia ishii* Wiebes was only negatively correlated with *S. maculafacies* (Table 7.6; Fig. 7.2). In addition, *Bruchophagus sensoriae* Chen and *P. okinavensis* strongly suppressed the abundance of *M. bicolor* and *Walkerella microcarpa* Bouček respectively (Table 7.6; Fig. 7.2). We failed to detect any evidence for negative impacts of parasitoids on any other parasitoids (Table 7.6). Seeds have a characteristic appearance. Only the seed predator, *P. taiwanensis*, and no parasitoids were recorded as emerging from seeds.

When phytophagous hosts were grouped together, *Eupristina* spp. was adversely influenced by *Philotrypesis* spp., *Sycoryctes* spp. and *Sycoscapter* spp., and *Meselatus*, *Odontofroggattia* spp. and *Otitesellinae* spp. had negative associations with the numbers of their presumed parasitoids, *Bruchophagus*, *Sycophila* spp. and *Philotrypesis* spp. respectively (Table 7.7; Fig. 7.3 a-f).

Table 7.6 Interactions between pairs of species where more than 20 figs contained both species (LMMs).

LR=Likelihood ratio. Species abbreviations and order are as in Table 7.4.

Species pairs (n figs)	Slope (mean \pm SE)	df	LR
<i>Ev</i> vs. <i>Sm</i> (n=60)	-0.013 \pm 0.033	1	0.168 ^{NS}
<i>Ev</i> vs. <i>Sp</i> (n=31)	0.111 \pm 0.122	1	0.831 ^{NS}
<i>Ev</i> vs. <i>Pe</i> (n=55)	-2.147 \pm 0.953	1	5.013 [*]
<i>Ev</i> vs. <i>Po</i> (n=106)	-0.101 \pm 0.045	1	5.044 [*]
<i>Ev</i> vs. <i>Srm</i> (n=110)	-0.023 \pm 0.010	1	4.927 [*]
<i>Ev</i> vs. <i>Scg</i> (n=144)	-0.050 \pm 0.015	1	10.760 ^{**}
<i>Es</i> vs. <i>Pe</i> (n=34)	0.013 \pm 0.024	1	0.303 ^{NS}
<i>Es</i> vs. <i>Srs</i> (n=20)	-1.814 \pm 0.485	1	11.509 ^{***}
<i>Es</i> vs. <i>Scg</i> (n=51)	-1.736 \pm 0.773	1	4.974 [*]
<i>Mb</i> vs. <i>Bs</i> (n=22)	-0.470 \pm 0.092	1	18.411 ^{***}
<i>Oc</i> vs. <i>Sm</i> (n=89)	-0.039 \pm 0.017	1	5.066 [*]
<i>Oc</i> vs. <i>Smp</i> (n=20)	-0.048 \pm 0.023	1	4.419 [*]
<i>Oc</i> vs. <i>Sp</i> (n=24)	-0.113 \pm 0.041	1	6.969 ^{**}
<i>Oc</i> vs. <i>Po</i> (n=44)	-0.023 \pm 0.015	1	2.258 ^{NS}
<i>Og</i> vs. <i>Sm</i> (n=158)	-0.058 \pm 0.022	1	6.739 ^{**}
<i>Og</i> vs. <i>Smp</i> (n=23)	-0.206 \pm 0.081	1	6.230 [*]
<i>Og</i> vs. <i>Sp</i> (n=124)	-0.068 \pm 0.023	1	7.924 ^{**}
<i>Og</i> vs. <i>Srm</i> (n=22)	-0.105 \pm 0.078	1	1.673 ^{NS}
<i>Og</i> vs. <i>Scg</i> (n=77)	0.010 \pm 0.018	1	0.323 ^{NS}
<i>Oi</i> vs. <i>Sm</i> (n=23)	-1.136 \pm 0.465	1	3.854 [*]
<i>Oi</i> vs. <i>Sp</i> (n=21)	0.145 \pm 0.076	1	2.537 ^{NS}
<i>Oi</i> vs. <i>Po</i> (n=26)	-0.003 \pm 0.031	1	0.012 ^{NS}
<i>Md</i> vs. <i>Scg</i> (n=76)	0.021 \pm 0.018	1	1.478 ^{NS}
<i>Wm</i> vs. <i>Sm</i> (n=29)	0.037 \pm 0.034	1	1.101 ^{NS}
<i>Wm</i> vs. <i>Po</i> (n=119)	-0.034 \pm 0.013	1	6.807 ^{**}
<i>Pt</i> vs. <i>Pe</i> (n=21)	0.028 \pm 0.423	1	0.005 ^{NS}
<i>Pt</i> vs. <i>Po</i> (n=43)	-0.008 \pm 0.032	1	0.065 ^{NS}
<i>Pt</i> vs. <i>Srm</i> (n=47)	-0.028 \pm 0.021	1	1.666 ^{NS}
<i>Pt</i> vs. <i>Scg</i> (n=59)	-0.018 \pm 0.023	1	0.602 ^{NS}
<i>Sm</i> vs. <i>Sp</i> (n=92)	0.019 \pm 0.017	1	1.211 ^{NS}
<i>Sm</i> vs. <i>Po</i> (n=24)	-0.024 \pm 0.035	1	0.536 ^{NS}
<i>Pe</i> vs. <i>Scg</i> (n=26)	-0.009 \pm 0.030	1	0.094 ^{NS}
<i>Po</i> vs. <i>Srm</i> (n=22)	0.025 \pm 0.026	1	1.036 ^{NS}
<i>Srm</i> vs. <i>Scg</i> (n=30)	0.015 \pm 0.028	1	0.328 ^{NS}

^{NS}: not significant; ^{*}: p<0.05; ^{**}: p<0.01; ^{***}: p<0.001.

Table 7.7 Pairwise interactions of parasitoid and phytophage groups using GLMMs that assumed Poisson distributions of residuals.

Group pairs (n figs with phyophages)/ Parasitoids)	β value (mean \pm SE)	df	Likelihood ratio
<i>Eupristina</i> spp. and <i>Philotrypesis</i> spp. (187/290)	-0.040 \pm 0.004	1	133.540 ***
<i>Eupristina</i> spp. and <i>Sycoryctes</i> spp. (134/136)	-0.034 \pm 0.003	1	200.360 ***
<i>Eupristina</i> spp. and <i>Sycoscapter</i> spp. (166/184)	-0.039 \pm 0.003	1	175.460 ***
<i>Meselatus</i> and <i>Bruchophagus</i> (22/22)	-0.088 \pm 0.010	1	70.374 ***
<i>Odontofroggatia</i> spp. and <i>Sycophila</i> spp. (323/348)	-0.051 \pm 0.005	1	135.040 ***
Otitesellinae spp. and <i>Philotrypesis</i> spp. (181/290)	-0.050 \pm 0.007	1	65.391 ***

***:p<0.001.

Spp. = more than one species

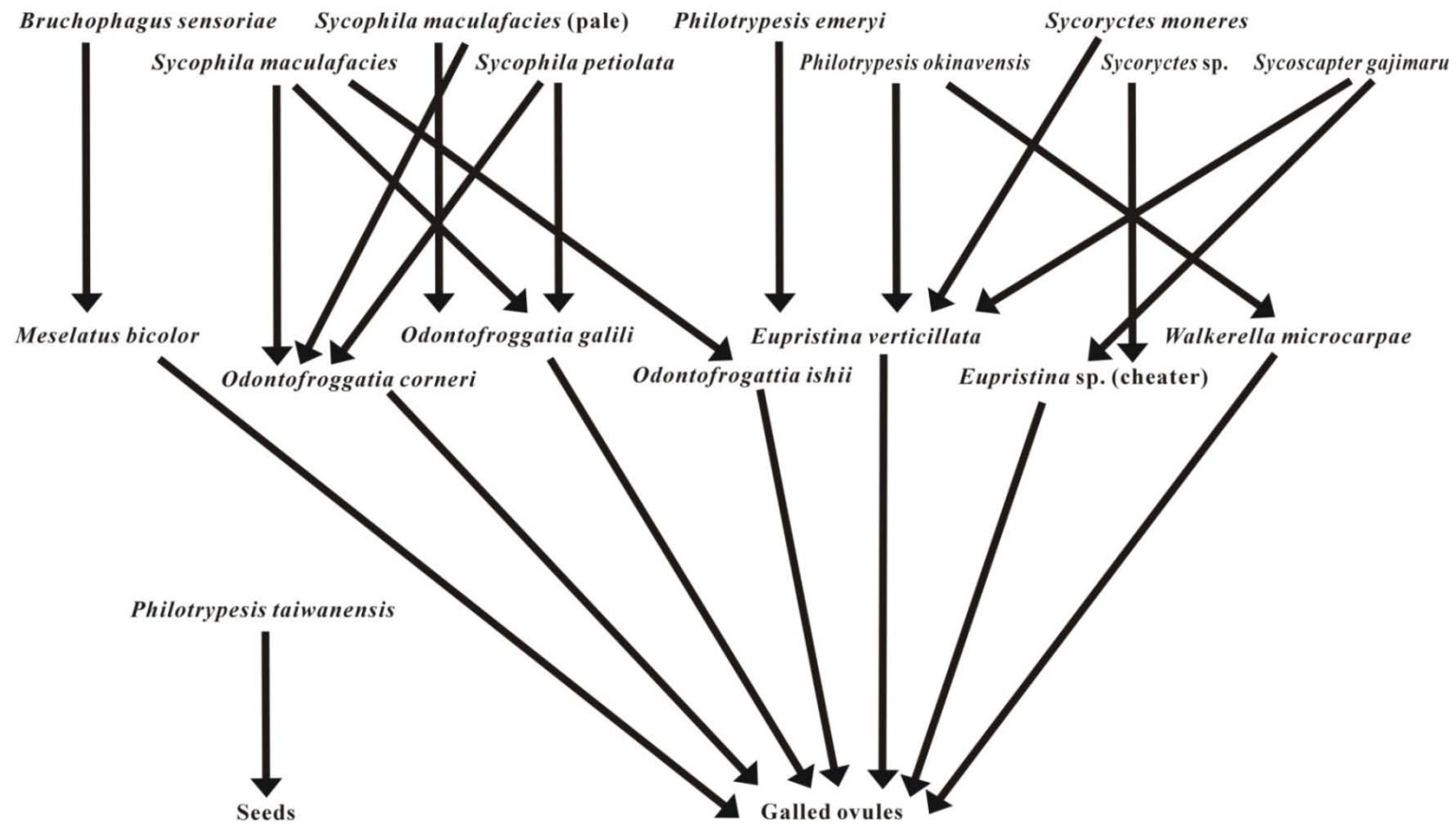


Fig. 7.2 Proposed major interactions in the fig wasp food web in figs *F. microcarpa*.

Additional interactions are highly likely, but were too infrequent to be detected in our analyses. For example *Sycoryctes* sp. had a localized distribution, where it had few opportunities to utilise *E. verticillata*.

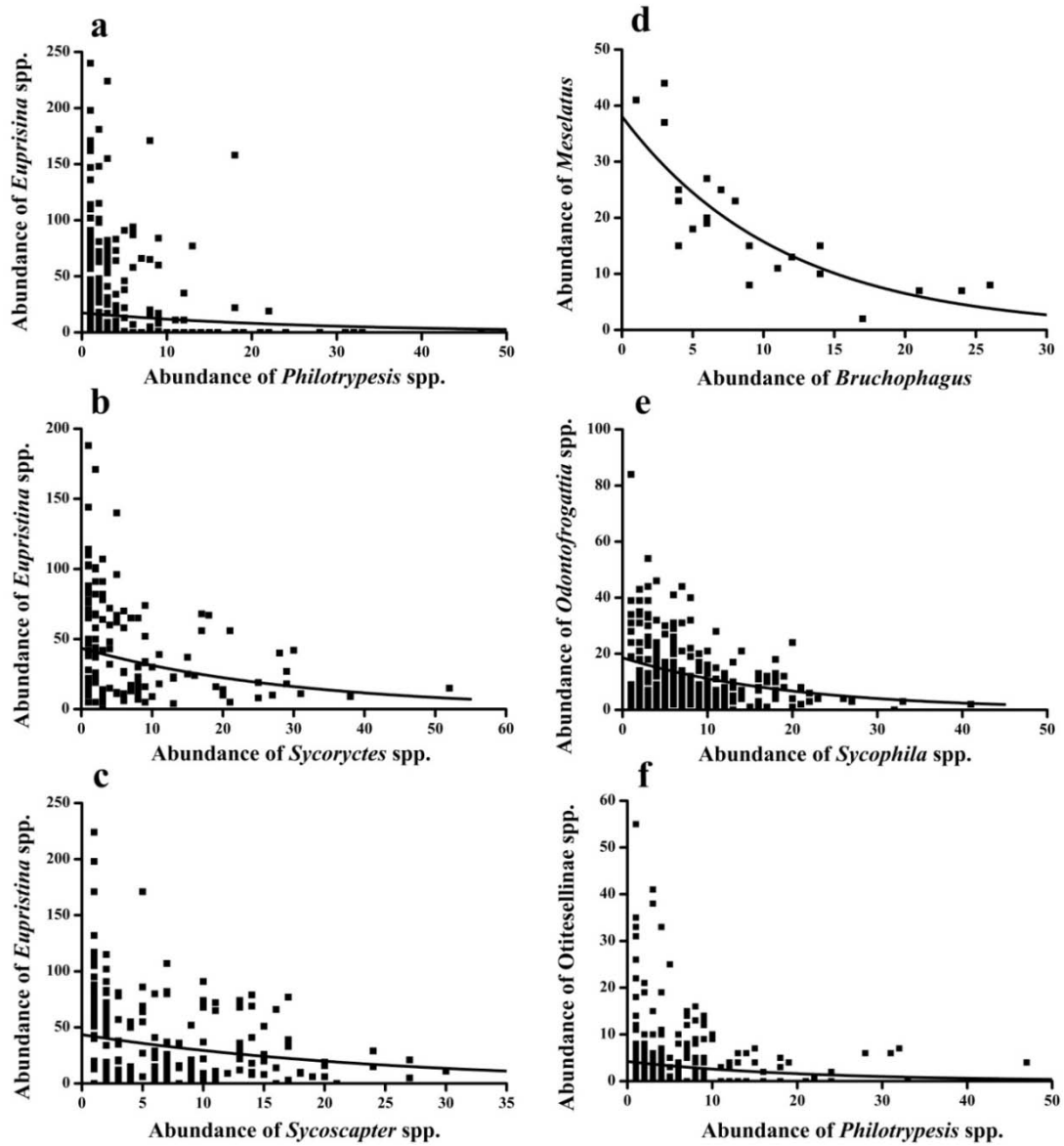


Fig. 7.3 Interactions between the numbers of galls per fig and their putative parasitoid groups using GLMMs assuming Poisson distribution of residuals (a-f).

7.4.3 Density-dependent responses of parasitoids

The 15 species pairs in which negative interactions between phytophages and parasitoids were detected were selected to test for density-dependent responses of parasitoids to their hosts. Phytophages at sites where their putative parasitoids were absent were excluded from the analyses.

When the density of putative parasitoids was included into the estimation of the density of their particular hosts, most parasitoid species were more likely to be recorded from the figs that contained high densities of their putative hosts, but exceptions were provided by *P. okinavensis* with *E. verticillata*, and *Sycoryctes* sp. and *S. gajimaru* with *Eupristina* sp. (cheater) (Table 7.8). Strong positive relationships were also detected between the proportion of figs where parasitoids emerged and the abundance of their putative host group(s) (Table 7.9; Fig. 7.4 a-f). When only the density of hosts was considered, we obtained similar results (Tables 7.8 & 7.9).

Table 7.8 Density-dependent responses of parasitoids to their putative host galler species (GLMMs assuming binomial distribution of residuals).

Species abbreviations and order are as in Table 7.4. Note that only the species pairs with significant negative interactions are included (Table 7.6). Sample size is presented as n figs with parasitoid group/n figs with galler group, i.e. n figs (P)/n figs (G).

Group pair (n figs (G)/ n figs (P))	Host density including putative parasitoids			Host density not including putative parasitoids		
	β value (mean \pm SE)	df	LR	β value (mean \pm SE)	df	LR
<i>Pe</i> vs. <i>Ev</i> (55/744)	0.017 \pm 0.005	1	11.360 ***	0.013 \pm 0.005	1	6.664 **
<i>Po</i> vs. <i>Ev</i> (106/773)	-0.001 \pm 0.006	1	0.005 NS	-0.007 \pm 0.007	1	1.189 NS
<i>Srm</i> vs. <i>Ev</i> (110/553)	0.024 \pm 0.006	1	20.673 ***	0.017 \pm 0.005	1	9.909 **
<i>Scg</i> vs. <i>Ev</i> (144/588)	0.027 \pm 0.007	1	20.363 ***	0.013 \pm 0.006	1	4.904 *
<i>Srs</i> vs. <i>Es</i> (20/180)	0.013 \pm 0.011	1	1.398 NS	0.002 \pm 0.009	1	0.037 NS
<i>Scg</i> vs. <i>Es</i> (51/180)	0.005 \pm 0.006	1	0.762 NS	-0.001 \pm 0.006	1	0.027 NS
<i>Bs</i> vs. <i>Mb</i> (22/31)	1.034 \pm 0.729	1	32.700 ***	0.115 \pm 0.068	1	4.329 *
<i>Sm</i> vs. <i>Oc</i> (89/263)	0.216 \pm 0.031	1	110.390 ***	0.102 \pm 0.021	1	31.077 ***
<i>Smp</i> vs. <i>Oc</i> (20/175)	0.094 \pm 0.026	1	14.111 ***	0.009 \pm 0.031	1	0.079 NS
<i>Sp</i> vs. <i>Oc</i> (24/208)	0.103 \pm 0.026	1	18.352 ***	0.051 \pm 0.023	1	4.198 *
<i>Sm</i> vs. <i>Og</i> (158/532)	0.150 \pm 0.023	1	62.330 ***	0.041 \pm 0.017	1	5.109 *
<i>Smp</i> vs. <i>Og</i> (24/324)	0.186 \pm 0.040	1	34.870 ***	0.108 \pm 0.030	1	12.383 ***
<i>Sp</i> vs. <i>Og</i> (124/354)	0.199 \pm 0.043	1	32.553 ***	0.071 \pm 0.032	1	5.027 *
<i>Sm</i> vs. <i>Oi</i> (23/50)	2.460 \pm 0.723	1	50.326 ***	0.670 \pm 0.391	1	19.890 ***
<i>Po</i> vs. <i>Wm</i> (119/255)	0.362 \pm 0.060	1	103.880 ***	0.122 \pm 0.033	1	18.108 ***

NS: not significant; *: p<0.05; **: p<0.01; ***: p<0.001.

Table 7.9 Density-dependent responses of parasitoid groups to their putative host galler groups using GLMMs with binomial distributions of residuals.

Sample sizes of figs containing phytophages and parasitoids are in parentheses.

Group pairs (n figs with parasitoids)/ Phytophages)	Host density including putative parasitoids			Host density not including putative parasitoids		
	β value (mean \pm SE)	df	Likelihood ratio	β value (mean \pm SE)	df	Likelihood ratio
<i>Philotrypesis</i> spp. and. <i>Eupristina</i> spp. (187/1048)	0.011 \pm 0.003	1	10.478 **	0.007 \pm 0.003	1	3.981 *
<i>Sycoryctes</i> spp. and. <i>Eupristina</i> spp. (134/629)	0.018 \pm 0.005	1	15.153 ***	0.010 \pm 0.004	1	4.477 *
<i>Sycoscapter</i> spp. and. <i>Eupristina</i> spp. (166/664)	0.020 \pm 0.005	1	17.856 ***	0.010 \pm 0.004	1	4.302 *
<i>Bruchophagus</i> and <i>Meselatus</i> (22/31)	1.034 \pm 0.729	1	32.700 ***	0.115 \pm 0.068	1	4.329 *
<i>Sycophila</i> spp. and <i>Odontofroggatia</i> spp. (323/797)	0.260 \pm 0.026	1	252.500 ***	0.116 \pm 0.016	1	66.508 ***
<i>Philotrypesis</i> spp. and Otitesellinae spp. (181/476)	0.214 \pm 0.029	1	94.181 ***	0.041 \pm 0.020	1	4.309 *

* p<0.05; ** p<0.01; *** p<0.001.

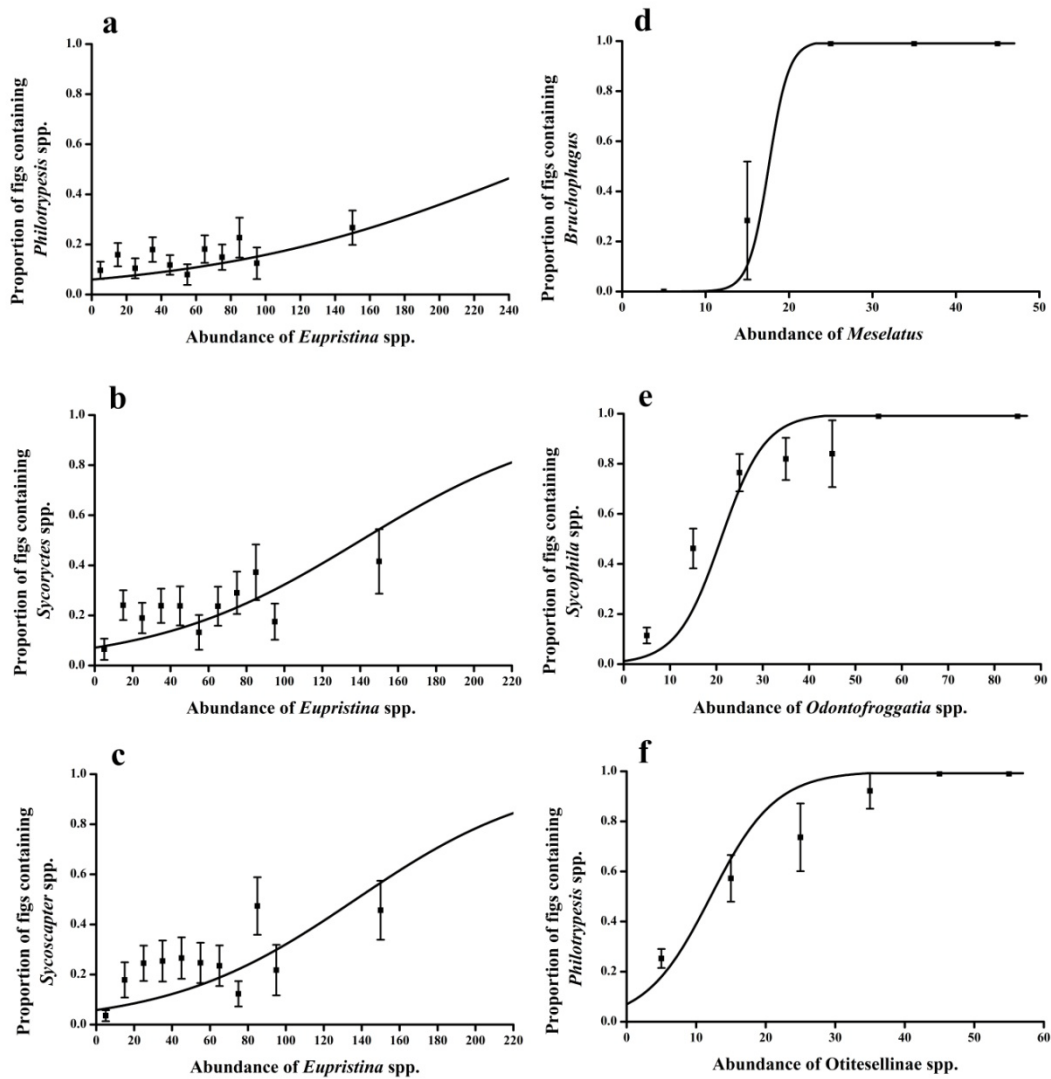


Fig. 7.4 Density-dependent responses in terms of the presence of parasitoid groups to the densities of their tentative host galler using GLMMs assuming binomial distributions of residuals (a-f).

The density of a host group (per fig) was estimated by the sum of its density and the density of its tentative parasitoid group. The proportion of figs containing each parasitoid group in each crop (mean \pm SE) was summarized with an interval of 10 individuals except that only one proportion was calculated for the figs containing more than 100 fig wasps.

7.4.4 Gall size

The volumes of 829 galls occupied by 18 fig wasp species were obtained from the 68 figs (Tables 7.10 and 7.11). *Meselatus* and *Bruchophagus* were reared from extremely large galls at least 2.5 times larger than those of any other species group (Tables 7.10 and 7.11). Significant variations in gall size were detected among the galls occupied by different phytophage groups and among those of parasitoid groups targeting distinct putative host groups, but parasitoid groups (*Philotrypesis* spp., *Sycoryctes* spp. and *Sycoscapter*) associated with *Eupristina* spp. emerged from galls of equal sizes (Tables 7.11 and 7.12; Fig. 7.5 a). We failed to detect any intra-group variation in gall size in all phytophage and parasitoid groups (Tables 7.10 and 7.13). There was no difference in sizes of galls containing any phytophage species or group and the galls supporting its putative parasitoid species/group(s) (Tables 7.14 and 7.15; Fig. 7.5 a).

Table 7.10 Gall sizes (mean \pm SE) and pedicel lengths (mean \pm SE, mm) of occupied flowers.

Species abbreviations and order are as in Table 7.4. Note that sizes of seeds containing *P. taiwanensis* and pedicel lengths of galls containing *M. bicolor* (*Mb*), *W. nigraabdomina* (*Wn*) and *B. sensoriae* (*Bs*) were not measured.

Biology	Species	Gall size (N galls) mm ³	Pedicel length (N galls or seeds)
Phytophage	<i>Ev</i>	0.353 \pm 0.011 (206)	0.637 \pm 0.015 (334)
Phytophage	<i>Es</i>	0.388 \pm 0.028 (40)	0.614 \pm 0.021 (130)
Phytophage	<i>Mb</i>	3.624 \pm 0.127 (52)	--
Phytophage	<i>Oc</i>	1.000 \pm 0.043 (43)	0.376 \pm 0.028 (60)
Phytophage	<i>Og</i>	1.006 \pm 0.038 (65)	0.394 \pm 0.027 (55)
Phytophage	<i>Oi</i>	1.059 \pm 0.046 (23)	0.349 \pm 0.029 (44)
Phytophage	<i>Md</i>	0.429 \pm 0.033 (16)	0.599 \pm 0.067 (12)
Phytophage	<i>Wm</i>	0.436 \pm 0.017 (43)	0.593 \pm 0.031 (58)
Phytophage	<i>Wn</i>	0.445 \pm 0.072 (13)	--
Phytophage	<i>Pt</i>	--	0.285 \pm 0.037 (27)
Parasitoid	<i>Bs</i>	3.801 \pm 0.176 (21)	--
Parasitoid	<i>Sm</i>	1.037 \pm 0.039 (38)	0.354 \pm 0.040 (20)
Parasitoid	<i>Smp</i>	0.984 \pm 0.042 (43)	0.347 \pm 0.054 (14)
Parasitoid	<i>Sp</i>	1.069 \pm 0.059 (20)	0.355 \pm 0.026 (38)
Parasitoid	<i>Pe</i>	0.396 \pm 0.030 (49)	0.686 \pm 0.039 (34)
Parasitoid	<i>Po</i>	0.401 \pm 0.020 (38)	0.613 \pm 0.039 (25)
Parasitoid	<i>Srm</i>	0.356 \pm 0.024 (52)	0.638 \pm 0.041 (43)
Parasitoid	<i>Srs</i>	0.404 \pm 0.046 (23)	0.664 \pm 0.054 (21)
Parasitoid	<i>Scg</i>	0.328 \pm 0.012 (41)	0.680 \pm 0.035 (48)

Table 7.11 Gall size (mean \pm SE, mm³) and pedicel length (mean \pm SE, mm) of species groups.

Note that the pedicel lengths of galls containing *Meselatus* spp. and *Bruchophagus* spp. were not measured.

Biology	Species group	Gall size (N galls)	Pedicel length (N galls)
Phytophage	<i>Eupristina</i> spp.	0.359 \pm 0.010 (246)	0.630 \pm 0.012 (464)
Phytophage	<i>Meselatus</i>	3.624 \pm 0.127 (52)	--
Phytophage	<i>Odontofroggatia</i> spp.	1.013 \pm 0.025 (134)	0.375 \pm 0.016 (159)
Phytophage	Otitesellinae spp.	0.436 \pm 0.018 (72)	0.594 \pm 0.028 (70)
Parasitoid	<i>Bruchophagus</i>	3.801 \pm 0.176 (21)	--
Parasitoid	<i>Sycophila</i> spp.	1.022 \pm 0.026 (101)	0.353 \pm 0.020 (72)
Parasitoid	<i>Philotrypesis</i> spp.	0.398 \pm 0.019 (87)	0.655 \pm 0.028 (59)
Parasitoid	<i>Sycoryctes</i> spp.	0.370 \pm 0.022 (75)	0.646 \pm 0.032 (64)
Parasitoid	<i>Sycoscapter</i>	0.328 \pm 0.012 (41)	0.680 \pm 0.035 (48)

Table 7.12 LMMs assessing differences in gall volumes and pedicel lengths among phytophage and parasitoid groups.

Response variable	Effect	df	LR	Pair-wise Comparison	df	t
Gall size	Phytophage groups	3	269.886 ***	<i>Eupristina</i> spp. vs. <i>Meselatus</i>	440	-35.922 ***
				<i>Eupristina</i> spp. vs. <i>Odontofroggatia</i> spp.	440	-22.873 ***
				<i>Eupristina</i> spp. vs. <i>Otitesellinae</i> spp.	440	-3.422 **
				<i>Meselatus</i> vs. <i>Odontofroggatia</i> spp.	440	18.364 ***
				<i>Meselatus</i> vs. <i>Otitesellinae</i> spp.	440	28.340 ***
				<i>Odontofroggatia</i> spp. vs. <i>Otitesellinae</i> spp.	440	14.593 ***
Gall size	Parasitoid groups	4	162.541 ***	<i>Bruchophagus</i> vs. <i>Sycophila</i> spp.	276	16.320 ***
				<i>Bruchophagus</i> vs. <i>Philotrypesis</i> spp.	276	28.198 ***
				<i>Bruchophagus</i> vs. <i>Sycoryctes</i> spp.	276	28.900 ***
				<i>Bruchophagus</i> vs. <i>Sycoscapter</i>	276	27.101 ***
				<i>Sycophila</i> spp. vs. <i>Philotrypesis</i> spp.	276	19.794 ***
				<i>Sycophila</i> spp. vs. <i>Sycoryctes</i> spp.	276	20.799 ***
				<i>Sycophila</i> spp. vs. <i>Sycoscapter</i>	276	17.939 ***
				<i>Philotrypesis</i> spp. vs. <i>Sycoryctes</i> spp.	276	1.922 NS
				<i>Philotrypesis</i> spp. vs. <i>Sycoscapter</i>	276	2.199 NS
<i>Sycoryctes</i> spp. vs. <i>Sycoscapter</i>	276	0.570 NS				
Pedicel length	Phytophage groups	2	102.621 ***	<i>Eupristina</i> spp. vs. <i>Odontofroggatia</i> spp.	672	10.995 ***
				<i>Eupristina</i> spp. vs. <i>Otitesellinae</i> spp.	672	1.109 NS
				<i>Odontofroggatia</i> spp. vs. <i>Otitesellinae</i> spp.	672	-6.053 ***
Pedicel length	Parasitoid groups	3	74.653 ***	<i>Sycophila</i> spp. vs. <i>Philotrypesis</i> spp.	220	-7.769 ***
				<i>Sycophila</i> spp. vs. <i>Sycoryctes</i> spp.	220	-7.699 ***
				<i>Sycophila</i> spp. vs. <i>Sycoscapter</i>	220	-7.910 ***
				<i>Philotrypesis</i> spp. vs. <i>Sycoryctes</i> spp.	220	0.231 NS
				<i>Philotrypesis</i> spp. vs. <i>Sycoscapter</i>	220	-0.565 NS
				<i>Sycoryctes</i> spp. vs. <i>Sycoscapter</i>	220	-0.793 NS

NS not significant; **:p<0.01; ***:p<0.001.

Table 7.13 Comparisons among species of gall size and pedicel length within each group using LMMs.

PL=Pedicel length. Note that *Meselatus* and *Bruchophagus* were excluded from the analyses as there was only one species in these ‘group’s. We also excluded the group of *Sycoscapter* spp. because gall size/pedicel length was only measured in one species.

Galler/parasitoid	Group	Species Number	Gall size		Pedicel length	
			df	LR	df	LR
Galler	<i>Eupristina</i> spp.	2	1	0.583 ^{NS}	1	0.254 ^{NS}
Galler	<i>Odontofroggatia</i> spp.	3	1	1.274 ^{NS}	1	2.120 ^{NS}
Galler	Otitesellinae spp.	3 (2 for PL)	1	1.149 ^{NS}	1	0.006 ^{NS}
Parasitoid	<i>Sycophila</i> spp.	3	1	2.050 ^{NS}	1	0.128 ^{NS}
Parasitoid	<i>Philotrypesis</i> spp.	2	1	0.476 ^{NS}	1	1.681 ^{NS}
Parasitoid	<i>Sycoryctes</i> spp.	2	1	0.633 ^{NS}	1	0.147 ^{NS}

^{NS}: not significant.

Table 7.14 Pair-wise comparisons of flowers occupied by pairs of gallers and parasitoids in terms of gall sizes and pedicel lengths (LMMs).

Species abbreviations and order are as in Table 7.4. Note that we excluded *W. nigrabdomina* (*Wn*) from the analyses relevant to gall size, and *S. maculafacies* pale (*Smp*) from the analyses relevant to pedicel length because of their small sample sizes (less than 20 galls occupied by each species).

Species pair	Gall size		Pedicel length	
	df	LR	df	LR
<i>Ev</i> vs. <i>Pe</i>	1	1.767 ^{NS}	1	1.010 ^{NS}
<i>Ev</i> vs. <i>Po</i>	1	1.673 ^{NS}	1	0.171 ^{NS}
<i>Ev</i> vs. <i>Srm</i>	1	3.629 ^{NS}	1	0.003 ^{NS}
<i>Ev</i> vs. <i>Scg</i>	1	0.968 ^{NS}	1	1.034 ^{NS}
<i>Es</i> vs. <i>Srs</i>	1	0.002 ^{NS}	1	0.662 ^{NS}
<i>Es</i> vs. <i>Scg</i>	1	2.004 ^{NS}	1	2.458 ^{NS}
<i>Mb</i> vs. <i>Bs</i>	1	0.500 ^{NS}		--
<i>Oc</i> vs. <i>Sm</i>	1	1.219 ^{NS}	1	0.028 ^{NS}
<i>Oc</i> vs. <i>Smp</i>	1	0.004 ^{NS}		--
<i>Oc</i> vs. <i>Sp</i>	1	1.262 ^{NS}	1	0.027 ^{NS}
<i>Og</i> vs. <i>Sm</i>	1	0.536 ^{NS}	1	0.488 ^{NS}
<i>Og</i> vs. <i>Smp</i>	1	0.001 ^{NS}		--
<i>Og</i> vs. <i>Sp</i>	1	1.261 ^{NS}	1	0.644 ^{NS}
<i>Oi</i> vs. <i>Sm</i>	1	0.189 ^{NS}	1	0.058 ^{NS}
<i>Wm</i> vs. <i>Po</i>	1	2.251 ^{NS}	1	0.139 ^{NS}

^{NS}: not significant.

Table 7.15 Results of pair-wise comparisons between galler groups and parasitoid groups in gall size and pedicel length using LMM.

Group pair	Gall size		Pedicel length	
	df	LR	df	LR
<i>Eupristina</i> spp. vs. <i>Philotrypesis</i> spp.	1	3.691 ^{NS}	1	0.474 ^{NS}
<i>Eupristina</i> spp. vs. <i>Sycoryctes</i> spp.	1	0.613 ^{NS}	1	0.198 ^{NS}
<i>Eupristina</i> spp. vs. <i>Sycoscapter</i> spp.	1	0.802 ^{NS}	1	1.472 ^{NS}
<i>Meselatus</i> spp. vs. <i>Bruchophagus</i> spp.	1	0.500 ^{NS}	1	--
<i>Odontofroggatia</i> spp. vs. <i>Sycophila</i> spp.	1	0.909 ^{NS}	1	0.637 ^{NS}
Otitesellinae spp. vs. <i>Philotrypesis</i> spp.	1	3.254 ^{NS}	1	2.324 ^{NS}

^{NS}: not significant.

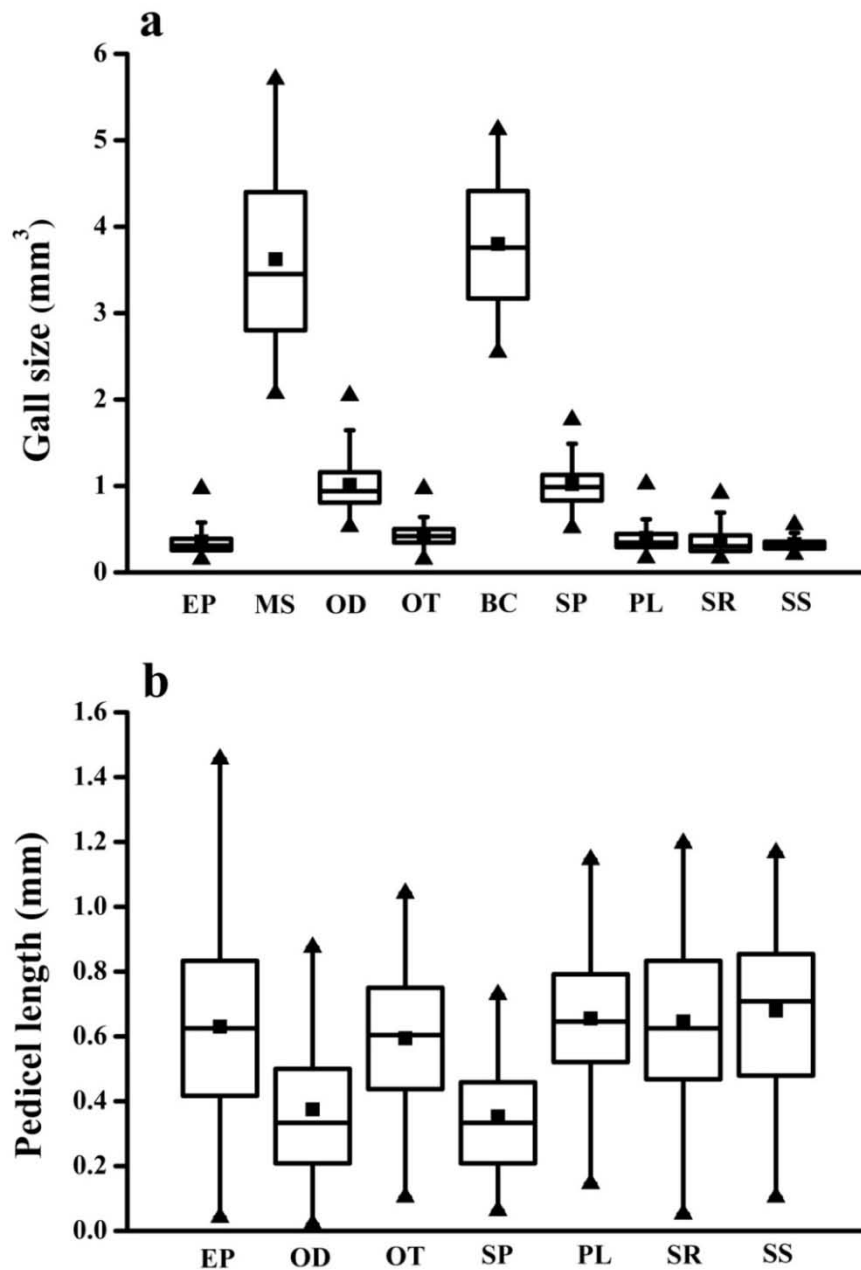


Fig. 7.5 Sizes (a) and pedicel lengths (b) of galls containing species or groups of species.

Line, box, whiskers, black squares and black triangles represent the median, the range from the first to third quartile, 1.5 times lower and upper quartiles, mean and minimum and maximum values of pedicel lengths in each utilization type. *Eupristina* spp.: **EP**; *Meselatus*: **MS**; *Odontofroggatia* spp.: **OD**; Otitesellinae spp.: **OT**; *Bruchophagus*: **BC**; *Sycophila* spp.: **SP**; *Philotrypesis* spp. excluding *P. taiwanensis*: **PL**; *Sycoryctes* spp.: **SR**; *Sycoscapter*: **SS**.

7.4.5 Spatial stratification of fig wasps

Pedicle lengths of 1314 ovules from 20 figs were measured. They included 317 seeds, 34 failed galls, 27 seeds occupied by *P. taiwanensis* and 936 galled ovules containing 15 fig wasp species representing all the species groups except *Meselatus* and *Bruchophagus* (Tables 7.10 and 7.11). Overall, agaonids and Otitesellinae spp. and their putative parasitoid groups (*Philotrypesis* spp., *Sycoryctes* spp. and *Sycoscapter* for agaonids; and *Philotrypesis* spp. for Otitesellinae) mainly emerged from more central galls with longer pedicels whereas *Odontofroggatia* spp. and their putative parasitoids (*Sycophila* spp.) tended to occupy ovules near to the fig wall (Tables 7.10 and 7.11; Fig. 7.5 b). The galls occupied by *Eupristina* spp. and Otitesellinae spp. had equal pedicel lengths, that were longer than those of galls containing *Odontofroggatia* spp., and significant differences in pedicel lengths were also detected among the galls supporting parasitoid groups associated with different putative host groups, but not among those sharing the same putative host group (Tables 7.11 and 7.12). Species assigned to the same group were reared from the galls with similar pedicel lengths (Tables 7.10 and 7.13). In summary, at both species and group levels, parasitoids generally occupied galls with equal pedicel lengths to those of their putative hosts (Tables 7.14 and 7.15; Fig. 7.5 b).

Eupristina spp. and their putative parasitoid groups were more likely to emerge from the galls towards the centre of figs (GLMM (binomial): *Eupristina* spp.: $\beta=2.742 \pm 0.247$, $z=11.104$, $p<0.001$; *Philotrypesis* spp.: $\beta=2.030 \pm 0.517$, $z=3.927$, $p<0.001$; *Sycoryctes* spp.: $\beta=1.949 \pm 0.458$, $z=4.253$, $p<0.001$; *Sycoscapter* spp.: $\beta=2.155 \pm 0.544$, $z=3.963$, $p<0.001$), and this trend did not vary among these species groups (Table 7.16; Fig. 7.6 a). A similar pattern also occurred in Otitesellinae spp. and their putative parasitoid group, *Philotrypesis* spp. (GLMM (binomial): Otitesellinae spp.: $\beta=1.126 \pm 0.537$, $z=2.095$, $p=0.036$; Table 7.16; Fig. 7.6 b). In contrast, *Odontofroggatia* spp. and their putative parasitoid group preferred to oviposit in the ovules closer to the fig wall (GLMM (binomial): *Odontofroggatia* spp.: $\beta=-2.017 \pm$

0.404, $z=-4.996$, $p<0.001$; *Sycophila* spp.: $\beta=-2.247 \pm 0.664$, $z=-3.385$, $p<0.001$), and this trend was similar in these two species groups (Table 7.16; Fig. 7.6 c).

Table 7.16 Spatial stratification of galls containing different groups of fig wasps using GLMM assuming binomial distribution of residuals.

Response variable	Effects	df	LR	Pair-wise Comparison of slopes (β)	Wald (z)
Absence/presence of species group	Pedicel length \times species group	3	5.139 ^{NS}	<i>Eupristina</i> spp. vs. <i>Philotrypesis</i> spp.	1.590 ^{NS}
				<i>Eupristina</i> spp. vs. <i>Sycoryctes</i> spp.	1.854 ^{NS}
				<i>Eupristina</i> spp. vs. <i>Sycoscapter</i> spp.	0.960 ^{NS}
				<i>Philotrypesis</i> spp. vs. <i>Sycoryctes</i> spp.	0.166 ^{NS}
				<i>Philotrypesis</i> spp. vs. <i>Sycoscapter</i> spp.	-0.408 ^{NS}
				<i>Sycoryctes</i> spp. vs. <i>Sycoscapter</i> spp.	-0.571 ^{NS}
Absence/presence of species group	Pedicel length \times species group	1	0.108 ^{NS}	<i>Odontofroggatia</i> spp. vs. <i>Sycophila</i> spp.	0.327 ^{NS}
Absence/presence of species group	Pedicel length \times species group	1	1.851 ^{NS}	Otitesellinae spp. vs. <i>Philotrypesis</i> spp.	-1.355 ^{NS}

^{NS}: not significant.

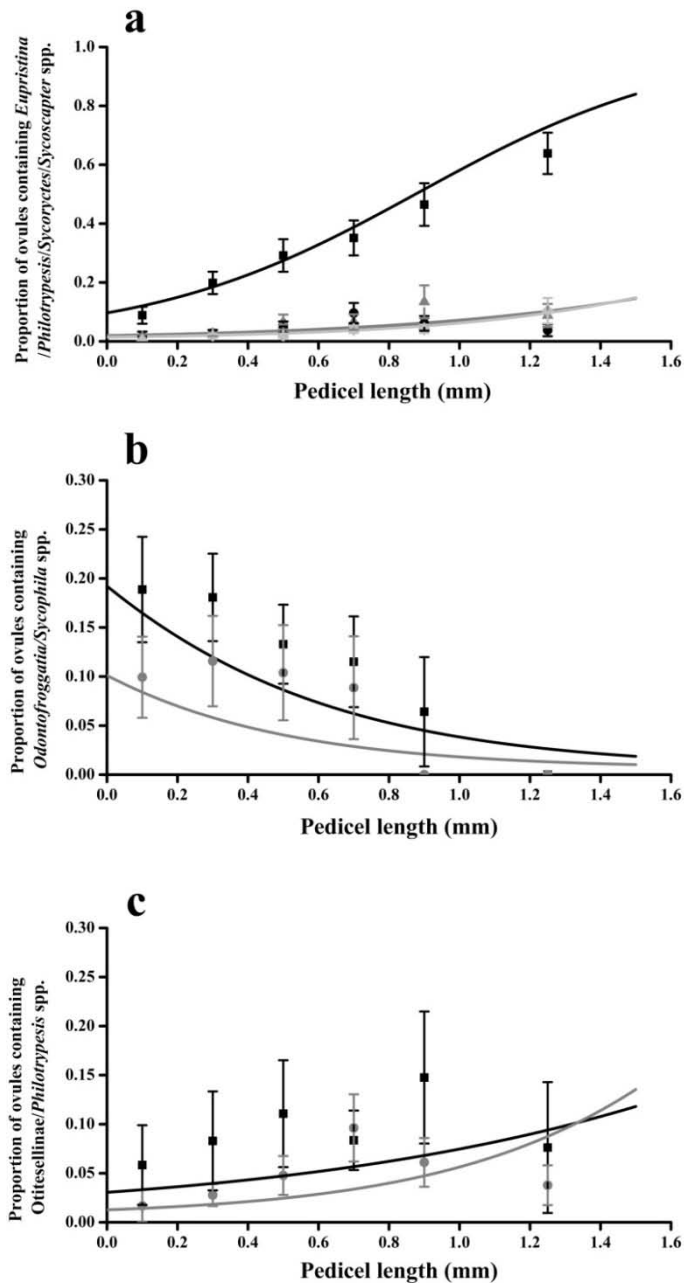


Fig. 7.6 Spatial stratification of ovules occupied by different groups of phytophages and their putative parasitoid groups.

(a) *Eupristina* spp. (black line with square data points), *Philotrypesis* spp. (dark grey line with circle data points), *Sycoryctes* spp. (grey line with triangle data points) and *Sycoscapter* (light grey line with diamond data points); (b) *Odontofroggattia* spp. (black line with square data points) and *Sycophila* spp. (grey line with circle data points); (c) *Otitesellinae* spp. (black line with square data points) and *Philotrypesis* spp. (grey line with circle data points). Proportion of ovules containing each species group to the total ovules that were occupied by either seeds or fig wasps (mean \pm SE) were shown in every 0.2 mm in pedicel length except the last one which included all data with pedicel lengths larger than 1mm (shown in 1.25 mm).

7.5 Discussion

Using a variety of sources of information, we attempted to explicitly delineate the fig wasp food web associated with the figs of *F. microcarpa*. We identified 32 morpho-species including 11 species that were not recorded in previous studies (Chen et al. 1999; Feng & Huang 2010; Li et al. 2013). These species were generally rare and/or highly local, and we know (in the case of African species) or suspect that *F. microcarpa* represents only an occasional resource for these species. We did not attempt to include them in our analyses.

The pollinator *Eupristina* sp. (Agaonidae) is the most widely distributed of the fig wasp species, but other galler species in the genera *Meselatus* and *Odontofroggatia* (Epichrysomallinae), *Walkerella* and *Micranisa* (Otitesellinae) can also act as pioneer species where *F. microcarpa* figs is introduced. The seed predator *P. taiwanensis* can also reach high densities, and it has also been introduced outside its native range. In contrast to these phytophagous species, the distribution and abundance of parasitoids was more localized, with far lower species richness and densities, and they were entirely absent from about half the sample sites in the plant's introduced range. The contrasting introduction and distribution patterns between species at different trophic levels is likely to reflect the additional difficulties faced by parasitoids, which must find both figs and host insects, compared with phytophages. Sycoryctines are almost all parasitoids, and they are poorly represented outside their native range. In addition to their trophic relationships, this might also reflect differences in dispersal ability among the different groups of fig wasps. Pollinators often disperse over very long distances, with the help of air currents (Ahmed et al. 2009), but the dispersal abilities of other fig wasps are largely unknown. Sycoryctinae females have extremely long exerted ovipositors (or greatly elongated gasters to support them) which can reach up to several times their body lengths. It seems likely that this will impede their flight ability, compared with agaonids, epichrysomallines and otitesellines, which either have much shorter ovipositors (Agaonidae) or

ovipositors that are largely coiled up with the bodies of the females (Galil & Copland 1981).

We are unlikely to have detected all the links in the *F. microcarpa* fig wasp food web, but the various lines of evidence (species interaction, density-dependent response of parasitoids to host abundance and spatial distribution of galls inside figs) provided consistent evidence for the major links and relationships between the species. Consistent with previous studies (Segar & Cook 2012), agaonids were attacked by several Sycoryctinae species that provided a major predation pressure that reduced the fig tree's male reproductive function. Compton (1993) reported that African epichrysomallines are the exclusive hosts of *Sycophila* species in figs. In the Asian *F. microcarpa*, the epichrysomalline *Odontofroggatia* spp. (a genus not represented in Africa) are again the hosts of *Sycophila*. There was no evidence for particular *Sycophila* species being associated with individual *Odontofroggatia* species, but *Sycophila* were not associated with the other abundant epichrysomalline *Meselatus bicolor*. This species forms extremely large galls and supported a specific parasitoid (*Bruchophagus sensoriae*), which belongs to the same family as *Sycophila*.

Otiteselline produce galls that are intermediate in size between those of epichrysomallines and agaonids. In *F. microcarpa* figs their galls appear to be too small to support *Sycophila* species, but they can act as hosts for some of the sycoryctines that are usually considered as parasitoids of agaonids. *Philotrypesis okinavensis* was present in figs where no agaonid was present at several sites, and utilises *Walkerella microcarpae* as its host. Individuals that emerge from *Walkerella* galls are noticeably larger than those that have attacked agaonids. This may be an example of niche broadening and host shifting from *Eupristina*, but the larger galls and their often more peripheral location within the figs probably make otitesellines more favorable hosts than the agaonids. In contrast, another prevalent parasitoid *Philotrypesis* species, *P. emeryi*, seems to be more specifically associated with agaonids, but we cannot rule out its use of other hosts. Given the high species richness of the genus *Philotrypesis* (Zhou et al. 2012) and their known trophic diversity (*P.*

taiwanensis is a seed predator), it is likely that this genus is quite flexible in its host relationships.

The *Philotrypesis* that attacks both agaonids and Otiteseallinae species appears to be an exception among the parasitoids, which in general attack either the larger galls generated by epichrysomallines or the smaller galls of agaonids. Within groups with similar sized galls there does not appear to be host specificity. Specificity to particular higher taxa has been confirmed in previous studies (Dunn et al. 2008; Segar & Cook 2012), and suggests a co-evolutionary history between parasitoids and their host gallers (Segar & Cook 2010; Segar et al. 2013), but insofar as related species tend to generate similar sized galls, it is hard to separate gall size effects from phylogenetic history. In *F. microcarpa* figs, the epichrysomalline that produces exceptionally large galls appears to now evade the *Sycophila* species that attack other epichrysomallines, but is host to a related eurytomid currently placed in the genus *Bruchophagus*, but which is possibly an unusual *Sycophila* (S.G. Compton, unpublished). The apparent widespread breakdown of host specificity at host species level indicates a lack of niche differentiation within each gall-size group. This pattern is not necessarily repeated in other fig wasp communities. The *Apocrypta* parasitoid NPFW in figs of the African *F. sur* (Compton and Robertson, 1988) utilizes both agaonids and sycophagines (an unplaced subfamily of mainly gall-forming NPFW not found in association with *F. microcarpa*). Its different hosts produce galls of varying sizes, and adult *Apocrypta* display a strikingly wide range of body sizes.

Various mechanisms that are likely to change food webs have been proposed, such as host shifts (Leppänen et al. 2013; Segar et al. 2013), but given that most of the fig wasps identified in the present study have only been recorded in figs of *F. microcarpa*, and there seems to be strong niche conservatism, adaptation of gallers to reduce parasitism is not apparent, though intraspecific adjustment to specifically protect males has been described (Yu & Compton 2012). A switch in feeding behavior from galls to seeds also seems to have allowed *P. taiwanensis* to enter 'enemy-free space'. Predator behavior can nonetheless help stabilise fig wasp populations, because their

foraging is often density-dependent (West et al. 1996; Suleman et al. 2013). The preference of parasitoids for figs with dense aggregations of hosts may be driven by a searching strategy in which they can easily find galled ovules when offspring of their hosts are aggregated, but host finding is more time-consuming when host larvae are rare, which increases the risk of them being attacked by ants, generalist natural enemies of many fig wasps that lay eggs from the outside of figs (Compton & Robertson 1988).

Specific natural enemies of invasive species are used routinely in biological control programmes (Richardson et al. 2000; Garren & Strauss 2009; Baraibar et al. 2011). Given that many *Ficus* species have numerous generalist seed dispersers (Shanahan et al. 2001; Caughlin et al. 2012), it is impossible to reduce the transportation of their seeds. Prevention of seed production, taking advantage of the trees' extremely specialized pollination system is a more viable option, though some previous studies have argued that attacking the reproductive systems of long-lived plants may also not be effective (Radford et al. 2001). Some sycoryctine parasitoids can be strongly detrimental to pollinating agaonids (Suleman et al. 2013), but in general the costs imposed by these NPFW on seed production are not large (Dunn et al. 2008; Segar & Cook 2012). Alternatively, competition among gallers may provide a promising way to improve biological control. For example, figs with *M. bicolor* always fail to contain any pollinator larvae and are seedless (R. Wang & S.G. Compton, unpublished). A combination of the parasitoids of pollinators, the seed predator and gall-forming NPFW that compete for ovules, as seen in the plant's native range, may nonetheless be capable of simultaneously depressing both male and female reproductive functions of *F. microcarpa*, and potentially contribute to its biocontrol in countries where it is invasive, especially if the parasitoids of these species are not introduced.

Chapter 8 The invasive fig tree *Ficus microcarpa* in its native and introduced ranges: enemy release benefits pollinators but fails to enhance plant reproductive success

8.1 Abstract

Escape from natural enemies can facilitate biological invasions by plants. Fig trees depend on host specific fig wasps for pollination, but their figs also support a diverse fauna of non-pollinating fig wasps (NPFW) that reduce numbers of female pollinator offspring and seeds (the plant's male and female reproductive success, respectively). Pollinators preferentially gall central ovules, whereas NPFW lay eggs from the outside of figs and mostly occupy more peripheral ovules. Avoidance of peripheral ovules by pollinators has been suggested as one factor that maintains the mutualism between pollinators and monoecious figs, allowing both seeds and pollinator offspring to develop. The Asian *Ficus microcarpa* is widely introduced and can be invasive when pollinators are present. Several NPFW have also been introduced. We compared the contents of *F. microcarpa* figs in its native and introduced ranges. Outside the native range, parasitoids of pollinator offspring were less diverse and abundant, the plant's relative reproductive function was more male-biased, and the location of pollinator offspring was less central, but the figs did not contain more seeds or pollinator offspring. The invasiveness of *F. microcarpa* cannot be attributed to natural enemy release of its pollinator, but depauperate natural enemy faunas favour pollinators, allowing it to utilize ovules that would otherwise have mainly supported seeds or NPFW though the survival rate of its larvae is lower outside the tree's native range.

8.2 Introduction

Plants are increasingly being transported outside their natural ranges via either

deliberate trans-national introductions for horticulture and agriculture or through unintentional transit associated with international travel and commerce (Hulme 2009; Catford et al. 2012). Some non-indigenous species became established or even invasive within their introduced areas, where they can alter soil nutrient cycling and hydrology, threaten indigenous species and disrupt ecosystem functions (Mack et al. 2000; Simberloff 2011). The success of invasive plants can be attributed to both evolutionary and ecological factors (Carroll 2011; Catford et al. 2012). Most introduced species do not become invasive. Expansion of these species may be restricted by poor adaptations to their novel environments, which depends on traits that are often tightly linked to their genetic background (Schlaepfer et al. 2010; Carroll 2011). Specific mutualists, such as pollinators, may also be rare or absent (Richardson et al. 2000). Conversely, invasive species may benefit from the absence of species with which they routinely interact in their native range (Wolfe & Klironomos, 2005). The enemy release hypothesis emphasizes that a paucity of natural enemies associated with non-indigenous species allows them to reach higher densities that they could attain in their native areas (Keane & Crawley 2002; Müller-Schärer & Schaffner 2008). This idea forms the basis of classical biological control programs, many of which have been highly successful, where natural enemies from the native range are introduced to aid control of invasive plants (Garren & Strauss 2009; Baraibar et al. 2011; Wilson et al. 2011).

Plants that require specific mutualistic partners are expected to be less likely to become invasive than more generalist species, but if their animal associates are also introduced, they can then benefit from the relative lack of natural enemies within their introduced ranges and become invasive (Richardson et al. 2000). *Ficus* species (fig trees, Moraceae) are highly diverse, and are often considered as keystone species in tropical and subtropical forests (Harrison 2005; Herre et al. 2008). They are also well known for their obligate mutualism with host species-specific pollinating fig wasps (Hymenoptera, Agaonidae), with each fig tree relying on one or a small number of agaonid species for pollination, and larvae of the pollinators developing only in galled

ovules of their particular host figs (Weiblen 2002; Cruaud et al. 2012). Fig trees are commonly grown outside their native ranges, where they normally fail to reproduce because of the absence of pollinators, but if their associated agaonids are also introduced they are then able to produce seeds that are rapidly spread by the many species of vertebrates that feed on figs, and can become invasive (McKey 1989; Shanahan et al. 2001; Starr et al. 2003; Miao et al. 2011).

In addition to pollinating fig wasps, figs are also utilized by numerous species of non-pollinating fig wasps (NPFW, Chalcidoidea) that induce galls (gallers), consume seeds (seed predators), or prey on the larvae of other fig wasps (parasitoids, that may also consume plant tissue) (Hawkins & Compton, 1992; Kerdelhué et al. 2000; Pereira et al. 2007; Segar & Cook 2012; Chen et al., 2013). Far less is known about the feeding biology of NPFW, but species from Pteromalidae subfamily Sycoryctinae are often parasitoids of pollinators (Tzeng et al. 2008; Segar & Cook 2012; Suleman et al. 2013) that reduce the male reproductive function of the figs. Typical seed predators are rare among NPFWs (Pereira et al. 2007; Wang et al. in press a) but ovule-galling NPFW from Pteromalidae subfamilies Epichrysomallinae and Oritesellinae reduce both male and female reproductive successes by competing for oviposition and pollination sites with pollinators and probably also via competition for nutrients within figs (Kobbi et al. 1996; Jandér & Herre 2010; R. Wang & S.G. Compton, unpublished). However, not all NPFW reduce fig tree reproductive successes, because some are parasitoids of galler NPFWs (Godfray 1988; Compton 1993; R. Wang & S.G. Compton, unpublished), or secondary parasitoids that destroy other parasitoids (Compton et al. 2009). Pollinators may become established without any associated NPFW (for example *F. rubiginosa* (R. Wang & S.G. Compton, unpublished)) or subsets of native-range NPFW communities may also be introduced. Many of the gall-forming NPFW do not require the figs to have been entered by pollinators. Their reduced diversity may result in lower ovule occupation rates in the figs, or density compensation may occur among the species that are present, including the pollinators of the trees. Fig trees in their introduced ranges may therefore benefit

from enemy release in terms of both seed and pollinator production, and this may increase their likelihood of becoming invasive (McPherson 2005).

In many figs a significant proportion of galled ovules fail to support development of fig wasps and in mature figs they appear as hollow empty galls ('bladders'). Limited evidence suggests that, in one fig tree species at least, pollinator eggs are laid in most of these empty galls (Ghana et al., 2012). Probing with their ovipositors by NPFW females and competition for nutrient supplies between galls may be responsible for the failure of these galls (Zavodna et al. 2005; Miao et al. 2012; Zhao et al. 2013).

In dioecious figs, functionally distinct male and female figs are responsible for pollinator and seed outputs respectively, whereas the figs of monoecious species contain both staminate flowers and pistillate flowers that can either develop into seeds or support fig wasp offspring. Each fig therefore contributes to both the plant's male and female reproductive success (Cook & Rasplus 2003; Herre et al. 2008). Factors responsible for constraining pollinator oviposition in monoecious figs, and thereby ensuring that not all the ovules are occupied by pollinator offspring, appear to vary between species, but a consistent pattern is for pollinator offspring to be concentrated in ovules located towards the centre of figs, whereas seeds are more likely to be located towards the periphery (Compton & Nefdt 1990; Yu et al. 2004; Dunn et al. 2008a, 2008b; Wang et al. 2013). This reflects a preference among pollinator females for shorter-styled flowers, which have more accessible ovules for females that oviposit from within the figs, and which may be more suitable physiologically for development of their offspring than more peripheral ovules (Compton et al., 1994; Nefdt & Compton 1996; Wang et al., 2013). In addition, most NPFW lay eggs from the outside of figs, and so are more likely to utilize outer ovules. More centrally located pollinator larvae are therefore less likely to be killed by parasitoids and occupy 'enemy free space' (Holt & Lawton, 1993; Dunn et al. 2008a; Yu & Compton, 2012). Fig trees growing outside their native range, where NPFW faunas are depauperate, would be expected to contain more seeds and more pollinator offspring, assuming that the number of pollinator foundresses remains similar. They also

provide an opportunity to assess the significance of parasitoids and other NPFW for ovule utilization patterns by pollinators, because selection pressures to avoid longer styled flowers are reduced or absent. Segar & Cook (2012) found that NPFW consistently have a greater impact on male than female reproductive function, suggesting that the balance between pollinator and seed production in figs outside their native range should also be tilted towards the former.

F. microcarpa L. is a monoecious fig tree with a natural distribution in tropical and subtropical forests of Southeast Asia and North Australasia (Berg & Corner 2005; Mifsud et al. 2012). Over the last 200 years it has been widely transplanted as an ornamental tree in many tropical and subtropical urban areas in East Asia, Hawaii, the Mediterranean, Middle East, Africa and also throughout warmer areas of the New World (Compton 1989; Lo Verde et al. 1991; Nadel et al. 1992; Figueiredo et al. 1995; Kobbi et al. 1996; Beardsley 1998; Burrows & Burrows 2003; Lo Verde & Porcelli 2010; Caughlin et al. 2012; Y-Q Peng & Y Chen, Pers. Comm.). The tree's specific pollinating agaonid, *Eupristina verticillata* Waterston has also colonized most of the introduced populations of *F. microcarpa*, allowing fertile seeds to be produced. Extensive seed dispersal by birds allows its seeds to be widely dispersed from fruiting trees (Kaufmann et al. 1991; Nadel et al. 1992; Shanahan et al. 2001; Corlett 2006). Seedlings cause damage to buildings in urban settings (Tan & Yeo 2009; Caughlin et al. 2012) and the tree can eventually become established in natural environments. *F. microcarpa* is now considered a significant invasive weed in Bermuda, Florida, and Hawaii (Nadel et al. 1992; Beardsley 1998; Starr et al. 2003).

Here we compare the fig wasp faunas and other contents of *F. microcarpa* figs across its native and introduced ranges. We hypothesized that: (1) fig wasp communities are distinct among different ranges of the plant; (2) figs outside the native range contain more pollinator offspring and more seeds than figs in the native range; (3) the balance between male and female reproduction is biased towards the pollinator; and (4) the spatial distribution of pollinator offspring within a fig also changes in the tree's introduced ranges.

8.3 Materials and methods

8.3.1 *Ficus microcarpa* and its associated fig wasps

F. microcarpa grows naturally as a strangler or ‘rock-splitter’ fig tree in tropical and subtropical forests, but has been widely planted as an ornamental and street tree in urban areas in both its native and introduced ranges (Mifsud et al. 2012; van Noort et al. 2013). The plant's native range is largely in areas with hot and humid climates (Berg and Corner, 2005), but it has been planted in a broader range of climatic conditions, including areas with more seasonal and dryer climates, as well as in areas such Rio de Janeiro with more tropical conditions typical of its native range,

Under natural conditions, *F. microcarpa* usually produces discrete synchronized crops, but tree's growing outside the native range often experience a slow rate of colonization by fig wasps (pollinators or gall formers) that results in figs of various developmental stages being present at the same time. The figs are located in the leaf axils. Mature figs ripen to a pink or purple colour and reach about 10.1 ± 0.3 mm in diameter ($n = 20$ figs). Crops can number up to several thousand figs, but are smaller under cold conditions (Yang et al. 2013). The figs attract a large number of seed dispersal agents, especially birds, and ants can serve as secondary seed dispersers (Kaufmann et al. 1991; Shanahan et al. 2001).

Sexual reproduction of *F. microcarpa* relies on its host specific pollinating agaonid, *Eupristina verticillata* (Agaonidae). Recent molecular evidence indicates that this morphospecies includes several distinct pollinator species with similar morphological characters, only one of which has been detected outside the plant's native range (Sun et al. 2011; A. Cruaud and J-Y Rasplus, Pers. Comm.). In addition there is a *Eupristina* species that is a ‘cheater’ that no longer actively pollinates the figs. It has only been recorded from Yunnan Province, China (J-Y Rasplus, Pers. Comm.; R. Wang & S.G. Compton, unpublished). The single pollinator taxon within *E. verticillata* recorded outside the host's native range is distributed almost wherever *F. microcarpa* is widely planted, with the exception of South Africa (van Noort et al.

2013).

In addition to agaonids, the figs of *F. microcarpa* are also utilized by over 20 NPFW species belonging to the families Eurytomidae, Ormyridae and Pteromalidae (Chen et al. 1999; Feng & Huang 2010; Li et al. 2013) that lay their eggs into ovules via the outer wall of the fig using their long ovipositors (Galil and Copland 1981). Species from subfamilies Epichrysomallinae and Otitesellinae are thought to all be gallers, whereas NPFW from the families Eurytomidae and Ormyridae and subfamilies Pireninae and Sycoryctinae in Pteromalidae are mainly parasitoids of other fig wasps. The only exception is *Philotrypesis taiwanensis* Chen (subfamily Sycoryctinae), an obligate seed predator whose larvae consume seeds (Wang et al., in press a). We allocated the species to two trophic levels: phytophages, which included all gallers and *P. taiwanensis*, and parasitoids whose larvae kill other fig wasps.

Several NPFW that utilise *F. microcarpa* within its native range have also become established outside the native range of *F. microcarpa*, with several species recorded from multiple countries and continents (Galil & Copland 1981; Nadel, et al. 1992; Beardsley 1998, Lo Verde & Porcelli 2010). In addition, small numbers of native African fig wasps, which normally develop in other species of fig trees, have also been reared from *F. microcarpa* figs in South Africa, where *E. verticillata* is absent (van Noort et al. 2013).

The hosts of common parasitoid NPFW within *F. microcarpa* figs have been identified (R. Wang & S.G. Compton, unpublished). Agaonids and gall formers from subfamily Otitesellinae (Pteromalidae) are the hosts of species from subfamily Sycoryctinae (except the seed-eating *P. taiwanensis*). Species from subfamily Epichrysomallinae (Pteromalidae) form large galls and are the hosts of species belonging to the genera *Sycophila* and *Bruchophagus sensu* Chen et al. (1999) (Eurytomidae). In addition, species from family Ormyridae are supposed to be parasitoids or hyper-parasitoids of fig wasps, but this relationship has not been verified. *Sirovena costallifera* (Pireninae, Pteromalidae) is likely to be a parasitoid of Cecidomyiidae (Diptera) (Li et al. 2013), but has been reared in very small numbers

from galled ovules of *F. microcarpa*. Gall forming NPFW, especially epichrysomallines, do not require the figs to be pollinated and their early arrival can prevent pollinator entry. In figs that are shared with pollinators, they compete for ovules and nutrients with *E. verticillata* and also occupy ovules that could have developed seeds (Kobbi et al. 1996; R. Wang & S.G. Compton, unpublished).

8.3.2 Sample sites

Figs of *F. microcarpa* were sampled across 14 countries, covering most of the plant's introduced and native ranges (Table 8.1; Fig. 8.1). The 27 sample sites were allocated into three areas: the plant's native range within the East and Southeast Asian native range of *F. microcarpa*, including 7 sample sites located in southern mainland China, Taiwan, Thailand and the Philippines; the Chinese introduced range included 6 sites from the extra-subtropical extension to the native range; and the non-Chinese introduced range containing 14 sites covering most of the countries where the tree has been widely introduced (Table 8.1; Fig. 8.1). The reason for the division of sampling sites in the plant's introduced range is that fig wasps can disperse directly to the figs in the Chinese introduced range by both their own efforts as well as with the help of human transportation (R. Wang, S.G. Compton & Y. Chen, unpublished data), whereas the colonisation by fig wasps elsewhere is presumed to depend exclusively on human activities (e.g. van Noort et al. 2013).

Humid and hot climates typify the plant's native range, but far colder climate characterise the Chinese introduced range and a broad range of climatic conditions can also be found in the non-Chinese introduced range, including areas with more seasonal and dryer climates as well as more tropical conditions, such as Rio de Janeiro (<http://www.weatherbase.com>).

Table 8.1 Sample sizes and contents of *F. microcarpa* figs at each site (means \pm SE).

Occupancy rate is the proportion of ovules containing fig wasp adult offspring, and gall failure rate is the proportion of galls that failed to support fig wasp larvae. All figs containing fig wasps were included.

Area	Site (abbreviation)	Location	Year(s)	N crops	N figs	Male flowers	Female flowers	Total wasp abundance	Occupancy rate (%)
Native range									
China mainland	Guangzhou (GZ)	N 23°11', E 113°22'	2011-2012	12	169	14.1 \pm 0.4	134.4 \pm 2.8	35.9 \pm 2.1	26.2 \pm 1.3
China mainland	Xishuangbanna (XS)	N 22°00', E 100°48'	2010-2013	17	279	16.0 \pm 0.4	192.9 \pm 2.7	78.1 \pm 2.3	40.4 \pm 1.0
Taiwan	Taipei (TB)	N 25°01', E 121°33'	2012	11	220	13.9 \pm 0.4	142.8 \pm 2.8	29.5 \pm 1.6	21.1 \pm 1.0
Thailand	Bangkok (BK)	N 13°44', E 100°33'	2012-2013	4	39	12.7 \pm 1.2	130.9 \pm 9.1	31.6 \pm 3.4	32.7 \pm 5.1
Thailand	Chiang Mai (CM)	N 18°46', E 98°59'	2012	4	47	22.8 \pm 1.1	202.8 \pm 3.8	68.6 \pm 5.8	33.5 \pm 2.7
Thailand	Kanchanaburi (KC)	N 14°04', E 99°32'	2012-2013	6	63	14.0 \pm 0.8	154.8 \pm 7.9	36.3 \pm 3.6	24.7 \pm 2.1
The Philippines	Manila (MN)	N 14°40', E 121°04'	2012	2	40	20.8 \pm 1.0	197.3 \pm 4.0	46.8 \pm 4.9	23.8 \pm 2.4
Overall	--	--	--	56	857	15.4 \pm 0.2	163.6 \pm 1.7	50.1 \pm 1.3	30.0 \pm 0.6
Chinese introduced range									
China mainland	Chengdu (CD)	N 30°40', E 104°06'	2012	3	20	15.6 \pm 1.0	165.5 \pm 4.4	10.6 \pm 1.6	6.6 \pm 1.2
China mainland	Kunming (KM)	N 24°53', E 102°50'	2010-2011	6	48	16.9 \pm 1.4	200.1 \pm 9.2	32.0 \pm 3.0	17.7 \pm 2.0
China mainland	Mianyang (MY)	N 31°28', E 104°41'	2012	5	33	2.6 \pm 1.0	43.0 \pm 6.6	21.1 \pm 2.2	61.6 \pm 4.9
China mainland	Panzhuhua (PZ)	N 26°35', E 101°43'	2012	10	136	11.6 \pm 0.7	159.1 \pm 7.0	19.7 \pm 1.3	23.7 \pm 2.5
China mainland	Sanming (SM)	N 26°16', E 117°38'	2013	4	71	15.0 \pm 0.6	178.7 \pm 5.0	45.2 \pm 3.1	26.2 \pm 1.6
China mainland	Xichang (XC)	N 27°53', E 102°17'	2012	12	181	16.7 \pm 0.4	173.1 \pm 3.5	16.7 \pm 0.9	12.7 \pm 1.1
Overall	--	--	--	40	489	14.1 \pm 0.3	163.6 \pm 3.1	23.2 \pm 0.9	21.3 \pm 1.1

Non-Chinese introduced range									
Australia	Brisbane (BR)	S 27°29', E 153°06'	2012	2	40	18.1 ± 0.9	160.5 ± 5.5	32.8 ± 2.4	22.1 ± 1.8
Brazil	Rio de Janeiro (RJ)	S 22°53', W 43°34'	2012	6	130	13.5 ± 0.5	106.7 ± 3.9	19.3 ± 1.9	15.6 ± 1.2
Canary Islands	Tenerife (TN)	N 28°29', W 16°19'	2013	1	30	15.7 ± 1.0	168.6 ± 6.1	41.6 ± 2.9	25.5 ± 1.9
Florida (USA)	Davie (DV)	N 26°04', W 80°14'	2012	4	100	17.2 ± 0.6	172.8 ± 4.1	35.7 ± 2.1	21.9 ± 1.4
Greece	Rhodes (RD)	N 36°10', E 27°58'	2011-2012	27	294	7.3 ± 0.5	93.3 ± 4.4	40.0 ± 2.0	53.7 ± 1.6
Greece	Symi (SY)	N 36°35', E 27°50'	2012	4	37	5.5 ± 1.3	76.0 ± 10.3	32.0 ± 3.7	59.7 ± 4.7
Italy	Sicily (SC)	N 38°07', E 13°22'	2012	10	99	20.8 ± 0.8	237.8 ± 4.6	64.3 ± 3.5	26.5 ± 1.3
Libya	Tripoli (TP)	N 32°51', E 13°12'	2011-2012	7	96	19.8 ± 0.6	190.6 ± 3.1	51.9 ± 3.2	27.6 ± 1.7
Malta	Malta (MT)	N 35°56', E 14°23'	2011	9	130	18.4 ± 0.5	190.8 ± 3.6	42.6 ± 2.3	22.1 ± 1.1
Puerto Rico	Puerto Rico (PR)	N 18°23', W 66°04'	2013	7	47	14.1 ± 0.9	173.0 ± 6.8	46.4 ± 6.5	23.6 ± 2.8
South Africa	Grahamstown (GH)	S 33°19', E 26°31'	2011	7	140	20.9 ± 0.5	168.4 ± 4.5	7.0 ± 0.6	4.4 ± 0.4
South Africa	Port Elizabeth (PE)	S 33°58', E 25°37'	2011	5	81	23.5 ± 0.8	194.6 ± 2.8	5.6 ± 0.6	2.9 ± 0.3
Spain	Majorca (MJ)	N 39°35', E 2°40'	2012	6	101	19.1 ± 0.6	199.3 ± 4.0	16.7 ± 1.1	8.6 ± 0.5
Turkey	Marmaris (MM)	N 36°51', E 28°15'	2012	1	10	26.3 ± 2.2	263.0 ± 6.1	93.1 ± 10.8	35.7 ± 4.3
Overall	--	--	--	96	1335	15.5 ± 0.2	156.8 ± 1.9	33.5 ± 0.9	27.0 ± 0.7
All figs									
Overall	--	--	--	192	2681	15.2 ± 0.2	160.3 ± 1.2	36.9 ± 0.6	26.9 ± 0.4

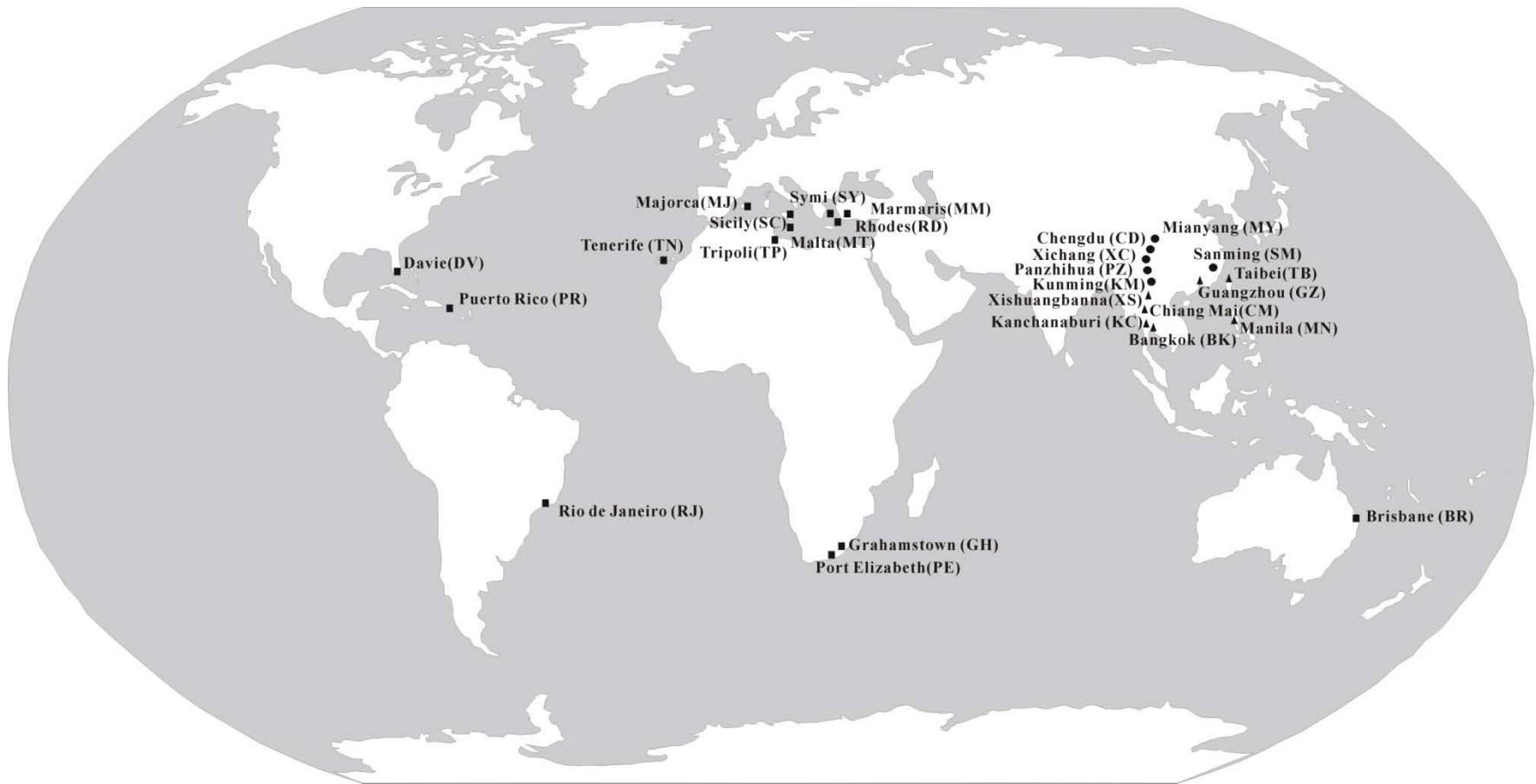


Fig. 8.1 The distribution of *F. microcarpa* sample sites (with abbreviations) in the plant's native (triangles), Chinese introduced (circles) and non-Chinese introduced (squares) ranges.

8.3.3 Fig contents

Between December 2010 and July 2013, collections of at least 10 mature figs (late C or early D phase, *sensu* Galil & Eisikowitch 1968) were taken haphazardly from *F. microcarpa* crops on different trees. The figs were stored in 70% ethanol prior to dissection and identification of their contents under a dissecting microscope. Any figs where some of the fig wasps had already emerged were discarded.

Each fig was cut into quarters and soaked in water for at least 10 minutes to soften the contents. All the flowers in each fig were sorted into the following categories: male flowers, seeds, unused female flowers (neither pollinated nor becoming galls), galls containing adult fig wasp offspring, and failed, empty galls that contained no adult offspring of fig wasps. The fig wasps were identified morphologically, based mainly on Chen et al. (1999) and Feng & Huang (2010), or scored as new morpho-species if necessary. It is hard to separate females of *Philotrypesis okinavensis* and *P. emeryi*, and we have segregated these two species on the basis of a line of hairs on the cheeks in males of *P. okinavensis*, which is absent in *P. emeryi* males. This character is a feature of *P. okinavensis sensu* Chen et al. (1999), but was also given as a character for *P. emeryi* not *P. okinavensis* by Bouček (1993). We have used the more recently published nomenclature here. Male pollinators could also not be distinguished from the males of the ‘cheater’ agaonid, and male abundance of both species was therefore estimated assuming equal sex ratios in those figs where females of both species were reared. Higher classification of the fig wasps followed Rasplus et al. (1998), Campbell et al. (2000), Cruaud et al. (2010) and Heraty et al. (2013) as shown in figweb (<http://www.figweb.org>). Figs that contained no fig wasp offspring were excluded from our analyses.

8.3.4 Spatial stratification of fig wasp offspring and seeds

Lengths of pedicels in late C phase figs reflect the spatial location of ovules, and can be used to delineate the spatial stratification of flowers with different contents (e.g.

Dunn et al. 2008a; Yu & Compton. 2012). We randomly selected 69 figs where adult pollinator offspring were present from 11 crops, including Guangzhou (7 figs from 1 crop), Xishuangbanna (11 figs from 3 crops), Bangkok (4 figs from 2 crops), Kanchanaburi (24 figs from 1 crop), Davie (6 figs from 1 crops), Tripoli (12 figs from 2 crops) and Majorca (5 figs from 1 crop). We here only specified two groups of sample sites: the plant's native (46 figs from 7 crops) and the non-Chinese introduced (23 figs from 4 crops) ranges. In these figs, we recorded the pedicel lengths and contents of all ovules that developed into either seeds or galls (including both those containing adult fig wasps and failed galls), and identified all the fig wasps. The Pedicel lengths were measured to the nearest 0.02 mm using an eyepiece graticule. Note that we did not measure the pedicel lengths of ovules supporting adult male agaonids (34 galls) in 7 figs from Xishuangbanna that were shared by both *Eupristina* species, because we could not distinguish the males of these two species. We however only collected 3 figs containing *E. verticillata* offspring (from 33 randomly selected figs) from the Chinese introduced range due to its low prevalence there, and thereby we did not include the Chinese introduced range into analyses.

8.3.5 Statistical analyses

Statistical analyses were carried out using either generalized linear mixed models (GLMMs) in R package lme4 version 1.0-5 (Bates et al. 2013) or generalized linear models (GLMs), in R 3.0.0 (R Development Core Team, 2013). Likelihood ratio tests were used to estimate the significance of both the random and fixed effects, and pair-wise comparisons between levels within fixed effects were achieved using multiple tests with Bonferroni correction. Crop identity nested in sampling sites was set as the random effect in all analyses except those relevant to species richness at crop level (where sampling site was the random effect), and pedicel lengths of ovules (where fig identity nested in crops was the random effect).

In order to compare fig wasp communities in the native and introduced ranges of *F. microcarpa*, we compared the numbers of male and female flowers per fig, the

abundance and occupancy rate of all fig wasps per fig, the prevalence and abundance of phytophagous NPFW and parasitoid per fig, and species richness of all fig wasps, and of only phytophages or parasitoids at both fig and crop levels using GLMMs that assumed either binomial (only for occupancy rate and the prevalence of phytophagous NPFW and parasitoid) or Poisson distributions of residuals. Occupancy rate was calculated as the proportion of ovules containing adult fig wasp offspring as a proportion of the total number of female flowers in a fig. In addition, differences in species richness at site level among the three areas of distribution were examined using GLMs, assuming Poisson distribution of residuals. All figs containing fig wasps were included in these analyses.

We examined variation in the prevalence (proportion of figs occupied) and abundance of total pollinator offspring, the plant's male reproductive success (female pollinator adult offspring), female reproductive success (seed numbers) and relative male reproductive success (the proportion of female pollinator offspring to the sum of female pollinator offspring and seeds in a fig) among the three areas of distribution using GLMMs that assumed either binomial (only for the prevalence of pollinator offspring and relative male reproductive success) or Poisson distributions of residuals. Only figs containing pollinator offspring were used in the analysis of relative male reproductive function of figs, and only the sample sites where pollinator offspring were present were included in the other analyses.

We specified three NPFW groups, namely the natural enemies (parasitoids) of pollinator offspring comprising all species from Sycoryctinae except *P. taiwanensis*, the only obligate seed predator, *P. taiwanensis*, and 'other NPFW' that included both other phytophagous NPFW (the non-pollinating agaonid, *Eupristina* sp., and species from subfamilies Epichrysomallinae and Otitesellinae) that are the potential competitors of both the pollinator and seeds, and parasitoids that do not attack pollinator offspring (species from families Eurytomidae and Ormyridae). The effects of these groups on pollinators/female pollinators or seeds in the three areas of distribution were estimated by comparing the prevalence and abundance of species in

these NPFW groups and ratios of these NPFW groups to pollinators/female pollinators/seeds per fig using GLMM assuming either binomial or Poisson (only for the abundance of NPFW groups) distributions of residuals. The ratio of a NPFW group to pollinator/seed was calculated as the proportion of its abundance to the sum of this abundance and adult pollinator offspring abundance/seed number in a fig (i.e. abundance of a NPFW group/ (abundance of a NPFW group + pollinator abundance/seed number)). Only figs where pollinator offspring were present were included in these analyses.

Not all galled ovules support the successful development of fig wasp larvae (Ghana et al 2012). Gall failure rates provide a potential means of assessing the quality of nutrition available to developing fig wasps, and the extent of competition between galls for these nutrients (Miao et al. 2012; Zhao et al. 2013). We examined differences in gall failure rates in all figs containing fig wasps, the figs where adult pollinator offspring or only adult pollinator offspring emerged, and those containing only 'Other NPFW' in the three areas using GLMMs that assumed binomial distributions of residuals. In addition, we also compared gall failure rates in figs with only adult pollinator offspring and those containing only 'Other NPFW' using GLMMs assuming binomial distributions of residuals. Only the sample sites where the pollinator was present were included in these analyses.

In figs where the pedicel lengths of all the ovules that developed seeds or galls were measured, we allocated all these ovules into the following categories: seeds, failed galls, galls containing adult pollinators, the parasitoids of pollinator offspring, the seed predator (*P. taiwanensis*) and 'Other NPFW'. GLMMs assuming a binomial distribution of residuals were used to test whether the spatial stratification of these categories of fig contents differed between the native and introduced ranges, i.e. the interaction between pedicel length and range on the proportion of ovules with a particular content. Considering only the ovules that developed into galls, we examined whether the proportion of failed galls to total galls with increasing pedicel length were different between the native and non-Chinese introduced ranges, using

GLMMs that assumed a binomial distribution of residuals.

8.4 Results

8.4.1 Fig wasp communities

We dissected 2681 figs that contained fig wasp offspring, from 192 crops, and identified a total of about 99038 fig wasps (Table 8.1). Overall, 32 fig wasp morpho-species were identified, comprising 14 phytophages and 18 parasitoids (Table 8.2). Among these species, 11 were not previously recorded from *F. microcarpa*. They include 10 possibly undescribed taxa and *Walkerella nigrabdomina* Ma & Yang, which had been reported from *Ficus pisocarpa* (Table 8.2; Ma et al. 2013). Eleven species were recorded only within the plant's native range and three from only the Chinese introduced range. Fig wasp communities elsewhere were composed of subsets of the species from the tree's native range, except in South Africa, where there are no pollinators present and small numbers of several native species colonize *F. microcarpa* figs (Table 8.2; van Noort et al. 2013).

Figs collected from the three groups of sample sites contained similar numbers of male and female flowers (Tables 8.1 and 8.3). Overall, they contained a mean (\pm SE) of 36.9 ± 0.6 fig wasp adult offspring, which were occupying an average of 26.9% of the ovules/female flowers available. Fig wasp abundance and ovule occupancy rates were similar in the three sample areas (Tables 8.1 and 8.3).

The pollinating agaonid was entirely absent from the figs collected at four sites (GH and PE in South Africa, KM, MY in the plant's introduced range in China). Elsewhere, it was the dominant species, with an average of 20.1 ± 0.6 adult offspring per fig and present in 55.9% (1330) of the figs (Table 8.3). The prevalence (proportion of figs occupied) of *E. verticillata* adult offspring at sites where it was present was similar in the three areas, but its abundance was significantly lower in the Chinese introduced range than elsewhere (Tables 8.4 and 8.5).

NPFW adult offspring were widespread and often abundant, occupying more than 80%

of all the figs and comprising about half the total number of fig wasp individuals (Table 8.6). Parasitoids were completely absent from two sites in the Chinese introduced range and seven sites where the plant is introduced outside China (Table 8.6), but phytophagous NPFW were present at all the sites. Phytophages were equally prevalent and abundant among the areas, but parasitoid NPFW were far less common in the non-Chinese introduced area than that in the native range. In addition, both the prevalence and abundance of parasitoids in the Chinese introduced range were intermediate among the three areas and were not significantly different from those in the other two areas (Tables 8.3 and 8.6).

The number of fig wasp species detected varied from 1 to 18 among different sample sites (Table 8.7). Totals of 29, 17 and 13 species were recorded from the native, Chinese and non-Chinese introduced ranges respectively. The mean species richness of all species, phytophages and parasitoids at all levels (fig, crop and site) were far higher in the plant's native range than in the non-Chinese introduced range with significant differences except the mean species richness of phytophages at fig level, whereas intermediate species richness were detected in the Chinese introduced range at all levels (Table 8.8). There were highly significant variations among crops within sites and among sites (crop identity nested in sampling sites) or among sites in all analyses (Random effect: $p < 0.001$ in all analyses).

Table 8.2 Summary of fig wasp morphospecies associated with *F. microcarpa*.

Note that the seed predator, *Philotrypesis taiwanensis*, was included in the phytophages, and calculations of prevalence were based on all figs containing any fig wasps.

Trophic level	Wasp taxon	Native range		Chinese introduced range		Non-Chinese introduced range	
		N sites	Prevalence	N sites	Prevalence	N sites present	Prevalence
		present	(%)	present	(%)		(%)
Putative phytophages							
	Agaonidae, Agaoninae						
<i>Ev</i>	<i>Eupristina verticillata</i> Waterston	7	66.6	4	19.6	12	49.7
<i>Es</i>	<i>Eupristina</i> sp. ('Cheater') *	1	21.0	1	3.7	0	0
	Pteromalidae, Epichrysomallinae						
<i>Aq</i>	<i>Acophila quinata</i> Zhang & Xiao	1	0.1	1	0.2	0	0
<i>Mb</i>	<i>Meselatus bicolor</i> Chen	2	1.2	5	13.5	2	15.1
<i>Oc</i>	<i>Odontofroggatia corneri</i> Wiebes	6	10.2	6	41.7	0	0
<i>Og</i>	<i>Odontofroggatia galili</i> Wiebes	3	24.0	5	36.8	10	38.1
<i>Oi</i>	<i>Odontofroggatia ishii</i> Wiebes	5	8.4	1	0.6	3	2.5
<i>Oq</i>	<i>Odontofroggatia quinifuniculus</i> Feng & Huang	4	2.9	0	0	1	0.6
<i>Sbs</i>	<i>Sycobia</i> sp. *	0	0	2	2.9	0	0
	Pteromalidae, Otitesellinae						
<i>Md</i>	<i>Micranisa degastris</i> Chen	5	6.8	2	1.2	4	9.7
<i>Wm</i>	<i>Walkerella microcarpae</i> Bouček	5	11.6	5	31.1	7	21.0
<i>Wn</i>	<i>Walkerella nigrabdomina</i> Ma & Yang	2	2.0	0	0	0	0
<i>Ws</i>	<i>Walkerella</i> sp. *	1	1.5	0	0	0	0
	Pteromalidae, Sycoryctinae						

<i>Pt</i>	<i>Philotrypesis taiwanensis</i> Chen	7	23.1	1	0.2	3	4.4
Putative parasitoids							
Eurytomidae							
<i>Bs</i>	<i>Bruchophagus sensoriae</i> Chen	0	0	2	4.5	0	0
<i>Sc</i>	<i>Sycophila curta</i> Chen	1	0.2	0	0	0	0
<i>Sm</i>	<i>Sycophila maculafacies</i> Chen	4	15.4	3	15.1	3	3.1
<i>Smp</i>	<i>Sycophila maculafacies</i> ('pale')	2	2.5	2	3.5	1	0.4
<i>Sp</i>	<i>Sycophila petiolata</i> Chen	3	17.0	2	3.3	0	0
<i>Ss</i>	<i>Sycophila</i> sp. *	1	0.6	0	0	0	0
Ormyridae							
<i>Ol</i>	<i>Ormyrus lini</i> Chen	2	0.8	0	0	0	0
<i>Os</i>	<i>Ormyrus</i> sp. *	0	0	1	0.2	0	0
Pteromalidae, Pireninae							
<i>Sic</i>	<i>Sirovena costallifera</i> Li, Xiao & Huang	1	0.1	0	0	0	0
Pteromalidae, Sycoryctinae							
<i>Pe</i>	<i>Philotrypesis emeryi</i> Grandi	5	7.7	0	0	5	1.0
<i>Po</i>	<i>Philotrypesis okinavensis</i> Ishii	5	8.3	3	10.6	3	5.4
<i>Ps1</i>	<i>Philotrypesis</i> sp.1 *	2	0.6	0	0	0	0
<i>Ps2</i>	<i>Philotrypesis</i> sp.2 *	1	0.1	0	0	0	0
<i>Ps3</i>	<i>Philotrypesis</i> sp.3 *	1	0.5	0	0	0	0
<i>Srm</i>	<i>Sycoryctes moneres</i> Chen	6	13.1	0	0	0	0
<i>Srs</i>	<i>Sycoryctes</i> sp. *	1	2.8	0	0	0	0
<i>Scg</i>	<i>Sycoscapter gajimaru</i> Ishii	5	15.9	0	0	1	3.6
<i>Scs</i>	<i>Sycoscapter</i> sp. *	1	0.5	0	0	0	0

*: newly recorded morphospecies.

Table 8.3 Comparisons of male and female flower number, total abundance of fig wasps, occupancy rates of female flowers, and the prevalence (proportion of figs where present) and abundance of phytophagous NPFW and parasitoids in the plant's native range, Chinese and non-Chinese introduced ranges using GLMMs that assumed either binomial (B) and Poisson (P) distributions of residuals.

All figs containing fig wasps were included in these analyses.

Responsible variable	Fixed effect	Model	df	Likelihood ratio	Pair-wise comparison	Z value
Male flower number	Area	GLMM (P)	2	2.218 ^{NS}	Chinese introduced vs. Non-Chinese introduced	-1.350 ^{NS}
					Chinese introduced vs. Native	-1.361 ^{NS}
					Non-Chinese introduced vs. Native	-0.194 ^{NS}
Female flower number	Area	GLMM (P)	2	0.577 ^{NS}	Chinese introduced vs. Non-Chinese introduced	-0.620 ^{NS}
					Chinese introduced vs. Native	-0.730 ^{NS}
					Non-Chinese introduced vs. Native	-0.210 ^{NS}
Total fig wasp abundance	Area	GLMM (P)	2	3.516 ^{NS}	Chinese introduced vs. Non-Chinese introduced	-0.827 ^{NS}
					Chinese introduced vs. Native	-1.897 ^{NS}
					Non-Chinese introduced vs. Native	-1.392 ^{NS}
Occupancy rate	Area	GLMM (B)	2	1.420 ^{NS}	Chinese introduced vs. Non-Chinese introduced	-0.284 ^{NS}
					Chinese introduced vs. Native	-1.104 ^{NS}
					Non-Chinese introduced vs. Native	-1.018 ^{NS}
Prevalence of phytophagous NPFW	Area	GLMM (B)	2	5.817 ^{NS}	Chinese introduced vs. Non-Chinese introduced	1.686 ^{NS}
					Chinese introduced vs. Native	2.251 ^{NS}
					Non-Chinese introduced vs. Native	1.178 ^{NS}
Phytophagous NPFW abundance	Area	GLMM (P)	2	1.016 ^{NS}	Chinese introduced vs. Non-Chinese introduced	0.523 ^{NS}
					Chinese introduced vs. Native	1.009 ^{NS}
					Non-Chinese introduced vs. Native	0.652 ^{NS}

Prevalence of parasitoids	Area	GLMM (B)	2	11.122 **	Chinese introduced vs. Non-Chinese introduced	1.266 ^{NS}
					Chinese introduced vs. Native	-1.839 ^{NS}
					Non-Chinese introduced vs. Native	-3.572 ^{**}
Parasitoid abundance	Area	GLMM (P)	2	10.000 **	Chinese introduced vs. Non-Chinese introduced	1.256 ^{NS}
					Chinese introduced vs. Native	-1.678 ^{NS}
					Non-Chinese introduced vs. Native	-3.359 ^{**}

^{NS}: not significant; ^{**}: p<0.01

Table 8.4 Prevalence (%) and abundance (means \pm SE) of pollinator adult offspring, female pollinator offspring, seeds and relative male reproductive successes of figs (female pollinators/(female pollinators + seeds)).

Relative male reproductive success of figs was calculated based only on figs containing adult pollinator offspring. We used all figs containing fig wasps in those sample sites where the pollinator was present. Order and abbreviations of sample sites are as in Table 8.1.

Site	Pollinator prevalence (N figs)	Pollinator abundance	Female pollinator abundance	Seeds	Relative male function
Native range					
GZ	75.1 (127)	24.2 \pm 2.1	18.4 \pm 1.6	16.7 \pm 1.3	0.505 \pm 0.019
XS	70.3 (196)	23.7 \pm 1.8	20.6 \pm 1.6	20.2 \pm 1.7	0.478 \pm 0.017
TB	45.5 (100)	11.8 \pm 1.3	9.9 \pm 1.1	11.4 \pm 1.1	0.440 \pm 0.019
BK	46.2 (18)	12.5 \pm 2.6	10.9 \pm 2.3	11.7 \pm 2.5	0.476 \pm 0.032
CM	100.0 (47)	67.6 \pm 5.8	54.6 \pm 4.5	42.4 \pm 3.7	0.544 \pm 0.032
KC	68.3 (43)	20.8 \pm 2.9	17.1 \pm 2.4	17.4 \pm 2.0	0.463 \pm 0.023
MN	100.0 (40)	35.0 \pm 5.2	27.9 \pm 4.4	35.2 \pm 2.6	0.372 \pm 0.027
Overall	66.6 (571)	23.0 \pm 1.0	18.9 \pm 0.8	18.6 \pm 0.8	0.473 \pm 0.009
Chinese introduced range					
CD	30.0 (6)	1.9 \pm 0.7	1.2 \pm 0.5	1.0 \pm 0.5	0.632 \pm 0.101
PZ	11.8 (16)	3.6 \pm 1.2	2.9 \pm 0.9	2.6 \pm 0.8	0.527 \pm 0.037
SM	100.0 (71)	42.7 \pm 3.3	37.5 \pm 2.7	30.5 \pm 1.7	0.560 \pm 0.017
XC	1.7 (3)	0.2 \pm 0.1	0.1 \pm 0.1	0.1 \pm 0.1	0.625 \pm 0.191
Overall	23.5(96)	8.8 \pm 1.0	7.6 \pm 0.9	5.7 \pm 0.6	0.585 \pm 0.017
Non-Chinese introduced range					
BR	95.0 (38)	26.2 \pm 2.6	21.1 \pm 2.2	16.8 \pm 1.8	0.564 \pm 0.010
RJ	77.7 (101)	15.5 \pm 1.9	10.2 \pm 1.5	21.9 \pm 2.4	0.355 \pm 0.031
TN	100.0 (30)	40.3 \pm 3.0	27.2 \pm 1.9	31.1 \pm 2.3	0.472 \pm 0.012
DV	75.0 (75)	13.0 \pm 2.0	9.3 \pm 1.5	3.7 \pm 0.5	0.661 \pm 0.028
RD	22.8 (67)	14.3 \pm 2.0	12.0 \pm 1.7	7.4 \pm 1.2	0.631 \pm 0.022
SY	24.3 (9)	9.1 \pm 4.1	7.8 \pm 3.7	2.9 \pm 1.1	0.667 \pm 0.049
SC	87.9 (87)	44.5 \pm 3.8	38.8 \pm 3.3	27.5 \pm 2.9	0.585 \pm 0.026
TP	78.1 (75)	39.4 \pm 3.9	29.8 \pm 3.3	25.0 \pm 2.3	0.520 \pm 0.023
MT	43.8 (57)	18.8 \pm 2.5	14.2 \pm 2.3	10.3 \pm 1.6	0.672 \pm 0.032
PR	93.6 (44)	42.0 \pm 6.9	35.3 \pm 6.2	24.3 \pm 4.5	0.580 \pm 0.053
MJ	69.3 (70)	9.7 \pm 1.0	7.2 \pm 0.8	7.7 \pm 1.2	0.652 \pm 0.032
MM	100.0 (10)	93.1 \pm 10.8	85.0 \pm 9.5	113.3 \pm 8.9	0.427 \pm 0.044
Overall	59.5 (663)	22.1 \pm 1.0	17.5 \pm 0.9	14.8 \pm 0.7	0.562 \pm 0.010
All figs					
Overall	55.9 (1330)	20.1 \pm 0.6	16.3 \pm 0.5	14.6 \pm 0.5	0.529 \pm 0.007

Table 8.5 Comparisons of total pollinator adult offspring and female pollinator offspring abundance, seed number and relative male reproductive success of figs among the three sample areas using GLMMs that assumed either binomial (B) and Poisson (P) distributions of residuals.

Relative male reproductive success of figs was calculated using only figs that contained adult pollinator offspring. The other analyses are based on all figs at those sites where pollinators were recorded.

Responsible variable	Fixed effect	Model	df	Likelihood ratio	Pair-wise comparison	Z value
Prevalence of pollinator offspring	Area	GLMM (B)	2	2.815 ^{NS}	Chinese introduced vs. Non-Chinese introduced	-1.610 ^{NS}
					Chinese introduced vs. Native	-1.666 ^{NS}
					Non-Chinese introduced vs. Native	-0.234 ^{NS}
Pollinator offspring abundance	Area	GLMM (P)	2	8.649 [*]	Chinese introduced vs. Non-Chinese introduced	-3.057 ^{**}
					Chinese introduced vs. Native	-2.954 ^{**}
					Non-Chinese introduced vs. Native	-0.130 ^{NS}
Female pollinator offspring abundance	Area	GLMM (P)	2	8.458 [*]	Chinese introduced vs. Non-Chinese introduced	-2.874 [*]
					Chinese introduced vs. Native	-3.066 ^{**}
					Non-Chinese introduced vs. Native	-0.498 ^{NS}
Seed number	Area	GLMM (P)	2	9.459 ^{**}	Chinese introduced vs. Non-Chinese introduced	-2.844 [*]
					Chinese introduced vs. Native	-3.407 ^{**}
					Non-Chinese introduced vs. Native	-0.987 ^{NS}
Relative male reproductive success of figs	Area	GLMM (B)	2	6.135 [*]	Chinese introduced vs. Non-Chinese introduced	-0.442 ^{NS}
					Chinese introduced vs. Native	2.852 [*]
					Non-Chinese introduced vs. Native	2.511 [*]

^{NS}: not significant; ^{*}: p<0.05; ^{**}: p<0.01.

Table 8.6 Prevalence (%) and abundance (means \pm SE) of total NPFW, phytophagous NPFW and parasitoids.

The seed predator, *Philotrypesis taiwanensis* was included in phytophagous NPFW. Order and abbreviations of sample sites are as in Table 8.1.

Site	Prevalence NPFW (%) (N figs)	NPFW abundance	Galler NPFW prevalence (%) (N figs)	Galler NPFW abundance	Parasitoid prevalence (%) (N figs)	Parasitoid abundance
Native range						
GZ	89.3 (151)	11.7 \pm 0.8	81.1 (137)	7.2 \pm 0.6	57.4 (97)	4.4 \pm 0.5
XS	83.2 (232)	54.4 \pm 2.7	81.7 (229)	51.3 \pm 2.7	39.1 (109)	2.8 \pm 0.3
TB	94.5 (208)	17.7 \pm 0.9	85.9 (189)	9.4 \pm 0.7	78.6 (173)	8.2 \pm 0.5
BK	89.7 (35)	19.1 \pm 3.3	76.9 (30)	17.1 \pm 3.4	25.6 (10)	1.5 \pm 0.6
CM	31.9 (15)	0.9 \pm 0.3	10.6 (6)	0.3 \pm 0.2	21.3 (10)	0.5 \pm 0.2
KC	76.2 (48)	15.6 \pm 1.8	73.0 (48)	11.9 \pm 1.5	42.9 (27)	3.4 \pm 0.9
MN	80.0 (32)	11.8 \pm 2.0	10.0 (4)	0.1 \pm 0.1	80.0 (32)	11.7 \pm 1.9
Overall	84.1 (721)	27.2 \pm 1.2	75.0 (643)	22.2 \pm 1.2	53.4 (458)	4.8 \pm 0.3
Chinese introduced range						
CD	85.0 (17)	8.7 \pm 1.8	85.0 (17)	8.7 \pm 1.8	0 (0)	0
KM	100.0 (48)	32.0 \pm 3.0	100.0 (48)	26.0 \pm 3.1	52.1 (25)	5.9 \pm 1.4
MY	100.0 (33)	21.1 \pm 2.2	100.0 (33)	21.1 \pm 2.2	0 (0)	0
PZ	98.5 (134)	16.1 \pm 1.0	98.5 (134)	12.5 \pm 0.8	45.4 (61)	3.6 \pm 0.5
SM	16.9 (12)	2.5 \pm 0.8	16.9 (12)	2.4 \pm 0.8	1.4 (1)	0.1 \pm 0.1
XC	99.4 (180)	16.6 \pm 0.9	99.4 (180)	12.7 \pm 0.8	39.2 (71)	3.9 \pm 0.5
Overall	86.7 (424)	15.9 \pm 0.6	86.7 (424)	12.9 \pm 0.6	32.3 (158)	3.0 \pm 0.3
Non-Chinese introduced range						
BR	62.5 (25)	6.6 \pm 1.3	62.5 (25)	4.5 \pm 0.9	32.5 (13)	2.2 \pm 0.6
RJ	79.2 (103)	3.8 \pm 0.4	64.6 (84)	2.3 \pm 0.3	44.6 (58)	1.5 \pm 0.3
TN	36.7 (11)	1.3 \pm 0.4	36.7 (11)	1.3 \pm 0.4	0 (0)	0
DV	99.0 (99)	22.7 \pm 0.9	99.0 (99)	19.2 \pm 0.8	53.0 (53)	3.5 \pm 0.5
RD	89.1 (262)	25.7 \pm 1.4	86.7 (255)	22.4 \pm 1.4	9.9 (29)	0.5 \pm 0.2
SY	89.2 (33)	22.9 \pm 2.5	83.8 (31)	20.9 \pm 2.6	8.1 (3)	1.4 \pm 0.9
SC	64.6 (64)	19.8 \pm 2.2	63.6 (63)	19.7 \pm 2.2	1.0 (1)	0.02 \pm 0.02
TP	76.0 (73)	12.5 \pm 1.3	76.0 (73)	12.5 \pm 1.3	0 (0)	0
MT	94.6 (123)	23.8 \pm 1.4	94.6 (123)	23.8 \pm 1.4	0 (0)	0
PR	63.8 (30)	4.5 \pm 0.8	55.3 (26)	3.8 \pm 0.8	27.7 (13)	0.7 \pm 0.2
GH	100.0 (140)	7.0 \pm 0.6	100.0 (140)	7.0 \pm 0.6	0 (0)	0
PE	100.0 (81)	5.6 \pm 0.6	100.0 (81)	5.6 \pm 0.6	0 (0)	0
MJ	53.5 (54)	7.1 \pm 0.9	53.5 (54)	7.1 \pm 0.9	0 (0)	0
MM	0 (0)	0	0 (0)	0	0 (0)	0
Overall	82.2 (1098)	15.0 \pm 0.5	79.8 (1065)	13.7 \pm 0.5	12.7 (170)	0.7 \pm 0.1
All figs						
Overall	83.7 (2243)	19.1 \pm 0.5	79.5 (2132)	16.3 \pm 0.5	29.2 (786)	2.4 \pm 0.1

Table 8.7 Global distributions and prevalence of fig wasps associated with *F. microcarpa* figs.

Order and abbreviations of sample sites are as in Table 8.1; Order and abbreviations of species are as in Table 8.2. **S_g**: species richness of phytophages; **S_p**: species richness of putative parasitoids; **S_t**: total fig wasp species richness. Prevalence of a species was calculated based on all figs with fig wasps present at each sampling site.

Site	Putative phytophages														Putative parasitoids														S _t								
	<i>Ev</i>	<i>Es</i>	<i>Aq</i>	<i>Mb</i>	<i>Oc</i>	<i>Og</i>	<i>Oi</i>	<i>Oq</i>	<i>Sbs</i>	<i>Md</i>	<i>Wm</i>	<i>Wn</i>	<i>Ws</i>	<i>Pt</i>	S_g	<i>Bs</i>	<i>Sc</i>	<i>Sm</i>	<i>Smp</i>	<i>Sp</i>	<i>Ss</i>	<i>Ol</i>	<i>Os</i>	<i>Sic</i>	<i>Pe</i>	<i>Po</i>	<i>Ps1</i>	<i>Ps2</i>		<i>Ps3</i>	<i>Srm</i>	<i>Srs</i>	<i>Scg</i>	<i>Scs</i>	S_p		
Native range																																					
GZ	75				2	28	12	6		2	24			36	8		20		8		1				4	28				20		16		7	15		
XS	70	65	0.4		11	0.4	6	4		9	14	5		21	11		1	3							15	6			4	9	19		7	18			
TB	45				2	72	9	1		10	3			21	8		39	7	58	2	2				3	3			10		22	2	10	18			
BK	46			21	21		10	8		3	5		33	18	9									3	13	3	10						4	13			
CM	100				2							9		2	4														11		13		2	6			
KC	68			3	24		17			10	14			35	7		6	10	8						8	2	2	2	6	16		3	10	17			
MN	100													10	2														80				1	3			
Chinese introduced range																																					
CD	30			1	1	65					5			5																				0	5		
KM		38	2	8	52	31			10	33				7	6		21						2			35								4	11		
MY				94	15									2																					0	2	
PZ	12			20	29	27		7		61				1	7	14		7	4	4						25								5	12		
SM	100				6	4	4			1	6			6												1								1	7		
XC	2			2	66	62		3		27				6			30	6	6															3	9		
Non-Chinese introduced range																																					
BR	95					43		20						3			33	15																2	5		

RJ	78		19		38	25	4			1	44			2	6
TN	100				37									0	2
DV	75		84		91	19	4		3		5		48	3	7
RD	23	60	26		1		4		9		2			2	6
SY	24	65	16	3			4				8			1	5
SC	88		63		1		3				1			1	4
TP	78		76											0	2
MT	44		95											0	2
PR	94		17		49	11	17	5			6	2		2	7
GH			18		93			2						0	2
PE			2		100			2						0	2
MJ	69		40		5	10		4						0	4
MM	100							1						0	1

Table 8.8 Comparisons of overall, phytophagous and parasitoid NPFW species richness at fig, crop and site levels among the three sample areas using GLMMs or GLMs assuming Poisson distributions of residuals.

All figs containing fig wasps were included in these analyses.

Responsible variable	Native range	Chinese introduced range	Non-Chinese introduced range	Model	df	Likelihood ratio	Pair-wise comparison	Z value
Overall species richness per fig	2.65 ± 0.05	1.88 ± 0.04	1.54 ± 0.02	GLMM	2	6.792 *	Chinese introduced vs. Non-Chinese introduced	0.807 ^{NS}
							Chinese introduced vs. Native	-1.611 ^{NS}
							Non-Chinese introduced vs. Native	-2.811 *
Species richness of phytophage per fig	1.79 ± 0.03	1.52 ± 0.03	1.41 ± 0.02	GLMM	2	1.254 ^{NS}	Chinese introduced vs. Non-Chinese introduced	0.260 ^{NS}
							Chinese introduced vs. Native	-0.707 ^{NS}
							Non-Chinese introduced vs. Native	-1.140 ^{NS}
Species richness of parasitoid per fig	0.86 ± 0.03	0.37 ± 0.03	0.13 ± 0.01	GLMM	2	10.053 **	Chinese introduced vs. Non-Chinese introduced	1.181 ^{NS}
							Chinese introduced vs. Native	-1.733 ^{NS}
							Non-Chinese introduced vs. Native	-3.381 **
Overall species richness per crop	6.50 ± 0.40	4.23 ± 0.34	2.73 ± 0.15	GLMM	2	13.439 **	Chinese introduced vs. Non-Chinese introduced	1.758 ^{NS}
							Chinese introduced vs. Native	-1.920 ^{NS}
							Non-Chinese introduced vs. Native	-4.418 ***
Species richness of phytophages per crop	3.75 ± 0.21	2.80 ± 0.20	2.30 ± 0.10	GLMM	2	11.122 **	Chinese introduced vs. Non-Chinese introduced	1.470 ^{NS}
							Chinese introduced vs. Native	-2.470 *
							Non-Chinese introduced vs. Native	-5.001 ***
Species richness of parasitoids per crop	2.75 ± 0.25	1.43 ± 0.22	0.43 ± 0.07	GLMM	2	12.263 **	Chinese introduced vs. Non-Chinese introduced	1.796 ^{NS}
							Chinese introduced vs. Native	-1.468 ^{NS}
							Non-Chinese introduced vs. Native	-3.906 ***

Overall species richness per site	12.86 ± 2.28	7.67 ± 1.54	3.93 ± 0.56	GLM	2	50.247 ^{***}	Chinese introduced vs. Non-Chinese introduced	3.346 ^{**}
							Chinese introduced vs. Native	-2.853 [*]
							Non-Chinese introduced vs. Native	-6.927 ^{***}
Species richness of phytophages per site	7.00 ± 1.15	5.50 ± 0.76	3.00 ± 0.31	GLM	2	17.434 ^{***}	Chinese introduced vs. Non-Chinese introduced	2.606 [*]
							Chinese introduced vs. Native	-1.071 ^{NS}
							Non-Chinese introduced vs. Native	-4.029 ^{***}
Species richness of parasitoids per site	5.86 ± 1.37	2.17 ± 0.87	0.93 ± 0.29	GLM	2	41.338 ^{***}	Chinese introduced vs. Non-Chinese introduced	2.160 ^{NS}
							Chinese introduced vs. Native	-3.124 ^{**}
							Non-Chinese introduced vs. Native	-5.786 ^{***}

^{NS}: not significant; ^{*}: p<0.05; ^{**}: p<0.01; ^{***}: p<0.001.

8.4.2 Reproductive success of figs

Pollinator adult offspring sex ratios were strongly female-biased, with >80% female (Table 8.4). Both the abundance of adult female offspring of *E. verticillata* per fig (the plant's male reproductive success) and also seed numbers per fig (the tree's female reproductive success) were significantly lower in the Chinese introduced range than in the other two areas, whereas there was no significant difference in both male and female reproductive successes between the plant's native and non-Chinese introduced ranges (Tables 8.4 and 8.5; Fig. 8.2 a & b). When only those figs that contained adult pollinator offspring are considered, a great increase in the relative male reproductive success was present in both the introduced ranges, relative to the native range (Tables 8.4 and 8.5; Fig. 8.2 c). Great variation among crops within sites and among sites was present (Random effect: $p < 0.001$ in all analyses).

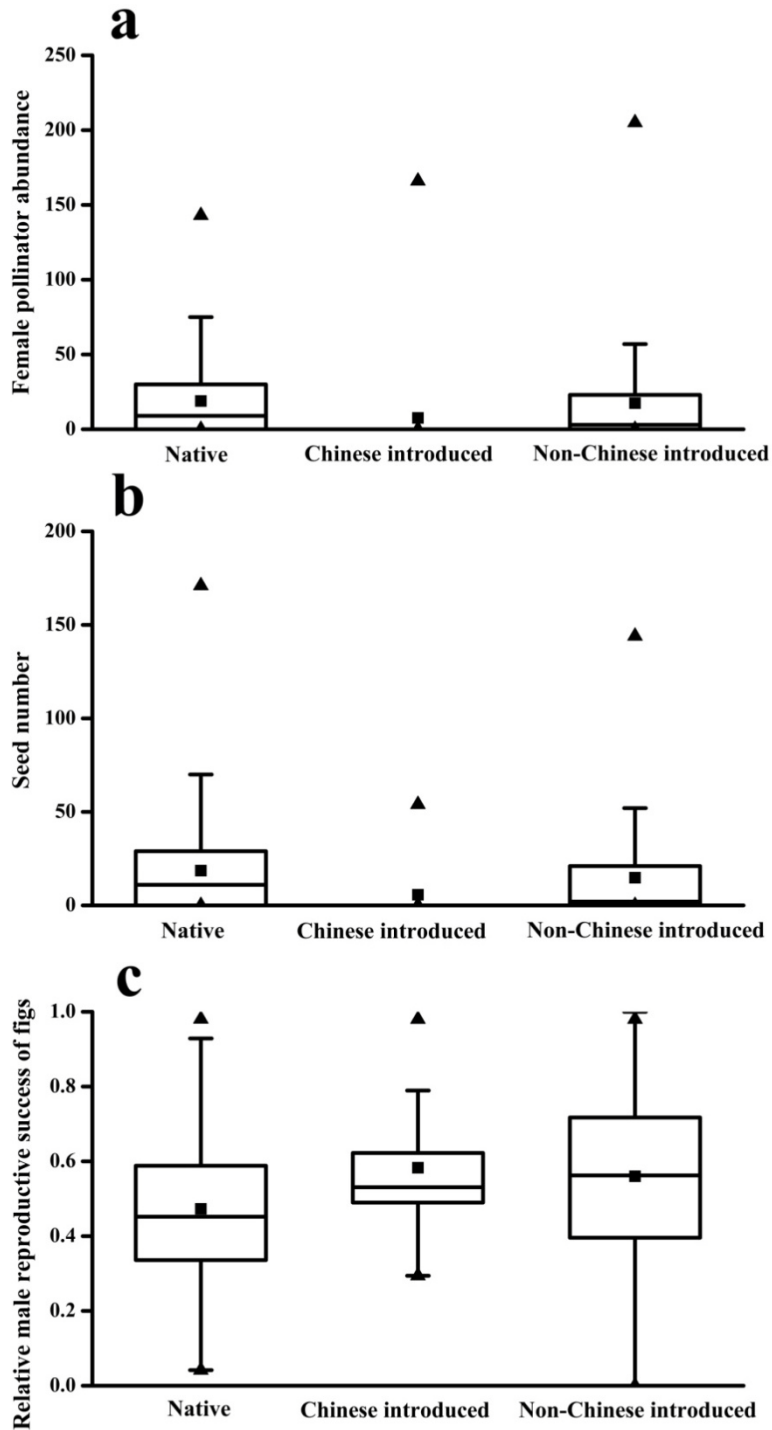


Fig. 8.2 Male (a), female (b) and relative male reproductive (c) successes of figs (per fig) in the plant's native, Chinese introduced and non-Chinese introduced ranges.

Line, box, whiskers, black squares and black triangles represent the median, the range from the first to third quartile, 1.5 times lower and upper quartiles, mean and minimum and maximum values. Only figs containing adult pollinator offspring were used.

8.4.3 Impacts of NPFW

NPFW frequently co-occurred with the pollinator, emerging in 67.1% (892 figs) of figs containing adult pollinator offspring with a mean abundance of 14.5 ± 0.6 individuals per fig. The parasitoids of pollinators were much more common in the plant's native range, occupying 42.2% of the figs where pollinator offspring were present, with a mean abundance of 3.6 ± 0.3 individuals per fig. The ratio of these parasitoids to pollinator/female pollinator offspring per fig in the native range was more than twice that recorded in the two areas of introduction (Tables 8.9 and 8.10; Fig. 8.3 a & b). The only known seed predator in *F. microcarpa* figs (*P. taiwanensis*) was present at far higher prevalence and densities in figs from the plant's native range and the ratio of *P. taiwanensis* to seeds per fig in the native range was at least twice that recorded elsewhere (Tables 8.9 and 8.10; Fig. 8.3 c). This seed predator was equally uncommon in the Chinese and non-Chinese areas of introduction.

The prevalence and abundance of 'Other NPFW' was highest in the plant's native range, but there was no significant differences among the three areas (Tables 8.9 and 8.10). In addition, neither the ratios of 'Other NPFW' to pollinator/female pollinator offspring or 'Other NPFW' to seeds per fig varied significantly among the three groups of sites, although the highest values for these two ratios were in the native range (Tables 8.9 and 8.10; Fig. 8.3 d-f). Great variation was detected among crops within sites and among sites (Random effect: $p < 0.001$ in all analyses).

Table 8.9 The prevalence (%) and abundance (mean \pm SE) of the three NPFW groups (parasitoids of the pollinator, seed predator and 'Other NPFW' (the non-pollinating agaonid, *Eupristina* sp. and species from subfamilies Epichrysomallinae and Otitesellinae and families Eurytomidae and Ormyridae)), and the ratios of these groups to either pollinators/female pollinators or seeds.

The ratio for a NPFW group was calculated as its proportion in relation to the total abundance of both this group and its hosts (either the pollinator or seeds) in a fig. All calculations were based only on figs containing adult pollinator offspring. Order and abbreviations of sample sites are as in Table 8.1.

Site	Parasitoids of pollinator prevalence (N figs)	<i>P. taiwanensis</i> prevalence (N figs)	'Other NPFW' prevalence (N figs)	Parasitoids of pollinator abundance	<i>P. taiwanensis</i> abundance	'Other NPFW' abundance	Parasitoids of pollinator to pollinator	'Other NPFW' to pollinator	Parasitoids of pollinator to female pollinator	'Other NPFW' to female pollinator	<i>P. taiwanensis</i> to seeds	'Other NPFW' to seeds
Native range												
GZ	48.0 (61)	42.5 (54)	64.6 (82)	2.5 \pm 0.4	2.8 \pm 0.4	4.1 \pm 0.6	0.099 \pm 0.016	0.162 \pm 0.020	0.121 \pm 0.019	0.192 \pm 0.022	0.156 \pm 0.025	0.194 \pm 0.023
XS	36.7 (72)	27.6 (54)	69.9 (137)	2.4 \pm 0.3	2.0 \pm 0.3	36.9 \pm 3.0	0.109 \pm 0.015	0.438 \pm 0.027	0.118 \pm 0.016	0.453 \pm 0.028	0.066 \pm 0.011	0.470 \pm 0.029
TB	50.0 (50)	38.0 (38)	71.0 (71)	6.6 \pm 0.9	4.9 \pm 1.1	8.2 \pm 1.0	0.200 \pm 0.027	0.266 \pm 0.028	0.217 \pm 0.028	0.288 \pm 0.028	0.127 \pm 0.024	0.253 \pm 0.024
BK	22.2 (4)	38.9 (7)	38.9 (7)	0.9 \pm 0.5	5.6 \pm 2.8	1.2 \pm 0.4	0.056 \pm 0.035	0.070 \pm 0.037	0.059 \pm 0.037	0.074 \pm 0.037	0.137 \pm 0.061	0.066 \pm 0.029
CM	21.3 (10)	2.1 (1)	10.6 (5)	0.5 \pm 0.2	0.04 \pm 0.04	0.3 \pm 0.1	0.011 \pm 0.006	0.006 \pm 0.003	0.013 \pm 0.007	0.007 \pm 0.003	0.001 \pm 0.001	0.004 \pm 0.002
KC	27.9 (12)	51.2 (22)	44.2 (19)	2.1 \pm 0.9	8.5 \pm 1.7	3.4 \pm 1.2	0.048 \pm 0.018	0.093 \pm 0.026	0.054 \pm 0.020	0.106 \pm 0.029	0.200 \pm 0.040	0.100 \pm 0.027
MN	80.0 (32)	10.0 (4)	0 (0)	11.7 \pm 1.9	0.1 \pm 0.1	0	0.296 \pm 0.042	0	0.346 \pm 0.048	0	0.004 \pm 0.002	0
Overall	42.2 (241)	31.5 (180)	56.2 (321)	3.6 \pm 0.3	3.0 \pm 0.3	15.3 \pm 1.2	0.122 \pm 0.009	0.243 \pm 0.013	0.137 \pm 0.010	0.260 \pm 0.014	0.100 \pm 0.009	0.259 \pm 0.014
Chinese introduced range												
CD	0 (0)	0 (0)	50.0 (3)	0	0	2.7 \pm 1.6	0	0.208 \pm 0.114	0	0.296 \pm 0.163	0	0.400 \pm 0.185
PZ	18.8 (3)	6.3 (1)	87.5 (14)	0.6 \pm 0.4	0.1 \pm 0.1	5.3 \pm 1.1	0.100 \pm 0.068	0.282 \pm 0.073	0.100 \pm 0.068	0.304 \pm 0.074	0.013 \pm 0.013	0.314 \pm 0.077
SM	1.4 (1)	0 (0)	16.9 (12)	0.1 \pm 0.1	0	2.4 \pm 0.8	0.001 \pm 0.001	0.061 \pm 0.019	0.002 \pm 0.002	0.064 \pm 0.020	0	0.085 \pm 0.026

XC	0 (0)	0 (0)	66.7 (2)	0	0	9.0 ± 4.6	0	0.513 ± 0.274	0	0.535 ± 0.279	0	0.515 ± 0.289
Overall	4.2 (4)	1.0 (1)	32.3 (31)	0.1 ± 0.1	0.01 ± 0.01	3.1 ± 0.7	0.018 ± 0.012	0.121 ± 0.023	0.018 ± 0.012	0.134 ± 0.025	0.002 ± 0.002	0.158 ± 0.029
Non-Chinese introduced range												
BR	0 (0)	0 (0)	60.5 (23)	0	0	6.2 ± 1.3	0	0.201 ± 0.038	0	0.234 ± 0.042	0	0.262 ± 0.046
RJ	39.6 (40)	29.7 (30)	37.6 (38)	1.0 ± 0.2	1.2 ± 0.3	0.9 ± 0.2	0.131 ± 0.024	0.122 ± 0.023	0.183 ± 0.031	0.163 ± 0.030	0.067 ± 0.021	0.156 ± 0.033
TN	0 (0)	0 (0)	36.7 (11)	0	0	1.3 ± 0.4	0	0.039 ± 0.012	0	0.052 ± 0.015	0	0.054 ± 0.016
DV	52.0 (39)	25.3 (19)	98.7 (74)	3.0 ± 0.4	2.3 ± 0.6	17.4 ± 0.8	0.175 ± 0.027	0.577 ± 0.024	0.239 ± 0.037	0.669 ± 0.028	0.231 ± 0.047	0.796 ± 0.021
RD	6.0 (4)	0 (0)	49.3 (33)	0.1 ± 0.1	0	5.9 ± 1.2	0.001 ± 0.001	0.116 ± 0.024	0.001 ± 0.001	0.127 ± 0.026	0	0.189 ± 0.035
SY	11.1 (1)	0 (0)	55.6 (5)	0.9 ± 0.9	0	5.4 ± 2.6	0.036 ± 0.036	0.176 ± 0.087	0.036 ± 0.036	0.187 ± 0.091	0	0.250 ± 0.107
SC	1.1 (1)	0 (0)	58.6 (51)	0.02 ± 0.02	0	18.1 ± 2.4	0.001 ± 0.001	0.297 ± 0.036	0.001 ± 0.001	0.317 ± 0.038	0	0.392 ± 0.041
TP	0 (0)	0 (0)	69.3 (52)	0	0	8.2 ± 1.1	0	0.201 ± 0.029	0	0.209 ± 0.030	0	0.263 ± 0.035
MT	0 (0)	0 (0)	87.7 (50)	0	0	18.5 ± 1.9	0	0.342 ± 0.034	0	0.285 ± 0.033	0	0.516 ± 0.047
PR	29.5 (13)	18.2 (8)	52.3 (23)	0.7 ± 0.3	0.5 ± 0.2	3.1 ± 0.7	0.078 ± 0.028	0.239 ± 0.045	0.137 ± 0.048	0.310 ± 0.057	0.098 ± 0.037	0.362 ± 0.058
MJ	0 (0)	0 (0)	32.9 (23)	0	0	3.5 ± 0.7	0	0.164 ± 0.032	0	0.193 ± 0.036	0	0.241 ± 0.044
MM	0 (0)	0 (0)	0 (0)	0	0	0	0	0	0	0	0	0
Overall	14.8 (98)	8.6 (57)	57.8 (383)	0.6 ± 0.1	0.5 ± 0.1	8.7 ± 0.5	0.046 ± 0.006	0.235 ± 0.011	0.068 ± 0.008	0.264 ± 0.013	0.042 ± 0.007	0.331 ± 0.015
All figs												
Overall	25.8 (343)	17.9 (238)	55.3 (735)	1.8 ± 0.1	1.5 ± 0.1	11.1 ± 0.6	0.076 ± 0.005	0.230 ± 0.008	0.095 ± 0.006	0.252 ± 0.009	0.065 ± 0.005	0.287 ± 0.010

Table 8.10 Comparisons of prevalence and abundance of the three NPFW groups, and the ratios of these groups to pollinator/female pollinator offspring or seeds in the three sample areas, using GLMMs assuming either binomial (B) or Poisson (P) distributions of residuals.

Only figs with adult pollinator offspring were included in these analyses.

Responsible variable	Fixed effect	Model	df	Likelihood ratio	Pair-wise comparison	Z value
Parasitoid prevalence	Area	GLMM (B)	2	10.963 **	Chinese introduced vs. Non-Chinese introduced	-0.103 ^{NS}
					Chinese introduced vs. Native	-3.182 **
					Non-Chinese introduced vs. Native	-3.335 **
Parasitoid abundance	Area	GLMM (P)	2	11.716 **	Chinese introduced vs. Non-Chinese introduced	-0.072 ^{NS}
					Chinese introduced vs. Native	-3.484 **
					Non-Chinese introduced vs. Native	-3.496 **
Parasitoid to pollinator	Area	GLMM (B)	2	10.317 **	Chinese introduced vs. Non-Chinese introduced	0.299 ^{NS}
					Chinese introduced vs. Native	-2.798 *
					Non-Chinese introduced vs. Native	-3.298 **
Parasitoid to female pollinator	Area	GLMM (B)	2	9.588 **	Chinese introduced vs. Non-Chinese introduced	0.274 ^{NS}
					Chinese introduced vs. Native	-2.729 *
					Non-Chinese introduced vs. Native	-3.148 **
<i>P. taiwanensis</i> prevalence	Area	GLMM (B)	2	8.861 *	Chinese introduced vs. Non-Chinese introduced	-0.228 ^{NS}
					Chinese introduced vs. Native	-2.723 *
					Non-Chinese introduced vs. Native	-2.676 *
<i>P. taiwanensis</i> abundance	Area	GLMM (P)	2	9.600 **	Chinese introduced vs. Non-Chinese introduced	-0.223 ^{NS}
					Chinese introduced vs. Native	-2.716 *
					Non-Chinese introduced vs. Native	-2.760 *
<i>P. taiwanensis</i> to seed	Area	GLMM (B)	2	7.497 *	Chinese introduced vs. Non-Chinese introduced	-0.062 ^{NS}

					Chinese introduced vs. Native	-2.619 *
					Non-Chinese introduced vs. Native	-2.740 *
'Other NPFW' prevalence	Area	GLMM (B)	2	1.198 ^{NS}	Chinese introduced vs. Non-Chinese introduced	-0.172 ^{NS}
					Chinese introduced vs. Native	0.600 ^{NS}
					Non-Chinese introduced vs. Native	1.095 ^{NS}
'Other NPFW' abundance	Area	GLMM (P)	2	1.527 ^{NS}	Chinese introduced vs. Non-Chinese introduced	-0.271 ^{NS}
					Chinese introduced vs. Native	0.655 ^{NS}
					Non-Chinese introduced vs. Native	1.244 ^{NS}
'Other NPFW' to pollinator	Area	GLMM (B)	2	1.530 ^{NS}	Chinese introduced vs. Non-Chinese introduced	0.298 ^{NS}
					Chinese introduced vs. Native	1.036 ^{NS}
					Non-Chinese introduced vs. Native	1.065 ^{NS}
'Other NPFW' to female pollinator	Area	GLMM (B)	2	1.648 ^{NS}	Chinese introduced vs. Non-Chinese introduced	0.454 ^{NS}
					Chinese introduced vs. Native	1.147 ^{NS}
					Non-Chinese introduced vs. Native	1.023 ^{NS}
'Other NPFW' to seed	Area	GLMM (B)	2	3.566 ^{NS}	Chinese introduced vs. Non-Chinese introduced	0.138 ^{NS}
					Chinese introduced vs. Native	1.419 ^{NS}
					Non-Chinese introduced vs. Native	1.805 ^{NS}

^{NS}: not significant; *: p<0.05; **: p<0.01

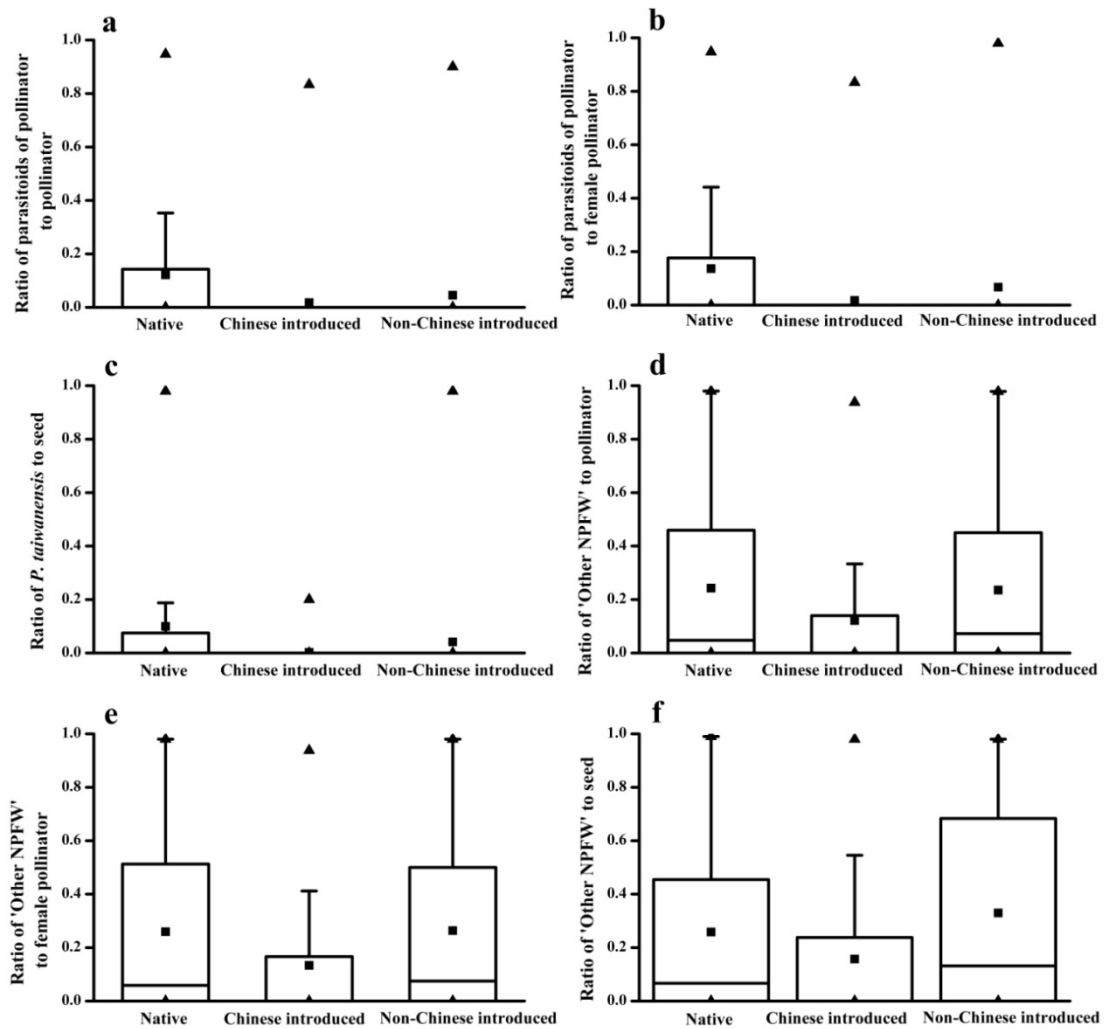


Fig. 8.3 Ratios of the three groups of NPFW (parasitoids of pollinator (a & b), seed predator (c) and 'Other NPFW' (d-f)) to pollinator/female pollinator abundance and seed number in the plant's native, Chinese introduced and non-Chinese introduced ranges.

Line, box, whiskers, black squares and black triangles represent the median, the range from the first to third quartile, 1.5 times lower and upper quartiles, mean and minimum and maximum values. 'Other NPFW' included the non-pollinating agaonid, *Eupristina* sp. and species from subfamilies Epichrysomallinae and Otitesellinae and families Eurytomidae and Ormyridae. Only figs containing adult pollinator offspring were used.

8.4.4 Gall failure rates

At sites where the pollinator was present, more than 10% of the galled ovules failed to support successful development of fig wasps in all three areas. Gall failure rates did not differ among areas (Tables 8.11 and 8.12; Fig. 8.4 a). However, when only figs that contained adult pollinator offspring are considered, there was a far lower gall failure rate in figs from the native range than elsewhere, with similarly high gall failure rates in both the Chinese and non-Chinese introduced areas (Tables 8.11 and 8.12; Fig. 8.4 b).

Gall failure rate in figs with only pollinators was far higher in both the introduced ranges than that in the native range, and this rate in figs containing only 'Other NPFW' was similar in the three areas (Tables 8.11 and 8.12; Fig. 8.4 c & d). Figs with only adult pollinator offspring typically had far higher gall failure rates than those containing only adult offspring of 'Other NPFW' (Tables 8.11 and 8.12). Response variables again varied strongly among crops within sites and among sites (Random effect: $p < 0.001$ in all analyses).

Table 8.11 Gall failure rates in all figs, the figs containing adult pollinator offspring or only adult pollinator offspring, and those where only offspring of 'Other NFPW' were present.

Only figs from sample sites where the pollinator was present are included. Order and abbreviations of sample sites are as in Table 8.1.

Site	Gall failure rates (%) in all figs	Gall failure rates in figs with pollinators (% (N figs))	Gall failure rates in figs with only pollinators (% (N figs))	Gall failure rates in figs with only 'Other NFPW' (% (N figs))
Native range				
GZ	12.8 ± 1.1	10.5 ± 1.0 (127)	6.2 ± 2.1 (18)	21.8 ± 4.8 (23)
XS	9.5 ± 0.7	10.5 ± 0.9 (196)	13.0 ± 2.3 (47)	4.6 ± 0.7 (47)
TB	11.7 ± 0.8	10.1 ± 0.7 (100)	9.9 ± 2.0 (12)	13.7 ± 1.4 (109)
BK	11.3 ± 2.2	7.6 ± 2.2 (18)	7.0 ± 4.5 (4)	12.0 ± 4.0 (15)
CM	9.9 ± 1.5	9.9 ± 1.5 (47)	10.3 ± 1.9 (32)	NA
KC	13.8 ± 2.2	7.5 ± 1.2 (43)	11.3 ± 2.8 (15)	26.4 ± 6.2 (16)
MN	9.0 ± 1.1	9.0 ± 1.1 (40)	2.2 ± 0.9 (8)	NA
Overall	11.1 ± 0.4	10.0 ± 0.4 (571)	10.2 ± 1.1 (136)	13.4 ± 1.2 (210)
Chinese introduced range				
CD	23.7 ± 4.6	38.1 ± 6.7 (6)	42.7 ± 7.9 (3)	17.4 ± 5.3 (14)
PZ	14.9 ± 1.3	17.6 ± 3.6 (16)	37.5 ± 9.4 (2)	15.8 ± 1.9 (89)
SM	17.4 ± 1.6	17.4 ± 1.6 (71)	16.4 ± 1.8 (59)	NA
XC	12.5 ± 1.0	37.4 ± 1.5 (3)	34.4 (1)	12.1 ± 1.0 (178)
Overall	14.7 ± 0.7	19.4 ± 1.3 (96)	18.5 ± 1.9 (65)	13.5 ± 0.9 (281)
Non-Chinese introduced range				
BR	15.4 ± 2.1	15.1 ± 2.2 (38)	11.0 ± 2.0 (15)	19.4 ± 7.9 (2)
RJ	47.7 ± 2.6	45.3 ± 2.9 (101)	49.2 ± 6.3 (27)	66.3 ± 9.9 (11)
TN	19.1 ± 1.8	19.1 ± 1.8 (30)	20.8 ± 2.1 (19)	NA
DV	22.0 ± 1.3	20.9 ± 1.5 (75)	57.6 (1)	26.6 ± 4.0 (14)
RD	5.6 ± 0.6	9.2 ± 1.2 (67)	8.5 ± 2.0 (32)	4.6 ± 0.6 (226)
SY	10.7 ± 2.2	16.0 ± 4.8 (9)	21.9 ± 9.2 (4)	9.2 ± 2.7 (26)
SC	21.7 ± 1.8	23.2 ± 1.8 (87)	24.0 ± 3.4 (35)	11.0 ± 6.5 (12)
TP	11.4 ± 1.3	13.9 ± 1.5 (75)	5.6 ± 1.5 (23)	2.5 ± 0.9 (21)
MT	5.6 ± 0.8	9.1 ± 1.5 (57)	14.5 ± 5.5 (7)	28.7 ± 0.8 (73)
PR	16.1 ± 2.7	14.6 ± 2.8 (44)	8.0 ± 3.3 (17)	38.8 ± 1.2 (3)
MJ	18.1 ± 1.9	24.4 ± 2.3 (70)	27.2 ± 3.0 (47)	3.6 ± 1.3 (31)
MM	5.9 ± 2.7	5.9 ± 2.7 (10)	5.9 ± 2.7 (10)	NA
Overall	16.4 ± 0.6	21.2 ± 0.8 (663)	20.5 ± 1.4 (237)	7.2 ± 0.8 (419)
All figs				
Overall	14.2 ± 0.3	16.2 ± 0.5 (1330)	17.0 ± 0.9 (438)	10.6 ± 0.5 (910)

NA: not available.

Table 8.12 Comparisons of gall failure rates in all figs, the figs containing adult pollinator offspring or only adult pollinator offspring, and those figs where only offspring of Other NFPW were present.

Only figs from sample sites where the pollinator was present are included in these analyses.

Responsible variable	Fixed effect	Model	df	Likelihood ratio	Pair-wise comparison	Z value
Gall failure rate	Area	GLMM (B)	2	2.864 ^{NS}	Chinese introduced vs. Non-Chinese introduced	0.877 ^{NS}
					Chinese introduced vs. Native	1.689 ^{NS}
					Non-Chinese introduced vs. Native	1.132 ^{NS}
Gall failure rate in the figs containing pollinators	Area	GLMM (B)	2	11.954 ^{**}	Chinese introduced vs. Non-Chinese introduced	1.651 ^{NS}
					Chinese introduced vs. Native	3.540 ^{**}
					Non-Chinese introduced vs. Native	2.864 [*]
Gall failure rate in the figs containing only pollinators	Area	GLMM (B)	2	8.795 [*]	Chinese introduced vs. Non-Chinese introduced	1.252 ^{NS}
					Chinese introduced vs. Native	2.786 [*]
					Non-Chinese introduced vs. Native	2.543 [*]
Gail failure rate in figs with only 'Other NFPW'	Area	GLMM (B)	2	0.717 ^{NS}	Chinese introduced vs. Non-Chinese introduced	0.857 ^{NS}
					Chinese introduced vs. Native	0.530 ^{NS}
					Non-Chinese introduced vs. Native	-0.333 ^{NS}
Gail failure rate	Fig types	GLMM (B)	1	127.910 ^{***}	Figs with only pollinators vs. Figs with only 'Other NFPW'	11.130 ^{***}

^{NS}: not significant; ^{*}: p<0.05; ^{**}: p<0.01; ^{***}: p<0.001.

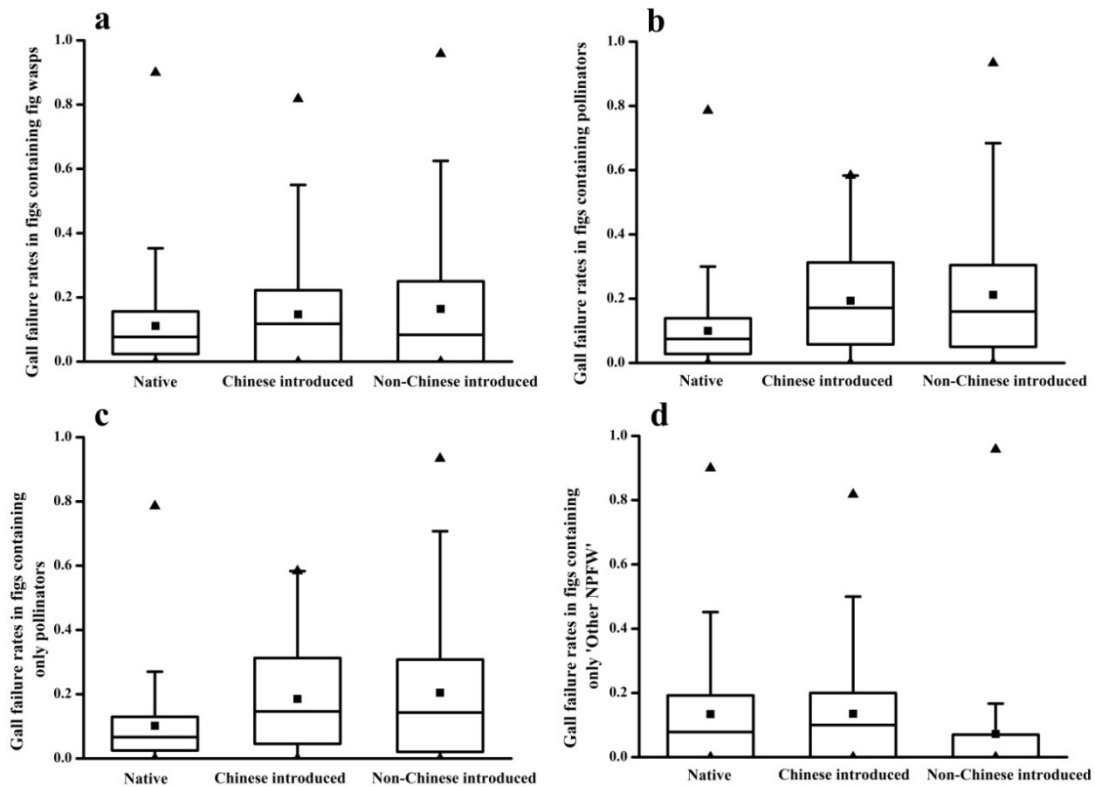


Fig. 8.4 Gall failure rates in figs containing fig wasps (a), pollinators (b), only pollinators (c) and 'Other NPFW' (d) in the plant's native, Chinese introduced and non-Chinese introduced ranges.

Line, box, whiskers, black squares and black triangles represent the median, the range from the first to third quartile, 1.5 times lower and upper quartiles, mean and minimum and maximum values. Only figs containing only adult pollinator offspring were used.

8.4.5 Spatial stratification of fig contents

We measured the pedicel lengths of 5163 ovules from 72 figs, of which 2037 supported adult pollinator offspring, 1467 developed into seeds, 1085 were galls of adult NPFW offspring and 574 were failed galls. A total of 15 NPFW species (7 phytophage and 8 parasitoid species) were in the figs from the plant's native range, with figs from the non-Chinese introduced range containing a subset of these species (Table 8.13). Most of the NPFW were 'Other NPFW' including other phytophages and parasitoids that do not attack pollinator offspring, emerging in 412 and 256 galls in the figs from the plant's native and non-Chinese introduced range respectively. Parasitoids of the pollinator and the only seed predator (*P. taiwanensis*) were common in figs from the native range, but these parasitoids were quite rare in figs from the non-Chinese introduced range (only 11 individuals from 0.6% of the total ovules measured in the non-Chinese introduced range) and *P. taiwanensis* was absent from these figs (Table 8.13).

Ovules containing pollinator offspring had significantly longer pedicels in the plant's native range, and had therefore been more likely to have been situated towards the central areas of the figs (significant difference between slopes of GLMMs in the two ranges (interaction)) (Table 8.14; Fig. 8.5 a & b). Seeds were significantly more likely to develop inside flowers with shorter pedicels, located towards the periphery of figs, but this trend was much stronger in the plant's non-Chinese introduced range than its natural range (Table 8.14; Fig. 8.5 c). Offspring of 'Other NPFW' tended to be located in ovules with shorter pedicels in the native range than the non-Chinese introduced range of *F. microcarpa*, reflected in a significant interaction between pedicel length and range (Table 8.14; Fig. 8.5 d). The proportion of ovules that become failed galls also increased with increasing pedicel length in both areas, with again a far stronger trend in the plant's non-Chinese introduced range (Table 8.14; Fig. 8.5 e). When only galled ovules are considered, the proportion of failed galls was significantly positively correlated with pedicel length in the non-Chinese introduced range but not in the

native range, where there was no hint of this effect (Table 8.14; Fig. 8.5 f).

We could not compare area effects on the spatial distributions of galls containing the parasitoids of pollinators, nor of seeds containing *P. taiwanensis*, because of their rarity or absence in the plant's introduced range. In the native range, parasitoids of pollinators were more likely to have developed in ovules with longer pedicels (GLMM: parasitoids of pollinator offspring: $\beta=2.357 \pm 0.296$, $z=7.972$, $p<0.001$), reflecting the concentration of hosts towards the centre of the figs. Conversely, the seed predatory *P. taiwanensis* was concentrated towards the periphery of the figs, where most of the seeds develop (GLMM: $\beta=-3.781 \pm 0.456$, $z=-8.285$, $p<0.001$). Great variation were present among figs within crops and among crops (fig identity nested in crops) (Random effect: $p<0.001$ in all analyses).

Table 8.13 Contents in the figs where pedicel lengths of all the ovules that developed seeds or galls were measured.

Abbreviations of species are as in Table 8.2.

Content	N figs	Pollinator <i>Ev</i>	Female pollinator	Seeds	Failed galls	Parasitoids of the pollinator						Seed predator	Other NPFW									
Species						<i>Pe</i>	<i>Po</i>	<i>Srm</i>	<i>Srs</i>	<i>Scg</i>	Sum	<i>Pt</i>	<i>Es</i>	<i>Oc</i>	<i>Og</i>	<i>Oi</i>	<i>Md</i>	<i>Wm</i>	<i>Sm</i>	<i>Smp</i>	<i>Sp</i>	Sum
Native range	46	1226	1025	960	371	39	2	49	21	54	165	241	130	46	54	107	12	31	14	11	7	412
Non-Chinese introduced range	23	811	658	507	203	0	5	0	0	6	11	0	0	0	227	0	12	12	5	0	0	256
Overall	69	2037	1683	1467	574	39	7	49	21	60	176	241	130	46	281	107	24	43	19	11	7	668

Table 8.14 Comparisons of spatial stratification patterns of ovules containing different contents between the native and non-Chinese introduced range of *F. microcarpa* using GLMM assuming binomial distribution in residuals.

Response variable	Effects	df	LR	Range	β (mean \pm SE)	Z value
Proportion of pollinators to all galled and pollinated flowers	Pedicel length \times Area	1	5.325 *	Native	2.036 \pm 0.145	14.065 ***
				Non-Chinese introduced	1.539 \pm 0.157	9.781 ***
Proportion of female pollinators to all galled and pollinated flowers	Pedicel length \times Area	1	13.618 ***	Native	1.900 \pm 0.145	13.099 ***
				Non-Chinese introduced	1.116 \pm 0.153	7.278 ***
Proportion of seeds to all galled and pollinated flowers	Pedicel length \times Area	1	38.866 ***	Native	-2.159 \pm 0.168	-12.850 ***
				Non-Chinese introduced	-4.054 \pm 0.267	-15.209 ***
Proportion of 'Other NPFW' to all galled and pollinated flowers	Pedicel length \times Area	1	5.353 *	Native	-0.918 \pm 0.209	-4.385 ***
				Non-Chinese introduced	-0.218 \pm 0.215	-1.014 NS
Proportion of failed galls to all galled and pollinated flowers	Pedicel length \times Area	1	24.830 ***	Native	0.433 \pm 0.208	2.075 *
				Non-Chinese introduced	1.946 \pm 0.222	8.754 ***
Proportion of failed galls to total galls	Pedicel length \times Area	1	18.588 ***	Native	-0.058 \pm 0.232	-0.252 NS
				Non-Chinese introduced	1.376 \pm 0.238	5.779 ***

NS: not significant; *: p<0.05; **: p<0.01; ***: p<0.001.

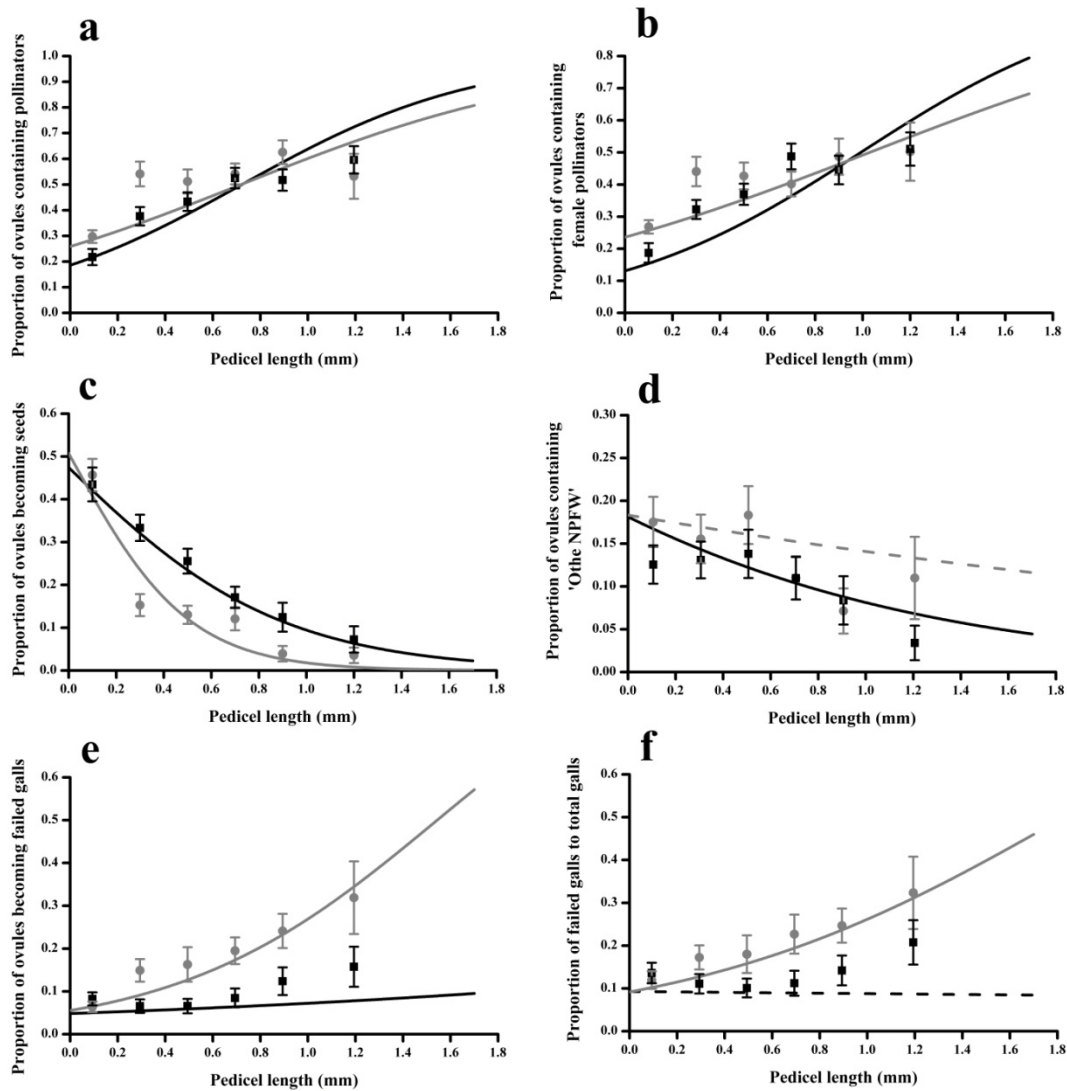


Fig. 8.5 Spatial stratification of ovules that became galls containing adult pollinator (a) and female pollinator offspring (b), developed seeds (c), adult offspring of 'Other NPFW' (d), or failed galls (e & f) in the native (black lines with square data points) and non-Chinese introduced ranges (grey lines with circle data points) ranges of *F. microcarpa*.

The proportion of ovules containing each kind of fig contents to the total ovules that were occupied by either seeds or fig wasps or the proportion of failed galls to total galls (mean \pm SE) are shown for every 0.2 mm in pedicel length except for the last one, which included all data with pedicel lengths larger than 1mm (shown in 1.20 mm, the maximum pedicel length of an ovule developing into either seed or gall was c. 1.70 mm). Note that the proportion of 'Other NPFW' offspring to all galled and pollinated flowers and that of the failed galls to total galls were independent of pedicel length in both the introduced and native ranges, as shown by dashed lines (d & f).

8.5 Discussion

The fig wasp community associated with *F. microcarpa* was far more species rich in the native range of the plant than in areas where the plant and insects have been introduced by man. The fig wasp fauna is of intermediate richness in the area of China where the range of *F. microcarpa* has been extended beyond its natural northern boundary by plantings in towns and cities. Species-poor fig wasp communities have been recorded before in some other areas where *F. microcarpa* is introduced (Compton 1989; Mifsud et al. 2012; van Noort et al. 2013), but they were more diverse in Hawaii (an archipelago we did not survey), where many NPFW might have accidentally immigrated in a deliberate introduction of the pollinator (Beardsley 1988).

The differences in species richness among the three sample area increased with spatial scales (figs, crops and sites), resulting in far higher turnover in community composition (beta diversity) in the areas where *F. microcarpa* is native (Lawton et al. 1993). The NPFW that are either parasitoids of pollinator offspring or eat seeds were rare in both areas of *F. microcarpa* introduction and consequently are expected to have much less impact on the plant's sexual reproductive successes. Like many other studies (Keane & Crawley 2002), this indicates that *F. microcarpa* should have benefitted from 'enemy release' when it was planted outside the native range, despite the spread of some of the plant's NPFW.

Like their host fig trees, fig wasps are presumed to have been dispersed around the world with the help of human activities (van Noort et al. 2013). This is in contrast to the fig wasps recorded from the plant's introduced range in China, where the fig wasps have had the opportunity to spread northwards to take advantage of the thousands (possibly millions) of *F. microcarpa* planted in towns and cities. There are no records of *F. microcarpa* becoming naturalized in these areas of China, presumably because the climatic and other conditions that prevented the natural establishment of *F. microcarpa* are unchanged. This is in contrast to areas such as Bermuda and Florida, where *F.*

microcarpa is invasive. The resources offered by the more northern-growing figs in China have nonetheless been utilized by fig wasps, some of which are clearly capable of persisting in strongly seasonal climates, both in China and elsewhere (Compton 1989; van Noort et al. 2013; Yang et al. 2013). Several NPFW were only recorded from outside the plant's native range in China, suggesting that they are more abundant there than within the native range, though the possibility that they also utilize other fig tree species cannot be excluded.

Many pollinators are capable of long-distance migration (e.g. Ahmed et al. 2009), but the dispersal abilities of NPFW are less well understood. The limited introduction and abundance of sycoryctine NPFW species (parasitoids and a seed predator) outside their native range may reflect a syndrome of relative rarity, restricted dispersal, difficulties with variable climates and their density-dependent foraging behaviour predation (Suleman et al. 2013; Yang et al. 2013; R. Wang & S.G. Compton, unpublished data). The relatively low densities of parasitoids, even in their native range (presumably as a result of feeding at a higher trophic level) means that they are less likely to be transported by unintended human activities. Females of Sycoryctinae species also have very long ovipositors, which may limit their flight abilities. More seasonal climates, with relatively cold winters, characterize many sites where *F. microcarpa* has been planted and result in large seasonal variation in the production and sizes of fig crops (Yang et al. 2013; R. Wang & S.G. Compton, unpublished data), generating fluctuations in the resources available to sycoryctines (pollinator larvae and seeds). Parasitoid NPFW can also show strong aggregations towards fig with high densities of hosts, and low densities of pollinator offspring in colder season may be problematic (Suleman et al. 2013).

The reduced diversity of NPFW in the introduced ranges of *F. microcarpa* failed to result in any increase in numbers of pollinator females or seeds that the figs contained – 'enemy release' did not translate into an increase in male and female reproductive successes for the plant. The former may be linked to elevated gall failure rates among

figs containing pollinator adult offspring collected from introduced areas, compared with the native range. At least in one fig tree species, eggs are laid in these failed galls (Ghana et al. 2012) and they represent a major mortality factor in many monoecious figs. The reasons that galls fail to support fig wasp development are unclear, but be at least partly attributable to competition for nutrients within the figs. *F. microcarpa* is often planted in areas outside its natural climatic zones, and perhaps this can result in a poorer supply of nutrients to the figs. Non-indigenous trees often lack pre-adaptations to novel environments, and founder effects can result in low population genetic diversity, both of which may have contributed to reduced vigour in the introduced range of *F. microcarpa* (Schlaepfer et al. 2010; Carroll 2011). Phytophagous NPFW (and their parasitoids) were less likely to produce failed galls in all three sample areas, suggesting that they can outperform the pollinator in terms of nutrition competition between galls. Failed galls were concentrated among ovules with longer pedicels, located towards the centre of figs. This is contrary to predictions that the survival rate of pollinator offspring ought to increase towards the center of figs (West & Herre 1994; Wang et al. 2013). Unlike the NPFW, pollinators concentrate their oviposition in these more central ovules. Most of the failed galls had therefore been galled by pollinators, which emphasizes their weakness compared with gall-forming NPFW, especially in figs shared by both groups, where NPFW appear to be superior competitors for nutrients within the figs. A specific example of differences in competitive ability is provided by the offspring of two galler NPFW (*Meselatus bicolor* Chen and *Odontofroggatia galili* Wiebes). Both are superior competitors for resources within figs of Mediterranean *F. microcarpa* (R. Wang & S.G. Compton, unpublished).

There was no increase in the numbers of seeds within figs in the introduced ranges of the plant. Seed numbers are correlated with the numbers of foundresses entering a fig, at least at lower foundress numbers, and foundress densities may simply be higher in the native range. Reduced vigour may also mean that fewer seeds develop, and this may again be linked to climatic or soil factors often being sub-optimal within thee

introduced range of the plant. Colder winters than would be experienced in the native range may be one particular problem for the plants

Although neither seed nor pollinator offspring numbers per fig increased outside the native range, the balance between male and female reproductive success was changed in favour of pollinators, probably in response to the scarcity of parasitoids. As recorded elsewhere (Dunn et al. 2008a; Yu & Compton 2012), there was a clear spatial stratification in the utilization of flowers within the figs, with galls containing pollinator larvae concentrated towards the centre and seeds towards the periphery. This pattern was present in both the native and introduced ranges of *F. microcarpa*, but in the non-Chinese introduced range there was a significant spatial expansion of ovules that were exploited for pollinator development. Fewer parasitoids in the introduced ranges has allowed more peripheral ovules to be exploited, either by more of the peripheral pollinator larvae surviving, or possible a greater willingness among foundresses to oviposit in flowers with longer styles. Other mechanisms that might generate this effect, such as a decline in the biochemical or physical defences of peripheral female flowers (West & Herre 1994) seem unlikely, given the much longer generation times of the trees and their relatively recent introductions.

In contrast to traditional 'enemy release' theory (Keane & Crawley 2002), we failed to detect enhanced male and female reproductive success among figs in the introduced ranges of *F. microcarpa*. Reduced competition for its seedlings and fewer post-dispersal seed predators (many insects and vertebrates eat fig seeds) are other factors that may be contributing to the establishment and spread of this species outside its native range. Control of invasive trees is unlikely ever to be achieved only by suppressing their sexual reproduction (Garren & Strauss 2009; Baraibar et al. 2011; Wilson et al. 2011), but the diverse communities of NPFW associated with fig trees around the world (Cook & Rasplus 2003; Cook & Segar 2010; Segar & Cook 2012), specifically those associated with *F. microcarpa*, will often include promising species that could be useful biological control agents, if required.

Chapter 9 General discussion

9.1 Species diversity

Diversity of species at higher trophic levels is predicted to be related to the diversity of their hosts (Futuyma & Moreno 1988; Forister et al. 2012). As to the insects associated with plants, their co-evolutionary history with their hosts is linked to characters of the plants, e.g. plant defence and floral scents (Schiestl 2010; Agrawal et al. 2012), that influence insect speciation. The outcome of such parallel co-evolution normally favours specialists, but is modified by host-shifts (Leppänen et al. 2013; Segar et al. 2013). The ovules inside figs are protected by a thick fig wall and have a strong defence, preventing most phytophagous insects from using them (Herre 2008). This is true for most fig trees, where only a small number of fig wasp species exploit them, though a maximum of more than 30 fig species (including both agaonid pollinators and NPFW) has been recorded to utilize a single fig species (Bouček 1988 & 1993; Cook & Segar 2010). In this study, a total of 43 morphospecies including one pollinator and 42 NPFW species were found in *F. microcarpa* figs (Chapter 2). If a leaf galling epichrysomallid (a fig wasp which does not exploit figs), *Josephiella microcarpae* Beardsley & Rasplus (Beardsley & Rasplus 2001), is added, the known number of fig wasp species associated with *F. microcarpa* rises to 44. As far as is known, this number represents the highest species richness recorded among fig plants. The extraordinarily wide native range of *F. microcarpa* must contribute to the high diversity of their related insects via creating both heterogeneous environments for species divergence and novel interactions with numerous plants whose herbivores may have the potential to utilize new hosts (Chen et al. 2012; Terborgh 2012). The intense sampling effort, and its introduction into so many novel environments has also contributed.

In *Ficus* species, host specificity is ensured by many barriers such as fig size and

specialized attractive compounds (Ware & Compton 1994; Wang et al. 2013a), whereas host-shifts of fig wasps among some genetically related fig trees had been reported recently (e.g. Zhou et al. 2012). In the present study, the dominant NPFW species varied among sites, suggesting species divergence in different parts of the native range of *F. microcarpa* (Chapter 2). In addition, host-shifts have also appeared in the plant's native range, such as with *W. nigrabdomina* (Chapter 2). Further, the fast expansion of the plant's introduced range has produced more chances for host-shifts, and several African fig wasps have been found in *F. microcarpa* figs in South Africa, though whether they can successfully establish populations on this host is still unknown (van Noort et al. 2013).

In addition to the total species diversity, recording of species distributions and community structure, which are influenced by numerous factors, are critical for a better understanding of bio-diversity patterns with major implications for the maintenance of plant-insect mutualism and biological invasion (Gaston 2000; He et al. 2005; Estes et al. 2011; Pillar et al. 2011; Segar et al. 2013). These are more fully discussed below.

9.2 Factors affecting species distributions

Current spatial patterns of species diversity reflect numerous biotic and abiotic factors, including dispersal, adaptation, inter-specific competition, defence from natural enemies, environmental conditions and latitudinal gradients (Gaston 2000; He et al. 2005; Benton 2009; Freely et al. 2012). Some generalists, usually with long life spans and a long-distance dispersal ability are likely to be more readily dispersed than others, and across-continent migration has been confirmed in some gall wasps (Stone et al. 2012). In fig wasps, pollinating agaonids also have great dispersal ability (Ahmed et al. 2009). They are also more likely to establish outside their native range with the help of anthropological activities than NPFW, because of their extremely high abundance relative to NPFW (Cook & Segar 2010; van Noort et al. 2013). The distribution of fig wasps associated with *F. microcarpa* at global scale does not reflect natural dispersal,

and instead the frequent transportation induced by international business via e.g. shipping and airline flights. For example, in the plant's introduced range, the fig wasp community in the new world was no less diverse than that in the Mediterranean (Chapters 2 and 8), and it is almost impossible for fig wasps to disperse across the Pacific by their own efforts, even if some of them can fly over 100 km (e.g. Ahmed et al. 2009). In addition, the high diversity of fig wasp in Hawaii may be linked to contamination (figs containing NPFW) at the time of the deliberate introduction of *E. verticillata* to Hawaii in 1930s (Pemberton 1939). Species at higher trophic level (parasitoids) and the single species of seed predator are far less common in the plant's introduced areas (Chapters 2, 4 and 8) probably because they rely on the presence of gallers and pollinators (Chapter 7) and their relative rarity even in the native range will also reduce the likelihood of them being transferred to the tree's introduced populations. After arrival in a novel environment, immigrant species faces challenges from inter-specific competition, predation and adaptation to their novel environments (He et al. 2005; Carroll 2011). Fig trees however represent a specific case in which all generalist herbivores and parasitoids are prevented from using figs, and competition and predation only occurs among a few fig wasps, and is commonly not intensive (Cook & Rasplus 2003; Dunn et al. 2008; Segar & Cook 2012). This was supported by our results with most ovules still remaining unused and very low prevalence of parasitoids even in the figs from the tree's native range (Chapters 7 and 8). In addition to the pollinator, gallers whose larvae can grow in the figs where pollinator offspring are absent are likely to be pioneer species because they can exploit all figs and may have superior efficiency of nutrition acquisition relative to the pollinator (Chapters 2 and 6).

Failure to adapt to novel biotic surroundings often frustrates the establishment of species (Carroll 2011). The small initial populations may suffer strong founder effects, restricting their genetic diversity and preventing the recovery of the species from inverse density dependence (the Allee effect) (Kanarek & Webb 2010). Unfortunately,

we could not test for the presence of founder effects because of a lack of DNA sequencing data, but given that many fig wasps, especially agaonids, are used to inbreeding (e.g. Herre 1987), low genetic diversity may not have a substantial impact on them.

Allee effects are common, and have been considered as one mechanism that detrimentally influences the sustainability of small populations as well as an important aspect for biological control (Tobin et al. 2011). Allee effects can be reflected in many life history traits such as mating chance (Courchamp et al. 2008). In the present study, it was found that Allee effects induced by the inherently female-biased sex ratio of most fig wasps can also impede their population growth at the early stages of colonization, when densities are low (Chapter 3). Generally, sex ratios of wasps are highly variable (Smart & Mayhew 2009). Nonetheless, it is surprising that fig wasp foundresses only lay eggs containing female offspring in a fig, because normally a foundress should have at least one male offspring (though the overall sex ratio can be extremely female-biased) to avoid strong location mating competition (Hamilton 1979; Herre 1985, 1987). Lack of male offspring has been reported in NFPW (Fellowes et al. 1999), but the underlying mechanisms are still unclear. The possible scenario is likely to reflect long-term interactions between NFPW and pollinators and the outbreeding behaviour of NFPW. In particular, many NFPW species are adapted for co-occurrence with the male pollinators that have been present throughout their co-evolutionary history. These pollinator males create exit holes for females with which they have mated, but they also help other species. Further, the fully winged males in some NFPW and the actively-walking wingless males of others indicate that they can mate outside of figs, and thus their female do not need to share figs with conspecific males, so long as other males are present.

Negative effects at the beginning of a colonisation phase can be mitigated by some genetic or community based traits e.g. pre-adaptation and inter-specific facilitation (Richardson et al. 2000; Carroll 2011; Schönrogge et al. 2012). Some common large

gallers like *O. galili* and *W. microcarpa* seem to be well-adapted to living outside the tree's native range, probably because they have evolved tolerance to variable environments, as reflected by their wide native ranges (Chapters 2 and 8). Inter-specific facilitation has been frequently reported in insects, for instance, the phloem-sucking *Brevicoryne brassicae* is able to weaken the chemical defence of its host trees and consequently facilitate the leaf-chewing *Pieris brassicae* (Soler et al. 2012). In South Africa, the co-occurrence of *O. galili* and *W. microcarpa* results in a significantly lower proportion of male-free figs, decreasing the risk of female fig wasps being trapped inside (Chapter 3), because they 'share' males for hole clearance.

Host plants strongly influence insect populations (Forister et al. 2012; Schönrogge et al. 2012), and their growth is often restricted by poor nutrient utilization, which must reflect the maladaptation to the novel environments in their introduced ranges. Such maladaptation is probably induced by the absence of mutualistic organisms in the local soil communities and abiotic conditions such as some latitude related factors (Richardson et al. 2000; Wolfe & Klironomos 2005; Yang et al. 2013). In the present study, host plant nutrition issues may be reflected in the higher mortality of pollinator offspring in the introduced range, because an insufficient nutrition supply from the host figs may have affected fig wasp (and fig tree) reproductive success. The larvae of large gallers and their parasitoids were apparently immune from such problems (Chapter 8). We did not carry out studies on the plant nutrition and soil organisms associated with *F. microcarpa*, but it is a possible area for field work in the future.

Latitudinal effects, which are often tightly correlated to abiotic environment gradients like climate, are one of the most common influences on species' distributions (Gaston 2000; Witman et al. 2004; Buckley et al. 2010). With insects, the species diversity of ichneumonid communities may display latitudinal trends (Sime & Bower 1998), but major latitudinal effects were not reported in African fig wasps (e.g. Hawkins & Compton 1992). In our study, species richness of fig wasps displayed a strong response to latitudinal outside their native range (Chapter 4). More seasonal climates in

subtropical areas appeared to contribute to the boundary of each species, with cold winters determining the thresholds (Chapter 4). Low temperatures will influence larval development times and the ability of the adult wasps to emerge from the figs and migrate between trees to look for oviposition sites (Yang et al. 2013; Zhang et al. in press). Other climate factors such as humidity can also affect the longevity of adult fig wasps (Dunn et al. 2008b), consequently contributing to their distribution patterns. These factors should be targets of further research.

Different introduced communities are predicted to become more similar through time (Cash et al. 2012), despite the barriers discussed above. In gall wasps, Stone et al. (2012) found that parasitoids trace their host herbivores far from their native ranges and are reintegrated into communities, but this can take thousands of years. An ongoing wave of species immigration has been detected in the fig wasp fauna outside the native range of *F. microcarpa* (Chapter 2), probably reflecting the increasing frequency of long-distance human transportation triggered by globalization, as in many other plants and animals (e.g. Hulme 2009). The fig wasp community in the plant's introduced range is predominantly composed of phytophages (subfamilies Agaoninae, Epichrysomallinae, Otitesellinae), but colonization of parasitoids (families Eurytomidae, Ormyridae and Sycoryctinae) has been reported more frequently in recent years (Chapter 2), indicating the reintegration of local fig wasp communities.

9.3 Community structure and niche divergence

Community structure reflects the food webs that involve component species, through which bottom-up and top-down regulations contribute to the sustainability of biodiversity and ecosystem function (Pace et al. 1999; Finke & Denno 2004; O'Corner et al. 2009; Estes et al. 2011; Yamaguchi et al. 2011). The stability of food webs tends to be positively linked with their complexity, and also the existence of the generalists that generate increased connectance (Pillar et al. 2011; Rooney & McCann 2012). The range of hosts of a particular predator (substructures in food web, or host specificity)

is compartmented mainly by niche diversification, which may be the outcome of co-evolution (Stouffer & Bascompte 2011; Segar et al. 2013), though host shifts may occur perhaps as a result of strong predation pressures and novel opportunities (Leppänen et al. 2013). For example, parasitoids of gall wasps may be restricted to a small number of host oak plants, or to galls of a certain form (Stone et al. 2012).

Unlike the pollinating agaonids, the host specificity of NPFW largely remains unknown, but with cases in which some NPFW were recorded in more than one fig species (Cook & Segar 2010). In *F. microcarpa* figs, we however detected very high host specificity of phytophages with only one galler fig wasp (*W. nigrabdomina*) known to utilize figs from two fig species in the plant's native range (Chapter 2). Further, based on several different forms of indirect evidence, for the first time we described a complex food web for the common fig wasps associated with a single fig species. Our results showed that parasitoids are often highly specific to their host phytophages at a genus level, with one genus of parasitoids preying on only one or at most two genera of gallers (Chapter 7).

Niche differentiation among phytophages and niche overlap between hosts and their parasitoids based on gall size and spatial distribution of galls inside figs contribute to shaping host specificity (Dunn et al. 2008a; Segar & Cook 2012; Yu & Compton 2012; Segar et al. 2013). In the present study, consistent results were obtained at genus level, but strong species competitions may be present within a genus and speciation may reflect other mechanisms like allopatric speciation (Chapter 7).

In contrast to many other fig tree species (e.g. Jandér et al. 2012), we did not find any evidence supporting sanctions by figs in *F. microcarpa* acting when no pollinators colonized a fig, and cheaters, galler NPFWs and their parasitoids therefore can be independent of the pollinator (Chapter 3). Such fig wasp community may be uncommon (Cook & Rasplus 2003; Herre 2008), and more work is needed to explain the independence from the pollinator. Species of NPFW belonging to the genus *Philotrypesis* were believed to all be the parasitoids of agaonids (Segar & Cook 2012;

Suleman et al. 2013; Chapter 7), but *P. taiwanensis* has adopted a novel niche (exploiting seeds). A consequence seems to be that it is currently immune to the parasitoids that attack galled ovules (Chapters 5 & 7).

9.4 Fig-fig wasp mutualism

In contrast to many other plant-pollinator symbionts, yucca moths and pollinating agaonids attack the reproductive system of their host plants (Cook & Rasplus 2003; Harrison 2005; Svensson et al. 2011). Mechanisms like selective abortion of heavily infested flowers contribute to the stability of yucca-yucca moth mutualism (Svensson et al. 2011). As to fig-pollinator mutualism, hypotheses have been proposed to explain why pollinating agaonids do not occupy all ovules in a fig, and thus leave some ovules to develop into seeds. These explanations include the impacts of parasitoids, 'optimal foraging' and 'unbeatable seeds' (Nefdt & Compton 1996; Yu et al. 2004; Dunn et al. 2008 a & b; Wang et al. 2013b).

In the present study, our results emphasised the impacts of parasitoids on pollinators ((Dunn et al. 2008a). Due to ovipositing from the outside of figs, parasitoids prefer to attack the galls containing pollinator offspring located closer to the fig wall, forcing pollinator foundresses to primarily utilize ovules located in the centre of figs and thus reserved outer ovules for seed production. Significant alteration of the spatial stratification of fig contents was detected in the tree's introduced range, where the parasitoids of the pollinator were rare, indicating that in the figs where natural enemies were almost absent, pollinator foundresses expanded the spatial range of ovules that were used and thus restricted the spatial distribution of seeds, causing higher relative male reproductive function of figs (Chapter 8). The more male-biased relative male reproductive function is not likely to be attributed to fewer pollinator offspring killed by parasitoids in the plant's introduced range because the pollinator was not more abundant, with higher mortality of pollinator offspring (gall failure rate) there (Chapter 8). Associated with the wide distribution of the pollinator, such expansion of

oviposition range may reflect rapid adaptation to novel environments, specifically to the enemy-free environment. The other NPFW did not seem to contribute to the change in the spatial stratification of fig contents because their oviposition does not depend on the presence of pollinator offspring and their effects were similar throughout both parts of the plant's range (Chapters 3 and 8). Nonetheless, we failed to detect any evidence supporting other hypotheses in the present study because many ovules were not exploited (Chapter 8) and we were not able to estimate the fitness of pollinator offspring, the life span of adult female pollinators and the oviposition handling times of pollinator foundresses. Deliberately designed experiments are therefore necessary to test them.

9.5 Biological control of invasive plants

Generally, species with high efficiency in resource utilization, i.e. more competitive (Vitousek 1990) or allelopathic capacities (He et al. 2009), and those that have generalist mutualists (e.g. seed dispersers and pollinators) (Richardson et al. 2000; Mack et al. 2000) are more likely to be invasive outside their native ranges. Many fig trees are able to survive under variable environments and produce a large number of seeds when they become mature (e.g. Dunn et al. 2008; Miao et al. 2011; Chen et al. 2012), and their seed disperser assemblage including thousands of animals, most of which can be long-distance dispersal agents (Shanahan et al. 2001; Herre 2008). It seems that a lack of host-specific pollinating agaonids is the only barrier that prevents these fig trees from becoming invasive. Actually, some fig species have been regarded as potential invasive species e.g. *Ficus benjamina* (Starr et al. 2003; Miao et al. 2011), but as far as known, only *F. microcarpa* whose pollinator has been dispersed and become well-established in most its introduced range has become invasive (Beardsley 1998; Caughlin et al. 2012). For prevention, quarantine therefore should focus on preventing both deliberate and unintended introduction of exotic pollinating agaonids. Both theoretical and empirical studies suggest that successful biological invasion is

likely to be facilitated by escape from natural enemies (Keane & Crawley 2002; Müller-Schärer & Schaffner 2008). We however did not detect enhanced seed production in the introduced populations of *F. microcarpa*, where the parasitoids of pollinator were almost absent (Chapter 8). Besides the natural enemies of the pollinators, plants may also be attacked by other kinds of enemies, e.g. phytophagous insects or other animals that feed on leaf and root tissues and seeds (Garren & Strauss 2009; Baraibar et al. 2011; Wilson et al. 2011). It is therefore likely that other factors which can affect the population recruitment of the tree might be decisive for the tree's invasion, for instance, lack of seed/seedling predators after seed dispersal and lack of specific phytophages that feed on *F. microcarpa*. In addition to the 'enemy release' hypothesis, lack of competent competitors could also facilitate biological invasion (Enge et al. 2012). For example, some plants that can co-exist with *F. microcarpa* in its native range may produce metabolites to avoid being strangled by this fig species, and hence the absence of such chemical weapon in the invaded communities may contribute to the plant's invasion. Nonetheless, from the outset, fast population expansion of *F. microcarpa* relies heavily on sexual reproduction. In addition, compared with the seed dispersal system involving numerous animals (Shanahan et al. 2001), the species-specific sexual reproductive system of the fig tree is more vulnerable and thereby could be the target for biological control.

Natural enemies of invasive species have been frequently applied to bio-control (Keane & Crawley 2002; Garren & Strauss 2009; Baraibar et al. 2011), for example *Torymus sinensis* has been used to control a pest gall wasp of chestnut, *Dryocosmus kuriphilus* (Gibbs et al. 2011). The parasitoids of the pollinator however are not the ideal agents for the control of *F. microcarpa* because (1) they exclusively rely on the pollinating and cheater agaonids (Chapter 7) and therefore they can't be used to prevent the immigration of *E. verticillata* in introduced populations of the plant before the pollinator has arrived; (2) density-dependent foraging behavior of these parasitoids will limit their effects on the host (Chapter 7), and the population density of pollinators can

rapidly recover even after serious interference (e.g. Yang et al. 2013); and (3) these parasitoids indirectly contribute to the protection of seeds (Chapter 8), as mentioned in previous studies (Dunn et al. 2008a; Segar & Cook 2012). Nonetheless, many species-specific parasitoids have been chosen as effective bio-control agents to control invasive insects despite their density-dependent foraging behaviour (e.g. Snyder & Ives 2003).

The seed predator, *P. taiwanensis*, specifically relies on the presence of the pollinator to generate seeds and it is able to strongly reduce seed production only when its larval density is very high (Chapter 5). We therefore cannot consider this species as an effective bio-control agent, though seed predators are commonly used bio-control agents of invasive plants (Baraibar et al. 2011).

Nevertheless, numerous NPFW provide a large species pool for agent selection to control invasive fig species. The large galler NPFW, *M. bicolor*, can be independent of the pollinator and can strongly suppress both male and female reproductive successes of figs via competition for nutrients and prevention of pollinator entry (Chapter 6). Further, as far as is known, this species has only been recorded in *F. microcarpa* figs, indicating great host specificity. Under these circumstances, *M. bicolor* is expected to be a promising bio-control agent.

9.6 Evolutionary implications and future work

Population genetic diversity is a key feature of newly colonised populations (Carroll 2011), and thus molecular data from fig wasp populations outside the tree's native range would be very useful to test founder effects. Phylogeographic studies at global scale are needed to detect cryptic species, delineate the dispersal routes of widespread species and examine whether multiple immigrations enriched population genetic diversity of these species and consequently facilitated rapid evolution (e.g. the apparent alteration of oviposition behavior of *E. verticillata*) outside their native ranges (e.g. Taylor & Keller 2007; Chen et al. 2012). Phylogenetics of fig wasps

associated with *F. microcarpa* will offer a better understanding of the structure and origins of fig wasp communities (e.g. Segar et al. 2013), such as host-parasitoid relationships. Further, we did not obtain direct evidence of parasitoid-host relationships in the present study because of the difficulties in identifying fig wasp larvae, but DNA barcoding techniques, which can rapidly identify insects irrespective of morphological identification (e.g. Zhou et al. 2012), are able to separate parasitoid larvae from their hosts, providing a straightforward way to study food webs.

In addition to the evolutionary history of fig wasps, it is necessary to find out the factors that facilitate the invasion of *F. microcarpa*. In order to achieve this purpose, a large amount of work needs to be carried out, including field observations to record and compare pre- and post-dispersal seed predators, soil organism communities and herbivore assemblages in the plant's native and introduced ranges. In addition, we also need to record the structure and components of the plant communities where *F. microcarpa* is either a native or an introduced species to test whether its successful invasion links to the species richness in a community (e.g. Sax et al. 2007; Catford et al. 2012). As for the biological control agent, *M. bicolor*, controlled experiments need to be carried out to quantify the relationship between its effects on seed production and its initial population density (e.g. at what initial population density (individuals per fig) can *M. bicolor* sterilize all/most figs) and to assess its tolerance to hot climates, in preparation for the future application of this species in the field.

Further, we need to carry out studies focusing on the fitness of pollinators, including the life span of adult female pollinators, the oviposition behavior and handling time of pollinator foundresses when their number is controlled in each fig to test whether other hypotheses like life-span constraints, 'optimal foraging' and 'unbeatable seeds' (Yu et al. 2004; Dunn et al. 2008b; Wang et al. 2013b) also contribute to stabilising the fig-pollinator mutualism in this monoecious fig.

Reference

- Ahmed, S., Compton, S.G., Butlin, R.K. & Gilmartin, P.M. (2009) Wind-borne insects mediate directional pollen transfer between desert fig trees 160 kilometers apart. *Proceedings of National Academy of Sciences of United States of American*, 106, 20342-20347.
- Allee, W.C., Emerson, A.E., Park, O. & Schmidt, K.P. (1949) *Principles of Animal Ecology*. W.B. Saunders.
- Agrawal, A.A., Hastings, A.P., Johnson, M.T.J., Maron, J.L. & Salminen, J-P. (2012) Insect herbivores drive real-time ecological and evolutionary change in plant populations. *Science*, 338, 113-116.
- Arnaud, J-F., Fénart, S., Cordellier, M. & Cuguen, J. (2010) Populations of weedy crop-wild hybrid beets show contrasting variation in mating system and population genetic structure. *Evolutionary Applications*, 3, 305–318.
- Baayen, R.H. (2011) *LanguageR: data sets and functions with ‘Analyzing Linguistic Data: A practical introduction to statistics’*. R package version 1.4.
- Baez, M. (1998) Presence in the Canary Islands of the laurel de Indias pollinator wasp. *Boletín de la Asociación Española de Entomología*, 22, 225.
- Baltazar, C.R. (1966) A catalogue of Philippine Hymenoptera (with a bibliography, 1758-1963). *Pacific Insects Monograph*, 8, 1-488.
- Bannerman, J.A., Shorthouse, J.D., Pither, J. & Lalonde, R.G. (2012) Variability in the parasitoid community associated with galls of *Diplolepis variabilis* (Hymenoptera: Cynipidae): a test of the distance decay hypothesis. *Canadian Entomologist*, 144, 635-644.
- Baraibar, B., Carrión, E., Recasens, J. & Westerman, P.R. (2011) Unravelling the process of weed seed predation: Developing options for better weed control. *Biological Control*, 56, 85-90.
- Barrett, S.C.H., Colautti, R.I. & Eckert, C.G. (2008) Plant reproductive systems and evolution during biological invasion. *Molecular Ecology*, 17, 373–383.
- Bartel, R.A., Oberhauser, K.S., de Roode, J.C. & Altizer, S.M. (2011) Monarch butterfly migration and parasite transmission in eastern North America. *Ecology*, 92, 342-351.
- Bates, D., Maechler, M. & Bolker, B. (2013) *lme4: Linear mixed-effects models using S4 classes*. R package version 0.999999-2.
- Beardsley, W.J. (1998) Chalcid wasps (Hymenoptera: Chalcidoidea) associated with fruit of *Ficus microcarpa* in Hawaii. *Proceedings of the Hawaiian Entomological Society*, 33, 19-34.
- Beardsley, J.W. & Rasplus, J-Y. (2001) A new species of *Josephiella* (Hymenoptera: Agaonidae) forming leaf galls on *Ficus microcarpa* L. (Moraceae). *Journal of Natural History*, 35, 33-40.
- Benton, M.J. (2009) The Red Queen and the Court Jester: species diversity and the role of biotic and abiotic factors through time. *Science*, 323, 728-732.
- Berg, C.C. & Corner, E.J.H. (2005) *Moraceae-Ficus*, *Flora Malesiana*, Ser. I, 17/2, Leiden, pp. 289-301.
- Bolker, B.M., Brooks, M.E., Clark, C.J., Geange, S.W., Poulsen, J.R., Stevens, M.H.H. & White, J.-S.S. (2009) *Generalized linear mixed models: a practical guide for ecology and evolution*. *Trends in Ecology and Evolution*, 24, 127-135.

- Bouček, Z., Watsham, A. & Wiebes, J.T. (1981) The fig wasp fauna of the receptacles of *Ficus thonningii* (Hymenoptera, Chalcidoidea). *Tijdschrift voor Entomologie*, 124, 149-233.
- Bouček Z (1988) Australian Chalcidoidea (Hymenoptera): a biosystematic revision of genera of fourteen families, with a reclassification of species. CAB International, Wallingford.
- Bouček, Z. (1993) The genera of chalcidoid wasps from *Ficus* fruit in the New World. *Journal of Natural History*, 27, 173–217.
- Buckley, L.B., Davies, T.J., Ackerly, D.D., Kraft, N.J.B., Harrison, S.P. et al. (2010) Phylogeny, niche conservatism and the latitudinal diversity gradient in mammals. *Proceedings of the Royal Society B: Biological Sciences*, 277, 2131-2138.
- Burnham, K.P. & Overton, W.S. (1978) Estimation of the size of a closed population when capture probabilities vary among animals. *Biometrika*, 65, 623-633.
- Burrows, J. & Burrows, S. (2003) *Figs of southern & south-central Africa*. Umdaus Press, Hatfield.
- Burstein, M., Wool, D. & Eshel, A. (1994) Sink strength and clone size of sympatric, gall-forming aphids. *European Journal of Entomology*, 91, 57–71.
- Campbell, B., Heraty, J., Rasplus, J.-Y., Chan, K., Steffan-Campbell, J. & Babcock, C. (2000) Molecular systematics of the Chalcidoidea using 28S-rDNA. In: Austin, A.D. & Dowton, M. (Ed) *The Hymenoptera: Evolution, Biodiversity and Biological Control*, CSIRO Publishing, Canberra, pp. 59-73.
- Carroll, S.P. (2011) Conciliation biology: the eco-evolutionary management of permanently invaded biotic systems. *Evolutionary Applications*, 4, 184-199.
- Cash, F.B.J., Conn, A., Coutts, S., Stephen, M., Mason, N.W.H., Anderson, B.J. & Wilson, J.B. (2012) Assembly rules operate only in equilibrium communities: Is it true? *Austral Ecology*, 37, 903-914.
- Catford, J.A., Vesk, P.A., Richardson, D.M. & Pyšek, P. (2012) Quantifying levels of biological invasion: towards the objective classification of invaded and invulnerable ecosystems. *Global Change Biology*, 18, 44-62.
- Caughlin, T., Wheeler, J.H., Jankowski, J. & Lichstein, J.W. (2012) Urbanized landscapes favored by fig-eating birds increase invasive but not native juvenile strangler fig abundance. *Ecology*, 93, 1571-1580.
- Chao, A. & Lee, S.-M. (1992) Estimating the number of classes via sample coverage. *Journal of the American Statistical Association*, 87, 210-217.
- Charnov, E.L. & Skinner, S.W. (1984) Evolution of Host Selection and Clutch Size in Parasitoid Wasps. *Florida Entomologist*, 67, 5-21.
- Chen, H.-H., Yang, D.-R., Gu, D., Compton, S.G. & Peng, Y.-Q. (2013) Secondary galling: a novel feeding strategy among 'non-pollinating' fig wasps from *Ficus curtipes*. *Ecological Entomology*, 38, 381-389.
- Chen, Y., Compton, S.G., Liu, M., & Chen, X.-Y. (2012) Fig trees at the northern limit of their range the distributions of cryptic pollinators indicate multiple glacial refugia. *Molecular Ecology*, 21, 1687-1701.
- Chen, Y.-R., Chuang, W.-C. & Wu, W.-J. (1999) Chalcid wasps on *Ficus microcarpa* L. in Taiwan (Hymenoptera: Chalcidoidea). *Journal of Taiwan Museum*, 52, 39-79.
- Civeyrel, L. & Simberloff, D. (1996) A tale of two snails: is the cure worse than the disease?

- Biodiversity and Conservation, 5, 1231–1252.
- Compton, S.G. (1989) The fig wasp, *Odontofroggattia galili* (Hymenoptera: Pteromalidae), in the Greek Isles. Entomologist's Gazette, 40, 183-184.
- Compton, S.G. (1993a) An association between epichrysomallines and eurytomids (Hymenoptera: Chalcidoidea) in southern African fig wasp communities. African Entomology, 1, 123–125.
- Compton, S.G. (1993b) One way to be a fig. African Entomology, 1, 151-158.
- Compton, S.G., Ball, A.D., Collinson, M.E., Hayes, P., Rasnitsyn, A.P. & Ross, A.J. (2010) Ancient fig wasps indicate at least 34 million years of stasis in their mutualism with fig trees. Biology Letters, 6, 838-842.
- Compton, S.G. & Disney, R.H.L. (1991) New species of *Megaselia* (Diptera: Phoridae) whose larvae live in figs (Urticales: Moraceae), and adults prey on fig wasps (Hymenoptera: Agaonidae). Journal of Natural History, 25, 203-219.
- Compton, S.G. & Hawkins, B.A. (1992) Determinants of species richness in southern African fig wasp assemblages. Oecologia, 91, 68-74.
- Compton, S.G., Holton, K.C., Rashbrook, V.K., Van Noort, S., Vincent, S.L. & Ware, A.B. (1991) Studies on *Ceratosolen galili*, a non-pollinating Agaonid fig wasp. Biotropica, 23, 188–194.
- Compton, S.G. & Nefdt, R.J.C. (1990) The figs and fig wasps of *Ficus butt-davyi*. Mitteilungen aus dem Institut für allgemeine Botanik in Hamburg, 23a, 441-450.
- Compton, S.G., Rasplus, J. & Ware, A.B. (1994) African fig wasp parasitoid communities. In: Hawkins BA, Sheehan W, editors. Parasitoid community ecology. Oxford University Press. pp. 343-370.
- Compton, S.G. & Robertson, H.G. (1988) Complex interactions between mutualisms: ants tending homopterans protect fig seeds and pollinators. Ecology, 69, 1302–1305.
- Compton, S.G., Thornton, I.W.B., New, T.R. & Underhill, L. (1988) Colonisation of the Krakatau Islands by fig wasps and other chalcids. Philosophical Transactions of the Royal Society of London B, 322, 459-470.
- Compton, S.G. & van Noort, S. (1992) Southern African fig wasp assemblages: host relationships and resource utilization. Proceedings of the Koninklijke Nederlandse Akademie van Wetenschappen, 95, 423-435.
- Compton, S.G., van Noort, S., Mcleish, M., Deeble, M. & Stone, V. (2009) Sneaky African fig wasps that oviposit through holes drilled by other species. African Nature History, 5, 9-15.
- Condamine, F.L., Sperling, F.A.H., Wahlberg, N., Rasplus, J-Y. & Kergoat, G.J. (2012) What causes latitudinal gradients in species diversity? Evolutionary processes and ecological constraints on swallowtail biodiversity. Ecology Letters, 15, 267-277.
- Cook, J.M. & Power, S.A. (1996) Effects of within-tree flowering asynchrony on the dynamics of seed and wasp production in an Australian fig species. Journal of Biogeography, 23, 487-493.
- Cook, J.M. & Rasplus, J-Y. (2003) Mutualists with attitude: coevolving fig wasps and figs. Trends in Ecology and Evolution, 18, 241-248.
- Cook, J.M. & Segar, S.T. (2010) Speciation in fig wasps. Ecological Entomology, 35, 54-66.
- Corlett, R.T. (2006) Figs (*Ficus*, Moraceae) in urban Hong Kong, South China. Biotropica, 38, 116-121.
- Courchamp, F., Berec, L. & Gascoigne, J. (2008) Allee Effects in Ecology and Conservation.

Oxford University Press, Oxford.

Courchamp, F., Clutton-Brock, T. & Grenfell, B. (1999) Inverse density dependence and the Allee effect. *Trends in Ecology and Evolution*, 14, 405-409.

Crawley, M.J. (2007) *The R Book*. John Wiley & Sons, Ltd.

Cruaud, A., Jabbour-Zahab, R., Genson, G., Cruaud, C., Couloux, A., Kjellberg, F., van Noort, S. & Rasplus, J-Y. (2010) Laying the foundations for a new classification of Agaonidae (Hymenoptera: Chalcidoidea), a multilocus phylogenetic approach. *Cladistics*, 26, 359-387.

Cruaud, A., Ronsted, N., Chantarasuwan, B., Chou, L-S., Clement, W.L., Couloux, A., Cousins, B., Genson, G., Harrison, R.D., Hanson, P.E., Hossaert-McKey, M., Jabbour-Zahab, R., Jousset, E., Kerdelhué, C., Kjellberg, F., Lopez-Vaamonde, C., Peebles, J., Peng, Y-Q., Pereira, R.A.S., Schramm, T., Ubaidillah, R., van Noort, S., Weiblen, G.D., Yang, D-R., Yodpinyanee, A., Libeskind-Hadas, R., Cook, J.M., Rasplus, J-Y. & Savolainen, V. (2012) An extreme case of plant-insect codiversification: figs and fig-pollinating wasps. *Systematic Biology*, 61, 1029-1047.

Deutsch, C.A., Tewksbury, J.J., Huey, R.B., Sheldon, K.S., Ghalambor, C.K., et al. (2008) Impacts of climate warming on terrestrial ectotherms across latitude. *Proceedings of National Academic of Sciences of United States of America*, 105, 6668-6672.

Dietz, H., & Edwards, P.J. (2006) Recognition that causal processes change during plant invasion helps explain conflicts in evidence. *Ecology*, 87, 1359-1367.

Doğanlar, M. (2012) Occurrence of fig wasps (Hymenoptera: Chalcidoidea) in *Ficus caria* and *F. microcarpa* in Hatay, Turkey. *Turkish Journal of Zoology*, 36, 721-724.

Dorchin, N., Cramer, M.D. & Hoffmann, J.H. (2006) Photosynthesis and sink activity of wasp-induced galls in *Acacia pycnantha*. *Ecology*, 87, 1781-1791.

Dunn, D.W., Segar, S.T., Ridley, J., Chan, R., Crozier, R.H., Yu, D.W. & Cook, J.M. (2008a) A role for parasites in stabilising the fig-pollinator mutualism. *PLoS Biology*, 6, e59.

Dunn, D.W., Yu, D.W., Ridley, J.R. & Cook, J.M. (2008b) Longevity, early emergence and body size in a pollinating fig wasp - implications for stability in a fig-pollinator mutualism. *Journal of Animal Ecology*, 77, 927-935.

Ehrensfield, J.G. (2010) Ecosystem consequences of biological invasions. *Annual Review of Ecology, Evolution, and Systematics*, 41, 59-80.

Enge, S., Nylund, G.M., Harder, T. & Pavia, H. (2012) An exotic chemical weapon explains low herbivore damage in an invasive alga. *Ecology*, 93, 2736-2745.

Estes, J.A., Terborgh, J., Brashares, J.S., Power, M.E., Berger, J., Bond, W.J., Carpenter, S.R., Essington, T.E., Holt, R.D., Jackson, J.B.C., Marquis, R.J., Oksanen, L., Oksanen, T., Paine, R.T., Pickett, E.K., Ripple, W.J., Sandin, S.A., Scheffer, M., Schoener, T.W., Shurin, J.B., Sinclair, A.R.E., Soulé, M.E., Virtanen, R. & Wardle, D.A. (2011) Trophic downgrading of planet earth. *Science*, 333, 301-306.

Farache, F.H.A., DO Ó, V.T. & Pereira, R.A.S. (2009) New occurrence of non-pollinating fig wasps (Hymenoptera: Chalcidoidea) in *Ficus microcarpa* in Brazil. *Neotropical Entomology*, 38, 683-685.

Fauvergue, X., Vercken, E., Malausa, T. & Hufbauer, R.A. (2012) The biology of small, introduced populations, with special reference to biological control. *Evolutionary Applications*, 5, 424-443.

- Feeley, K.J., Malhi, Y., Zelazowski, P. & Silman, M.R. (2012) The relative importance of deforestation, precipitation change, and temperature sensitivity in determining the future distributions and diversity of Amazonian plant species. *Global Change Biology*, 18, 2636-2647.
- Feng, G. & Huang, D-W. (2010) Description of a new species of *Odontofroggattia* (Chalcidoidea, Epichrysomallinae) associated with *Ficus microcarpa* (Moraceae) with a key to species of the genus. *Zootaxa*, 2335, 40–48.
- Fellowes, M.D.E., Compton, S.G. & Cook, J.M. (1999) Sex allocation and local mate competition in Old World non-pollinating figs wasps. *Behavioral Ecology and Sociobiology*, 46, 95-102.
- Figueiredo, R.A.de. & Motta, J.C. Jr. (1993) Biology of *Walkerella yashiroi* (Hymenoptera, Chalcidoidea). *Naturalia* (Sao Paulo), 18, 27-32.
- Figueiredo, R.A.de., Motta, J.C. Jr. & Vasconcellos, L.A.S. (1995) Pollination, seed dispersal, seed germination and establishment of seedlings of *Ficus microcarpa*, Moraceae, in southeastern Brazil. *Revista Brasileira de Biologia*, 55, 233-239.
- Finke, D.L. & Denno, R.F. (2004) Predator diversity dampens trophic cascades. *Nature*, 429, 407-410.
- Forister, M.L., Dyer, L.A., Singer, M.S., Stireman, J.O. & Lill, J.T. (2012) Revisiting the evolution of ecological specialization, with emphasis on insect-plant interactions. *Ecology*, 93, 981-991.
- Freestone, A.L., Osman, R.W., Ruiz, G.M. & Torchin, M.E. (2011) Stronger predation in the tropics shapes species richness patterns in marine communities. *Ecology*, 92, 983-993.
- Futuyma, D.J. & Moreno, G. (1988) The evolution of ecological specialization. *Annual Review of Ecology and Systematics*, 19, 207-233.
- Galil, J. & Copland, J.W. (1981) *Odontofroggattia galili* Wiebes in Israel, a primary fig wasp of *Ficus microcarpa* with a unique ovipositor mechanism (Epichrysomallinae, Chalcidoidea). *Proceedings of the Koninklijke Nederlandse Akademie van Wetenschappen, Series C: Biological and Medical Sciences*, 84, 183-195.
- Galil, J. & Eisikowitch, D. (1968) Flowering cycles and fruit types of *Ficus sycomorus* in Israel. *New Phytologist*, 67, 745-758.
- Garren, L.M. & Strauss, S.Y. (2009) Population-level compensation by an invasive thistle thwarts biological control from seed predators. *Ecological Applications*, 19, 709-721.
- Gaston, K.J. (2000) Global patterns in biodiversity. *Nature*, 405, 220-227.
- Ghalambor, C.K., McKay, J.K., Carroll, S.P. & Reznick, D.N. (2007) Adaptive versus non-adaptive phenotypic plasticity and the potential for adaptation to new environments. *Functional Ecology*, 21, 394–407.
- Ghana, S., Suleman, N. & Compton, S.G. (2012) Factors influencing realized sex ratios in fig wasps: double oviposition and larval mortalities. *Journal of Insect Behavior*, 25, 254-263.
- Gibbs, M., Schönrogge, K., Alma, A., Melika, G., Quacchia, A., Stone, G.N. & Aebi, A. (2011) *Torymus sinensis*: a viable management option for the biological control of *Dryocosmus kuriphilus* in Europe? *BioControl*, 56, 527-538.
- Gill, R. J. (1994) California Plant Pest and Disease Report, pp62-64.
- Gluckman, P.D., Low, F.M., Bukijasa, T., Hanson, A. & Beedle, A.S. (2011) How evolutionary principles improve the understanding of human health and disease. *Evolutionary Applications*, 4, 249-263.

- Godfray, H.C.J. (1988) Virginity in haplodiploid populations: a study on fig wasps. *Ecological Entomology*, 13, 283–291.
- Godfray, H.C.J. (1994) *Parasitoids: behavioural and evolutionary ecology*. Princeton, New Jersey: Princeton University Press.
- Gomulkiewicz, R., Holt, R.D., Barfield, M. & Nuismer, S.L. (2010) Genetics, adaptation, and invasion in harsh environments. *Evolutionary Applications*, 3, 97–108.
- Grandi, G. (1926) Hyménoptères sycophiles récoltés à Sumatra et à Java par E. Jacobson. *Descriptions préliminaires*. *Treubia*, 8, 352-364.
- Greeff, J.M. (1997) Offspring allocation in externally ovipositing fig wasps with varying clutch size and sex ratio. *Behavioral Ecology*, 8, 500-505.
- Greeff, J.M. & Compton, S.G. (2002) Can seed protection lead to dioecy in *Ficus*? *Oikos*, 96, 386-388.
- Gurevitch, J., Fox, G.A., Wardle, G.M., Inderjit & Taub, D. (2011) Emergent insights from the synthesis of conceptual frameworks for biological invasions. *Ecology Letters*, 14, 407-418.
- Hadfield, J. (2012) MCMCglmm: MCMC Generalized linear Mixed Models. R package version 2.17.
- Hamilton, W.D. (1979) Wingless and fighting males in fig wasps and other insects. In Blum, M.S. & Blum, N.A. (eds): *Sexual selection and reproductive competition in insects*. Academic Press, London, pp 167-220.
- Hancock, A.M., Witonski, D.B., Ehler, E., Alkorta-Aranburu, G., Beall, C., Gebremedhin, A., Sukernik, R. et al. (2010) Human adaptations to diet, subsistence, and ecoregion are due to subtle shifts in allele frequency. *Proceedings of the National Academy of Sciences of the USA*, 107, 8924-8930.
- Harrison, R.D. (2005) Figs and the diversity of tropical rainforests. *Bioscience*, 55, 1053-1064.
- Harrison, R.D. & Rasplus, J-Y. (2006) Dispersal of fig pollinators in Asian tropical forests. *Journal of Tropical Ecology*, 22, 631-639.
- Hartley, S.E. (1998) The chemical composition of plant galls: are levels of nutrients and secondary compounds controlled by the gall-former? *Oecologia*, 113, 492-501.
- Hawkins, B.A. & Compton, S.G. (1992) African fig wasp communities: undersaturation and latitudinal gradients in species richness. *Journal of Animal Ecology*, 61, 361-372.
- He, F-L., Gaston, K.J., Connor, E.F. & Srivastava, D.S. (2005) The local-regional relationship: immigration, extinction and scale. *Ecology*, 86, 360-365.
- He, W-M., Feng, Y., Ridenour, W.M., Thelen, G.C., Pollock, J.L., Diaconu, A. & Callaway, R.M. (2009) Novel weapons and invasion: biogeographic differences in the competitive effects of *Centaurea maculosa* and its root exudate (\pm)-catechin. *Oecologia*, 159, 803-815.
- Heimpel, G.E. (1994) Virginity and the cost of insurance in highly inbred Hymenoptera. *Ecological Entomology*, 19, 299-302.
- Heltsh, J. & Forrester, N.E. (1983) Estimating species richness using the jackknife procedure. *Biometrics*, 39, 1-11.
- Heraty, J.M., Burks, R.A., Cruaud, A., Gibson, G.A.P., Liljeblad, J., Munro, J., Rasplus, J-Y., Delvare, G., Janšta, P., Gumovsky, A., Huber, J., Woolley, J.B., Krogmann, L., Heydon, S., Polaszek, A., Schmidt, S., Darling, D.C., Gates, M.W., Mottern, J., Murray, E., Dal Molin, A.,

- Triapitsyn, S., Baur, H., Pinto, J.D., van Noort, S., George, J. & Yoder, M. (2013) A phylogenetic analysis of the megadiverse Chalcidoidea (Hymenoptera). *Cladistics*, 29, 466-542.
- Herre, E.A. (1985) Sex ratio adjustment in fig wasps. *Science*, 228, 896-898.
- Herre, E.A. (1987) Optimality, plasticity, and selective regime in fig wasp sex ratios. *Nature*, 329, 627-629.
- Herre, E.A. (1993) Population structure and the evolution of virulence in nematode parasites of fig wasps. *Science*, 259, 1442-1445.
- Herre, E.A., Jandér, K.C. & Machado, C.A. (2008) Evolutionary ecology of figs and their associates: recent progress and outstanding puzzles. *Annual Review of Ecology, Evolution and Systematics*, 39, 439-458.
- Hilburn, D.J., Marsh, P.M. & Schauff, M.E. (1990) Hymenoptera of Bermuda. *The Florida Entomologist*, 73, 161-176.
- Hill, D.S. (1967) Figs (*Ficus* spp.) and fig-wasps (Chalcidoidea). *Journal of Natural History*, 1, 413-434.
- Hill, R.L., Gourlay, A.H. & Fowler, S.V. (2000) The biological control program against gorse in New Zealand. *Proceedings of the X International Symposium on Biological Control of Weeds*, 4-14 July 1999, Montana State University, Bozeman, Montana, USA. Neal R. Spencer [ed.]. pp. 909-917.
- Hillebrand, H. (2004) On the generality of the latitudinal diversity gradient. *The American Naturalist*, 163, 192-211.
- Holt, R.D. & Lawton, J.H. (1993) Apparent competition and enemy-free Space in insect host-parasitoid Communities. *The American Naturalist*, 142, 623-645.
- Horning, M. & Mellish, J-A.E. (2012) Predation on a upper trophic marine predator, the Steller sea lion: evaluating high juvenile mortality in a density-dependent conceptual framework. *PLoS ONE*, 7, e30173.
- Hulme, P.E. (2009) Trade, transport and trouble: managing invasive species pathways in an era of globalization. *Journal of Applied Ecology*, 46, 10-18.
- Ishii, T. (1934) Fig chalcidoids of Japan. *Kontyû*, 8, 84-100.
- Jandér, K.C., Herre, E.A. & Simms, E.L. (2012) Precision of host sanctions in the fig tree-fig wasp mutualism: consequences for uncooperative symbionts. *Ecology Letters*, 15, 1362-1369.
- Jauharlina, J. (2014) Fig trees and fig wasps: their interactions with non-mutualists. University of Leeds.
- Jauharlina, J., Lindquist, E.E., Quinnell, R.J., Robertson, H.G. & Compton, S.G. 2012. Fig wasps as vectors of mites and nematodes. *African Entomology*, 20, 101-110.
- Jiang, Z-F., Huang, D-W., Chen, L-L., Zhen, W-Q., Fu, Y-G. & Peng, Z-Q. (2006) Rampant host switching and multiple female body color transitions in *Philotrypesis* (Hymenoptera: Chalcidoidea: Agaonidae). *Journal of Evolutionary Biology*, 19, 1157-1166.
- Joseph, K.J. (1957) Le parasitisme de *Philotrypesis caricae* L. (Hym., Chalcidiens Callimomidae) et l'influence de la vie parasitairesur le parasite. *Comptes Rendus de l'Academie des Sciences*, 244, 1269-1272.
- Jousselin, E., van Noort, S., Berry, V., Rasplus, J-Y., Ronsted, N., Erasmus, J.C. & Greeff, J.M. (2008) One fig to bind them all: host conservatism in a fig wasp community unraveled by

- cospeciation analyses among pollinating and non-pollinating fig wasps. *Evolution*, 62, 1777-1797.
- Jousselin, E., van Noort, S. & Greeff, J.M. (2004) Labile male morphology and intraspecific male polymorphism in the *Philotrypesis* fig wasps. *Molecular Phylogenetics and Evolution*, 33, 706-718.
- Kanarek, A.R. & Webb, C.T. (2010) Allee effects, adaptive evolution, and invasion success. *Evolutionary Applications*, 3, 122-135.
- Kaufmann, S., Mckey, D.B., Hossaert-Mckey, M. & Horvitz, C.C. (1991) Adaptations for a two phase seed dispersal system involving vertebrates and ants in a hemiepiphytic fig (*Ficus microcarpa*: Moraceae). *American Journal of Botany*, 78, 971-977.
- Keane, R.M. & Crawley, M.J. (2002) Exotic plant invasions and the enemy release hypothesis. *Trends in Ecology and Evolution*, 17, 164-170.
- Kerdelhué, C. & Rasplus, J-Y. (1996) The evolution of dioecy among *Ficus* (Moraceae): an alternative hypothesis involving non-pollinating fig wasp pressure on the fig-pollinator mutualism. *Oikos*, 77, 163-166.
- Kerdelhué, C., Rossi, J.P. & Rasplus, J.Y. (2000) Comparative community ecology studies on old world figs and fig wasps. *Ecology*, 81, 2832-2849.
- Knoche, D.G., Flagg, C. & Seastedt, T.R. (2010) Effects of plant competition, seed predation, and nutrient limitation on seedling survivorship of spotted knapweed (*Centaurea stoebe*). *Biological Invasions*, 12, 3771-3784.
- Kobbi, M., Chaieb, M., Edelin, C. & Michaloud, G. (1996) Relationship between a mutualist and a parasite of the laurel fig, *Ficus microcarpa* L. *Canadian Journal of Zoology*, 74, 1831-1833.
- Kolbe, J.J., Glor, R.E., Schettino, L.R., Lara, A.C., Larson, A. & Losos, J.B. (2004) Genetic variation increases during biological invasion by a Cuban lizard. *Nature*, 431, 177-181.
- Koponen, M. & Askew, R.R. (2002) Chalcids from Madeira, Canary Islands and Azores (Hymenoptera, Chalcidoidea). *Vieraea*, 30, 115-145.
- Kruess, A. & Tscharntke, T. (1994) Habitat fragmentation, species loss, and biological control. *Science*, 264, 1581-1584.
- Lande, R. (1998) Demographic stochasticity and Allee effect on a scale with isotropic noise. *Oikos*, 83, 353-358.
- László, Z. & Tothmeresz, B. (2008) Optimal clutch size of the gall wasp *Diplolepis rosae* (Hymenoptera: Cynipidae). *Entomologica Fennica*, 19, 168-175.
- Lawton, J.H., Lewinsohn, T. & Compton, S.G. (1993) Patterns of diversity for the insect herbivores on bracken. In *Species Diversity in Ecological Communities: Historical and Geographical perspectives* (Ricklefs, R.E. & Schluter, D. eds.), pages 178-184, University of Chicago Press, Chicago.
- Leppänen, S.A., Altenhofer, E., Liston, A.D. & Nyman, T. (2013) Ecological versus phylogenetic determinants of trophic associations in a plant-leafminer-parasitoid food web. *Evolution*, 67, 1493-1502.
- Li, Y-W., Zhou, X., Feng, G., Hu, H-Y., Niu, L., Hebert, P.D.N. & Huang, D-W. (2010) COI and ITS2 sequences delimit species, reveal cryptic taxa and host specificity of fig-associated *Sycophila* (Hymenoptera, Eurytomidae). *Molecular Ecology Resources*, 10, 31-40.
- Li, Z., Xiao, H. & Huang, D-W. (2013) *Sirovena* Bouček (Pteromalidae: Pireninae), a new

member of the fig wasp community associated with *Ficus microcarpa* (Moraceae). *Zootaxa*, 3619, 581-588.

Lo Verde, G. & Porcelli, F. (2010) First record of the non-pollinating fig wasp *Odontofroggattia galili* Wiebes, 1980 from Malta (Hymenoptera, Chalcidoidea, Agaonidae). *Bulletin of the Entomological Society of Malta*, 3, 5-8.

Lo Verde, G., Porcelli, F., Bella, S. & Rasplus, J-Y. (2007) Imenotteri Agonidi nuovi per l'Europa e loro ruolo nella naturalizzazione di *Ficus* spp. in Italia. *Atti XXI Congresso Nazionale Italiano di Entomologia*, Campobasso, 11-16 giugno 2007, 60.

Lo Verde, G., Porcelli, F. & Sinacori, A. (1991) Presenza di *Parapristina verticillata* (Waterst.) e *Odontofroggattia galili* Wiebes (Hymenoptera: Chalcidoidea Agaonidae) in Sicilia. *Atti della Congresso Nazionale Italiana Di Entomologia*, 16, 139-143.

Ma, Y-C., Peng, Y-Q. & Yang, D-R. (2013) Description of two new species of *Walkerella* (Pteromalidae, Otitesellinae). *Zootaxa*, 3702, 473-482.

Mack, R.N., Simberloff, D., Lonsdale, W.M., Evans, H., Clout, M & Bazzaz, F. (2000) Biotic invasions: causes, epidemiology, global consequences and control. *Ecological Applications*, 10, 689-710.

McGeoch, M.A., Butchart, S.H.M., Spear, D., Marais, E., Kleynhans, E.J., Symes, A., Chanson, J. & Hoffman, M. (2010) Global indicators of biological invasion: species numbers, biodiversity impact and policy responses. *Diversity and Distribution*, 16, 95-108.

McGeoch, M.A. & Chown, S.L. (1997) Evidence of competition in a herbivorous, gall-inhabiting moth (Lepidoptera) community. *Oikos*, 78, 107-115.

McKey, D. (1989) Population biology of figs: applications for conservation. *Experientia*, 45, 661-673.

Mcleish, M., Guo, D., van Noort, S. & Midgley, G. (2011) Life on the edge: rare and restricted episodes of a pan-tropical mutualism adapting to drier climates. *New Phytologist*, 191, 210-222.

McPherson, J.R. (2005) A recent expansion of its Queensland range by *Eupristina verticillata*, Waterston (Hymenoptera, Agaonidae, Agaoninae), the pollinator of *Ficus microcarpa* l.f. (Moraceae). *Proceedings of the Linnean Society of New South Wales*, 126, 197-201.

Miao, B-G., Yang, D-R., Liu, C., Peng, Y-Q. & Compton, S.G. (2011) The impact of a gall midge on the reproductive success of *Ficus benjamina*, a potentially invasive fig tree. *Biological Control*, 59, 228-233.

Mifsud, S. www.maltawildplants.com. Accessed April 2014.

Mifsud, D., Falzon, A., Malumphy, C., de Lillo, E., Vovlas, N. & Porcelli, F. (2012) On some arthropods associated with *Ficus* species (Moraceae) in the Maltese Islands. *Bulletin of the Entomological Society of Malta*, 5, 5-34.

Molbo, D., Machado, C.A., Sevenster, J.G., Keller, L. & Herre, E.A. (2003) Cryptic species of fig-pollinating wasps: Implications for the evolution of the fig-wasp mutualism, sex allocation, and precision of adaptation. *Proceedings of the National Academy of Sciences of the United States of America*, 100, 5867-5872.

Moore, J.C., Zavodna, M., Compton, S.G. & Gilmartin, P. (2005) Sex ratio strategies and the evolution of cue use. *Proceedings of the Royal Society B: Biological Sciences*, 272, 1287-1294.

Murray, M.G. (1987) The closed environment of the fig receptacle and its influence on male

- conflict in the old World fig wasp, *Philotrypesis pilosa*. *Animal Behaviour*, 35, 488-506.
- Müller-Schärer, H. & Schaffner, U. (2008) Classical biological control: exploiting enemy escape to manage plant invasions. *Biological Invasion*, 10, 859-874.
- Nadel, H., Frank, J.H. & Knight Jr., R.J. (1992) Escapees and accomplices: The naturalisation of exotic *Ficus* and their associated faunas in Florida. *Florida Entomologist*, 75, 29-38.
- Navntoft, S., Wratten, S.D., Kristensen, K. & Esbjerg, P. (2009) Weed seed predation in organic and conventional fields. *Biological Control*, 49, 11-16.
- Nedft, R.J. & Compton, S.G. (1996) Regulation of seed and pollinator production in the fig-fig wasp mutualism. *Journal of Animal Ecology*, 65, 170-182.
- O'Connor, M.I., Piehler, M.F., Leech D.M., Anton, A. & Bruno, J.F. (2009) Warming and resource availability shift food web structure and metabolism. *PLoS Biology*, 7, e1000178.
- Otero, J.T. & Ackerman, J.D. (2002) Flower style length and seed production in two species of *Ficus* (Moraceae) in Puerto Rico. *Caribbean Journal of Science*, 38, 249-251.
- Pace, M.L., Cole, J.J., Carpenter, S.R. & Kitchell, J.F. (1999) Trophic cascades revealed in diverse ecosystems. *Trends in Ecology and Evolution*, 14, 483-488.
- Pearson, D.E., Gallaway, R.M. & Maron, J.L. (2011) Biotic resistance via granivory: establishment by invasive, naturalized, and native asters reflects generalist preference. *Ecology*, 92, 1748-1757.
- Pemberton, C.E. (1939) Note on introduction and liberation of *Eupristina verticillata* Waterston in Honolulu. *Proceedings of the Hawaiian Entomological Society*, 10, 182.
- Peng, Y-Q., Compton, S.G & Yang, D-R. (2010) The reproductive success of *Ficus altissima* and its pollinator in a strong seasonal environment: Xishuangbanna, Southwestern China. *Plant Ecology*, 209, 227-236.
- Peng, Y-Q., Duan, Z-B., Yang, D-R. & Rasplus, J-Y. (2008) Co-occurrence of two *Eupristina* species on *Ficus altissima* in Xishuangbanna, SW China. *Symbiosis*, 45, 9-14.
- Pereira, R.A.S., Teixeira, S. de P. & Kjellberg, F. (2007) An inquiline fig wasp using seeds as a resource for small male production: a potential first step for the evolution of new feeding habits? *Biological Journal of the Linnean Society*, 92, 9-17.
- Pheloung, P.C., Williams, P.A. & Halloy, S.R. (1999) A weed risk assessment model for use as a biosecurity tool evaluating plant introductions. *Journal of Environmental Management*, 57, 239-251.
- Pillai, P., Gonzalez, A. & Loreau, M. (2011) Metacommunity theory explains the emergence of food web complexity. *Proceedings of National Academy of Sciences of United States of American*, 108, 19293-19298.
- Pimentel, D. (2005) Environmental consequences and economic costs of alien species. *Invasive Plants: Ecological and Agricultural Aspects*, 269-276.
- Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D. & the R Development Core Team (2013) nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-110.
- Priyadarsanan, D.R. (2000) Fig insects of Kerala. *Records of the Zoological Survey of India, Occasional Paper, No 182: iv + 175pp.*
- Pyšek, P., Jarošík, V., Hulme, P.E., Pergl, J., Hejda, M., Schaffner, U. & Vilà, M. (2012) A global assessment of invasive plant impacts on resident species, communities and ecosystems: the

interaction of impact measures, invading species' traits and environment. *Global Change Biology*, 18, 1725-1737.

R Development Core Team (2012) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN3-900051-07-0.

Radford, I.J., Nicholas, D.M. & Brown, J.R. (2001) Assessment of the Biological Control Impact of Seed Predators on the Invasive Shrub *Acacia nilotica* (Prickly Acacia) in Australia. *Biological Control*, 20, 261-268.

Ramírez, B.W. & Montero, S.J. (1988) *Ficus microcarpa* L., *F. benjamina* L. and other species introduced in the New World, their pollinators (Agaonidae) and other fig wasps. *Revista de Biología Tropical*, 36, 441-446.

Ramsey, D.S.L., Parkes, J. & Morrison, S.A. (2008) Quantifying eradication success: the removal of feral pigs from Santa Cruz Island, California. *Conservation Biology*, 23, 449-459.

Ramya, K.T., Fiyaz, R.A., Shaanker, R.U. & Ganeshiah, K.N. (2011) Pollinators for a syconium: How do wasps choose among syconia? *Current Science*, 101, 520-527.

Rasplus, J-Y., Kerdelhué, C., Le Clainche, I., & Mondor, G. (1998) Molecular phylogeny of fig wasps (Hymenoptera). Agaonidae are not monophyletic. *Compte Rendu de l'Académie des Sciences de Paris*, 321, 517-527.

Richardson, D.M., Allospp, N., D'Antonio, C.M., Milton, S.J. & Rejmánek, M. (2000) Plant invasions - the role of mutualisms. *Biological Reviews*, 75, 65-93.

Romdal, T.S., Araújo, M.B., Rahbek, C. (2013) Life on a tropical planet: niche conservatism and global diversity gradient. *Global Ecology and Biogeography*, 22, 344-350.

Rooney, N. & McCann, K.S. (2012) Integrating food web diversity, structure and stability. *Trends in Ecology and Evolution*, 27, 40-46.

Santos, A.M.C. & Quicke, D.L.J. (2011) Large-scale diversity patterns of parasitoids insects. *Entomological Science*, 14, 371-382.

Sasua, M.A., Ferraria, M.J., Dua, D., Winsor, J.A. & Stephenson, A.G. (2009) Indirect costs of a nontarget pathogen mitigate the direct benefits of a virus-resistant transgene in wild *Cucurbita*. *Proceedings of the National Academy of Sciences of the USA*, 106, 19067-19071.

Sax, D.F., Stachowicz, J.J., Brown, J.H., Bruno, J.F., Dawson, M.N., Gaines, S.D., Grosberg, R.K., Hastings, A., Holt, R.D., Mayfield, M.M., O'Connor, M.I. & Rice, W.R. (2007) Ecological and evolutionary insights from species invasions. *Trends in Ecology and Evolution*, 22, 465-471.

Schlaepfer, D.R., Glättli, M., Fischer, M. & van Kleunen, M. (2010) A multi-species experiment in their native range indicates pre-adaptation of invasive alien plant species. *New Phytologist*, 185, 1087-1099.

Schönrogge, K., Begg, T., Williams, R., Melika, G., Randle, Z. & Stone, G.N. (2012) Range expansion and enemy recruitment by eight alien gall wasp species in Britain. *Insect Conservation and Diversity*, 5, 298-311.

Seaby, R.M. & Henderson, P.A. (2006) *Species Diversity and Richness Version 4*. Pisces Conservation Ltd., Lymington, England.

Segar, S.T. & Cook, J.M. (2012) The dominant exploiters of the fig/pollinator mutualism vary across continents, but their costs fall consistently on the male reproductive function of figs. *Ecological Entomology*, 37, 342-349.

- Segar, S.T., Dunn, D.W., Darwell, C.T. & Cook, J.M. (In press) How to be a fig wasp down under: The diversity and structure of an Australian fig wasp community. *Acta Oecologica*.
- Segar, S.T., Pereira, R.A.S., Compton, S.G. & Cook, J.M. (2013) Convergent structure of multitrophic communities over three continents. *Ecology Letters*, 16, 1436-1445.
- Shanahan, M., So, S., Compton, S.G. & Corlett, R. (2001) Fig-eating by vertebrate frugivores: a global review. *Biological Reviews*, 76, 529-572.
- Schiestl, F.P. (2010) The evolution of floral scent and insect chemical communication. *Ecology Letters*, 643-656.
- Sime, K.R. & Brower, A.V. (1998) Explaining the latitudinal gradient anomaly in ichneumonid species richness: evidence from butterflies. *Journal of Animal Ecology*, 67, 387-399.
- Simberloff, D. (2003) How much information on population biology is needed to manage introduced species? *Conservation Biology*, 17, 83-92.
- Simberloff, D. (2009) The role of propagule pressure in biological invasions. *Annual Review of Ecology, Evolution, and Systematics*, 40, 81-102.
- Simberloff, D. (2011) How common are invasion-induced ecosystem impact? *Biological Invasions*, 13, 1255-1268.
- Simberloff, D., Martin, J-L., Genovesi, P., Maris, V., Wardle, D.A., Aronson, J., Courchamp, F., Galil, B., García-Berthou, E., Pascal, M., Pyšek, P., Sousa, R., Tabacchi, E. & Vila, M. (2013) Impacts of biological invasions: what's what and the way forward. *Trends in Ecology and Evolution*, 28, 58-66.
- Skelhorn, J., Rowland, H.M., Delf, J., Speed, M.P. & Ruxton, G.D. (2011) Density-dependent predation influences the evolution and behavior of masquerading prey. *Proceedings of National Academy of Sciences of United States of American*, 108, 6532-6536.
- Smart, C.B. & Mayhew, P.J. (2009) A comparative study of sex ratio and clutch size in gregarious ichneumonoid wasps. *Journal of Insect Behavior*, 22, 273-288.
- Snyder, W.E. & Ives, A.R. (2003) Interactions between specialist and generalist natural enemies: parasitoids, predators, and pea aphid biocontrol. *Ecology*, 84, 91-107.
- Soler, R., Badenes-Pérez, F.R., Broekgaarden, C., Zheng, S-J., David, A., Boland, W. & Dicke, M. (2012) Plant-mediated facilitation between a leaf-feeding and a phloem-feeding insect in a brassicaceous plant: from insect performance to gene transcription. *Functional Ecology*, 26, 156-166.
- Stange, L.A. & Knight, R.J. Jr. (1987) Fig pollinating wasps of Florida (Hymenoptera: Agaonidae). *Entomology Circular, Division of Primary Industry, Florida Department of Agriculture and Consumer Service*. No. 296: 4pp.
- Starr, F., Starr, K. & Loope, L. (2003) *Ficus microcarpa* Chinese banyan Moraceae. Available from: http://www.hear.org/starr/hiplants/reports/pdf/ficus_microcarpa.pdf.
- Stone, G.N., Nee, S. & Felsenstein, J. (2011) Controlling for non-independence in comparative analysis of patterns across populations within species. *Philosophical Transactions of the Royal Society: Biological Sciences*, 366, 1410-1424.
- Stouffer, D.B. & Bascompte, J. (2011) Compartmentalization increases food-web persistence. *Proceedings of National Academy of Sciences of United States of American*, 108, 3648-3652.

- Suleman, N., Raja, S. & Compton, S.G. (2013) Parasitism of a pollinator fig wasp: mortalities are higher in figs with more pollinators, but are not related to local densities of figs. *Ecological Entomology*, 38, 478-484.
- Suleman, N., Raja, S. & Compton, S.G. (2012) Only pollinator fig wasps have males that collaborate to release their females from figs of an Asian fig tree. *Biology Letters*, 8, 344-346.
- Suleman, N., Raja, S., Zhang, Y. & Compton, S.G. (2011) Sexual differences in the attractiveness of figs to pollinators: females stay attractive for longer. *Ecological Entomology*, 36, 417-424.
- Sun, X-J., Xiao, J-H., Cook, J.M., Feng, G. & Huang, D-W. (2011) Comparisons of host mitochondrial, nuclear and endosymbiont bacterial genes reveal cryptic fig wasp species and the effects of *Wolbachia* on host mtDNA evolution and diversity. *BMC Evolutionary Biology*, 11, 86-93.
- Svensson, G.P., Pellmyr, O. & Raguso, R.A. (2011) Pollinator attraction to volatiles from virgin and pollinated host flowers in a yucca/moth obligate mutualism. *Oikos*, 120, 1577-1583.
- Tan, H.T.W. & Yeo, C.K. (2009) The potential of native woody plants for enhancing the urban waterways and water bodies environment in Singapore. Raffles Museum of Biodiversity Research and Singapore-Delft Water Alliance National, University of Singapore.
- Taylor, D.R. & Keller, S.R. (2007) Historical range expansion determines the phylogenetic diversity introduced during contemporary species invasion. *Evolution*, 61, 334-345.
- Terborgh, J. (2012) Enemies maintain hyperdiverse tropical forests. *The American Naturalist*, 179, 303-314.
- Tobin, P.C., Berec, L. & Liebhold, A.M. (2011) Exploiting Allee effects for managing biological invasions. *Ecology Letters*, 14, 615-624.
- Toscano, B.J. & Griffen, B.D. (2013) Predator size interacts with habitat structure to determine the allometric scaling of the functional response. *Oikos*, 122, 454-462.
- Tylianakis, J.M., Didham, R.K., Bascompte, J. & Wardle, D.A. (2008) Global change and species interactions in terrestrial ecosystems. *Ecology Letters*, 11, 1351-1363.
- Tylianakis, J.M., Tscharntke, T. & Lewis, O.T. (2007) Habitat modification alters the structure of tropical host-parasitoid food webs. *Nature*, 445, 202-205.
- Tzeng, H-Y., Tseng, L-J., Ou, C-H., Lu, K-C. & Lu, F-Y. (2008) Confirmation of the parasitoid feeding habit in *Sycoscapter*, and their impact on pollinator abundance in *Ficus formosana*. *Symbiosis*, 45, 129-134.
- van Lenteren, J.C. (2012) The state of commercial augmentative biological control: plenty of natural enemies, but a frustrating lack of uptake. *BioControl*, 57, 1-20.
- van Noort, S. & Compton, S.G. (1996) Convergent adaptations of fig wasps to the ostiolar morphology of their host figs. *Journal of Biogeography*, 23, 415-424.
- van Noort, S. & Rasplus, J-Y. (2010) Order Hymenoptera, Chalcidoidea associated with figs (families Agaonidae and Pteromalidae). *Arthropod Fauna of the UAE*, 3, 325-355.
- van Noort, S., Wang, R. & Compton, S.G. (2013) Fig wasps (Hymenoptera: Chalcidoidea: Agaonidae, Pteromalidae) associated with Asian fig trees (*Ficus*, Moraceae) in South Africa: Asian followers and African colonists. *African Invertebrates*, 54, 381-400.
- Vitousek, P.M. (1990) Biological invasions and ecosystem processes: towards an integration of population biology and ecosystem studies. *Oikos*, 57, 7-13.

- Wang, G., Compton, S.G. & Chen, J. (2013a) The mechanism of pollinator specificity between two sympatric fig varieties: a combination of olfactory signals and contact cues. *Annals of Botany*, 111, 173-181.
- Wang, H., Ridley, J., Dunn, D.W., Wang, R-W., Cook, J.M. & Yu, D.W. (2013b) Biased oviposition and biased survival together help resolve a fig-wasp conflict. *Oikos*, 122, 533–540.
- Wang, R., Ai, B., Gao, B-Q., Yu, S., Li, Y-Y. & Chen, X-Y. (2009) Spatial genetic structure and restricted gene flow in a functionally dioecious fig, *Ficus pumila* L. var. *pumila* (Moraceae). *Population Ecology*, 51, 307-315.
- Wang, R., Matthews, A., Ratcliffe, J., Barwell, L., Peng, Y-Q., Chou, L-S., Yu, H., Yang, H-W. & Compton, S.G. (In press a) First record of an apparently rare fig wasp feeding strategy: obligate seed predation. *Ecological Entomology*.
- Wang, R-W., Dunn, D.W. & Sun, B-F. (In press b) Discriminative host sanctions in a fig-wasp mutualism. *Ecology*.
- Ware, A.B. & Compton, S.G. (1994) Response of fig wasps to host plant volatile cues. *Journal of Chemical Ecology*, 20, 785-802.
- Warren, M., Robertson, M.P. & Greeff, J.M. (2010) A comparative approach to understanding factors limiting abundance patterns and distributions in a fig tree–fig wasp mutualism. *Ecography*, 33, 148-158.
- Waterston, J. (1921) On some Bornean fig-insects (Agaonidae - Hymenoptera Chalcidoidea). *Bulletin of Entomological Research*, 12, 35-40.
- Weiblen, G.D. (2002) How to be a fig wasp. *Annual Review of Entomology*, 47, 299-330.
- West, S.A. & Herre, E.A. (1994) The ecology of the New World fig-parasitizing wasps *Idarnes* and the implication for the evolution of the fig-pollinator mutualism. *Proceedings of the Royal Society B: Biological Sciences*, 258, 67-72.
- West, S.A., Herre, E.A., Compton, S.G., Godfray, H.C.J. & Cook, J.M. (1997) A comparative study of virginity in fig wasps. *Animal Behaviour*, 54, 437-450.
- West, S.A., Herre, E.A., Windsor, D.M. & Green, P.R.S. (1996) The ecology and evolution of the New World non-pollinating fig wasp communities. *Journal of Biogeography*, 23, 447–458.
- Wiebes, J.T. (1979) Co-evolution of figs and their insect pollinators. *Annual Review of Ecology and Systematics*, 10, 1-12.
- Wiebes, J.T. (1980) The genus *Odontofroggattia* Ishii (Hymenoptera Chalcidoidea, Pteromalidae Epichrysomallinae). *Zoologische Mededelingen Leiden*, 56, 1-6.
- Willig, M.R., Kaufman, D.M. & Stevens, R.D. (2003) Latitudinal gradients of biodiversity: pattern, process, scale, and synthesis. *Annual Review of Ecology, Evolution, and Systematics*, 34, 273-309.
- Wilson, J.R.U., Gairifo, C., Gibson, M.R., Arianoutsou, M., Bakar, B.B., Baret, S., Celesti-Grapow, L., DiTomaso, J.M., Dufour-Dror, J-M., Kueffer, C., Kull, C.A., Hoffmann, J.H., Impson, F.A.C., Loope, L.L., Marchante, E., Marchante, H., Moore, J.L., Murphy, D.J., Tassin, J., Witt, A., Zenni, R.D. & Richardson, D.M. (2011) Risk assessment, eradication, and biological control: global efforts to limit Australian *acacia* invasions. *Diversity and Distribution*, 17, 1030-1046.
- Witman, J.D., Etter, R.J. & Smith, F. (2004) The relationship between regional and local species

- diversity in marine benthic communities: A global perspective. *Proceedings of the National Academy of Sciences of the United States of America*, 101, 15664-15669.
- Wolfe, B.E. & Klironomos, J.N. (2005) Breaking new ground: soil communities and exotic plant invasion. *BioScience*, 55, 477-487.
- Yamaguchi, W., Kondoh, M. & Kawata, M. (2011) Effects of evolutionary changes in prey use on the relationship between food web complexity and stability. *Population Ecology*, 53, 59-72.
- Yang, H-W., Tzeng, H-Y. & Chou, L-S. (2013) Phenology and pollinating wasp dynamics of *Ficus microcarpa* L.f.: adaptation to seasonality. *Botanical Studies*, 54, 1-11.
- Yasuhara, M., Hunt, G., Dowsett, H.J., Robinson, M.M. & Stoll, D.K. (2012) Latitudinal species diversity gradient of marine zooplankton for the last three million years. *Ecology Letters*, 15, 1174-1179.
- Yokoyama, J. & Iwatsuki, K. (1998) A faunal survey of fig-wasps (Chalcidoidea: Hymenoptera) distributed in Japan and their associations with figs (*Ficus*: Moraceae). *Entomological Science*, 1, 37-46.
- Yu, H. & Compton, S.G. (2012) Moving your sons to safety: galls containing male fig wasps expand into the centre of figs, away from enemies. *PLoS ONE*, 7, ARTN e30833.
- Yu, D.W., Ridley, J., Jouselin, E., Herre, E.A., Compton, S.G., Cook, J.M., Moore, J.C. & Weiblen, G.D. (2004) Oviposition strategies, host coercion and the stable exploitation of figs by wasps. *Proceedings of the Royal Society B: Biological Sciences*, 271, 1185-1195.
- Zavodna, M., Compton, S.G., Biere, A., Gilmartin, P.M. & van Damme, J.M.M. (2005) Putting your sons in the right place: the spatial distribution of fig wasp offspring inside figs. *Ecological Entomology*, 30, 210-219.
- Zhang, L-S., Compton, S.G., Xiao, H., Lu, Q. & Chen, Y. (In press) Living on the edge: Fig tree phenology at the northern range limit of monoecious *Ficus* in China. *Acta Oecologica*. Manuscript ID: ACTOEC 2962.
- Zhang, R. & Xiao, H. (2008) A new species of the genus *Acophila* on *Ficus microcarpa* L. from China (Hymenoptera, Agaonidae). *Acta Zootaxonomica Sinica*, 33, 505-507.
- Zhao, J-B., Peng, Y-Q., Quinell, R.J., Compton, S.G. & Yang, D-R. (In press) A switch from mutualist to exploiter is reflected in smaller egg loads and increased larval mortalities in a 'cheater' fig wasp. *Acta Oecologica*.
- Zhou, M-J., Xiao, J-H., Bian, S-N., Li, Y-W., Niu, L-M., Hu, H-Y., Wu, W-S., Murphy, R.W. & Huang, D-W. (2012) Molecular approaches identify known species, reveal cryptic species and verify host specificity of Chinese *Philotrypesis* (Hymenoptera: Pteromalidae). *Molecular Ecology Resources*, 12, 598-606.
- Zimmermann, H.G. & Neser, S. (1999) Trends and prospects for biological control of weeds in South Africa. *African Entomology Memoir* 1, 165-174.