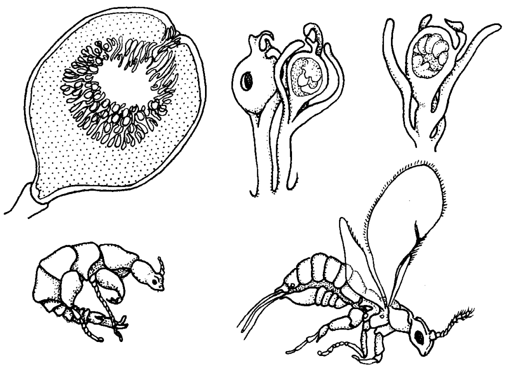
Investigations into stability in the fig/fig-wasp mutualism



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Declaration

I hereby declare that this submission is my own work, or if not, it is clearly stated and fully acknowledged in the text.

Sarah Al-Beidh

Abstract

Fig trees (Ficus, Moraceae) and their pollinating wasps (Chalcidoidea, Agaonidae) are involved in an obligate mutualism where each partner relies on the other in order to reproduce: the pollinating fig wasps are a fig tree's only pollen disperser whilst the fig trees provide the wasps with places in which to lay their eggs. Mutualistic interactions are, however, ultimately genetically selfish and as such, are often rife with conflict. Fig trees are either monoecious, where wasps and seeds develop together within fig fruit (syconia), or dioecious, where wasps and seeds develop separately. In interactions between monoecious fig trees and their pollinating wasps, there are conflicts of interest over the relative allocation of fig flowers to wasp and seed development. Although fig trees reap the rewards associated with wasp and seed production (through pollen and seed dispersal respectively), pollinators only benefit directly from flowers that nurture the development of wasp larvae, and increase their fitness by attempting to oviposit in as many ovules as possible. If successful, this oviposition strategy would eventually destroy the mutualism; however, the interaction has lasted for over 60 million years suggesting that mechanisms must be in place to limit wasp oviposition.

This thesis addresses a number of factors to elucidate how stability may be achieved in monoecious fig systems. Possible mechanisms include: 1) a parasitoid-centred short ovipositor hypothesis in *Ficus rubiginosa*, which suggests that a subset of flowers are out of reach to parasitoid ovipositors making these ovules the preferred choice for ovipositing pollinators and allowing seeds to develop in less preferred ovules; 2) the presence of third-party mutualists such as non-pollinating fig wasps (*F. burkei*) and patrolling green tree ants on the fig surface (*F. racemosa*) that limit pollinator and parasitoid oviposition respectively; and 3) selection on fig morphology which constrains the size (and therefore fecundity) of the associated pollinators. I discuss the lack of evidence for a single unifying theory for mutualism stability and suggest that a more likely scenario is the presence of separate, and perhaps multiple, stabilising strategies in different fig/ fig-wasp partnerships.

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I would first like to thank my supervisors, Professor James Cook and Dr. Sally Power for their guidance and perseverance throughout the course of my PhD. Dr. Derek Dunn has also provided helpful support and advice from the beginning and was a good companion- especially during the many months of fieldwork in a very strange homestay in Townsville. Research in Townsville was only possible through the generosity of the late Ross Crozier who, along with his group at the James Cook University, gave me ample lab space and state-of-the-art dissecting microscopes as well as providing me with many a happy evening spent consuming cheese and wine.

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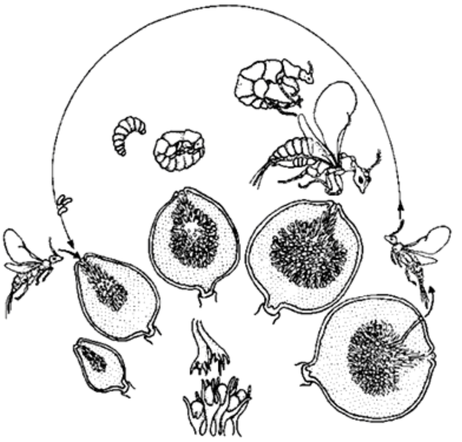
Chapter 1

Introduction



The fig/ fig-wasp life cycle :
http://biodidac.bio.uottawa.ca/thumbnails/filedet.htm?File_name=Hyme015b&File_type=gif

1.1 The study of mutualisms

"No man is an island, entire of itself". Written by John Donne in 1624 (Donne 2001), this statement is also applicable in a wider context as no organism is an island either. Try and imagine a species that lives in isolation from any other and you will find it very hard- if not impossible, because in reality species interact, often forming close associations, or 'symbioses', with one another during their lifetimes (Seckback 2002). These symbiotic relationships can cover a broad spectrum of interactions that can be loosely defined by the degree of benefit each partner receives, ranging from antagonistic interactions where neither partner benefits (competition) or only one does at the other's expense (parasitism), to mutualistic ones where the beneficial services are traded between partners as a kind of 'biological bartering' (mutualism) (Bronstein 2001).

That the natural world was dominated by antagonistic rather than mutualistic interactions was an idea that was, until recently, supported by many influential ecologists (Fox *et al.* 2001). Nature described as being 'red in tooth and claw' reinforced this belief at the time and in 1986, the ratio of papers covering competition, exploitation and mutualism was 4:4:1 (May and Seger 1986). Classic Lotka-Volterra models for antagonistic interactions and Gause's competitive exclusion principle helped keep these ideas at the forefront of ecology-related publications for over half a decade (Boucher 1985; Orians 1985; but see Bronstein 1994). Cultural bias (that cooperative models were absent in the socioeconomic environment), taxonomic bias (an emphasis on charismatic taxonomic groups not involved in mutualisms) and the idea that studies on cooperation were boring, were all proposed as potential explanations for the underrepresentation of positive interactions in the literature (Keddy 1989). This bias is slowly being readdressed and more attention has been paid to mutualisms in recent years (Boucher 1985; Kawanabe *et al.* 1993; Bronstein 1994; Duchateau-Nguyen *et al.* 1995; Dugatkin 1997; Bronstein 2001; Stachowicz 2001; Holland *et al.* 2004). It is now clear that few organisms are exempt from being involved in at least one mutualism during their lifetimes (Janzen 1985). Such interactions have driven the evolution of much of the biological diversity we observe today, and are critically important to ecosystem functioning. To get an idea of the extent of their impact, let us consider a world suddenly without three well known mutualisms; trees and mycorrhizal fungi, flowers and their pollinators and legumes and nitrogen-fixing bacteria. Without these interactions, there would be no animal-pollinated plants and no pollinators. Ninety percent of the land plants that depend on mycorrhizal fungi would disappear and the remainder would be restricted to high nutrient soils. Given that the vast majority of mammalian and insect herbivores are unable to digest plant cellulose (Minkoff and Baker 2001), these too would disappear. It is easy to see that even a cursory scan of a world without mutualisms would be unrecognisable and, if we are to agree with Margulis and Fester's (1991) suggestion that all eukaryotes originated as mutualistic associations, all organisms with the exception of bacteria, viruses and cyanobacteria would fail to exist.

1.2 Examples of mutualisms

Mutualistic interactions vary along a continuum of partner dependence with some partners able to survive without the other (facultative mutualisms) whilst others are prevented from reproducing in their partner's absence (obligate mutualisms) (Janzen 1985). The exact nature of the benefits received in a mutualism (e.g. protection, nutrients, shelter, and dispersal) can also vary considerably (Boucher 1985).

1.2.1 Pollination and seed dispersal

Pollination involves the movement of male gametes (pollen) to female reproductive organs (the style and ovary) for seed development, whilst seed dispersal involves the movement of seeds some distance from the parent plant to limit competition for space and nutrients. Both pollen and seeds can be dispersed by wind or by animal pollinators. Where the latter are the agents of dispersal, the plant has its pollen and seeds dispersed and the animal is often rewarded with food (nectar, pollen or fruit).

1.2.2 Ants and acacias

The interaction between the bull's horn acacia, *Acacia cornigera*, and the acacia ant, *Pseudomyrmex ferruginea*, is one example of a mutualism which involves defence. Unlike other acacia, the leaves of *A. cornigera* do not contain the bitter tasting alkaloids that are often used as herbivore deterrents. Instead, *P. ferruginea* workers fill this role. The tree provides the ants with year round housing (swollen thorns) and food (nectaries and Beltian bodies), whilst the ants provide the tree with 24-hour protection. So effective is their protection, which involves stinging intruders and cutting back encroaching seedlings (Janzen 1966, 1967), that their removal results in increased herbivory and the eventual death of unoccupied trees (Janzen 1966).

1.2.3 The anemone fish and the anemone

The mutualism between the anemone fish, *Amphiprion bicinctus*, and the anemone, *Entacmaea quadricolor*, also involves protection. In the presence of large (>7cm) and aggressive *A. bicinctus*, the anemone gains defence from its main predator, the butterfly fish (*Chaetodon fasciatus*) whilst *A. bicinctus* receives enemy-free space from its predators and a nest site to lay its eggs (Porat and Chadwish-Furman 2004). In the absence of *A. bicinctus*, anemone retreat completely into reef cavities which minimises attack from predators, but also limits the anemone's feeding and photosynthesis which can lead to its shrinkage and eventual death (Porat and Chadwish-Furman 2004).

1.3 The paradox of the mutualism

Despite differences in the precise nature of individual interactions, all mutualisms depend on maintained cooperation from both parties. Natural selection, however, acts selfishly (Krebs and Davies 1993) and favours individuals that reap the rewards associated with a mutualism whilst reciprocating little, or nothing, in return. Individuals involved in mutualistic interactions should therefore be under strong selection to cheat, destabilising the interaction and transforming it into one that is parasitic. Despite this, mutualisms are often stable over evolutionary timescales. How such long-term stability is maintained is a topic of continued and considerable debate (e.g. Pellmyr and Huth 1994; West *et al*. 2002; Holland *et al*. 2004; Dunn *et al*. 2008b).

1.4 The fig/ fig-wasp interaction

The 60 million year old interaction between fig trees (Ficus, Moraceae) and their pollinating wasps (Hymenoptera, Agaonidae) (Rønsted *et al.* 2005) is the archetypal example of an obligate mutualism. The trees produce fruit (figs or 'syconia'), each one an enclosed inflorescence. The inner cavity of each syconium is lined with tens or hundreds of tiny flowers (depending on the species) which never see daylight, but provide fig wasps with nest sites and nurseries for their developing larvae. The wasps in turn, are the fig tree's exclusive pollen vectors. The life-cycle begins when a syconium reaches a receptive period early on in its development ('B stage', sensu Galil and Eisikowitch 1968a), triggered by the opening of a small hole (the ostiole) located on the underside of each fruit. Female pollinators, attracted by volatile chemicals released from the ostiole (Grison-Pigé *et al*. 2002; Ware *et al.* 1993) crawl through the hole (Figure 1.1(1)), and travel through the narrow tunnel that connects the ostiole to the inner syconium cavity, often losing their wings and their antennae as they do so

(Grison-Pigé *et al.* 2002; Grafen and Godfray 1991; van Noort 2004). Once entered, syconia become less receptive to wasp entry (Khadari *et al.* 1995) and the closing of the ostiole limits the total numbers of females (now called 'foundresses') gaining entry whilst also entombing those that have been successful. With the end of their lives drawing near, foundresses work quickly to pollinate flowers and oviposit in as many ovules as possible before their death (Figure 1.1(2)). Their offspring are herbivorous and grow within galled ovules (C stage), surviving on the seed tissue as they develop (Figure 1.1(3)). Weeks later (D stage), male pollinators emerge from their galls (Figure 1.1(4)) and search for those containing females. Biting holes in the tops of these galls, they mate through the holes, later enlarging them as females are unable to emerge unaided (Zammit and Schwarz 2000). The wall of the syconium is then punctured with one or more emergence holes (depending on the species) chewed out by the male pollinators. The mated females then collect pollen (actively or passively) released from male flowers (Figure 1.1 (5)) and disperse, restarting the cycle (Figure 1.1 (6)). After wasp emergence, the syconium ripens completely (E stage) often changing in colour and scent and becoming more attractive to its seed-dispersing frugivores (Bonacorrso 1979; Milton *et al.* 1982; Kalko *et al.* 1996).

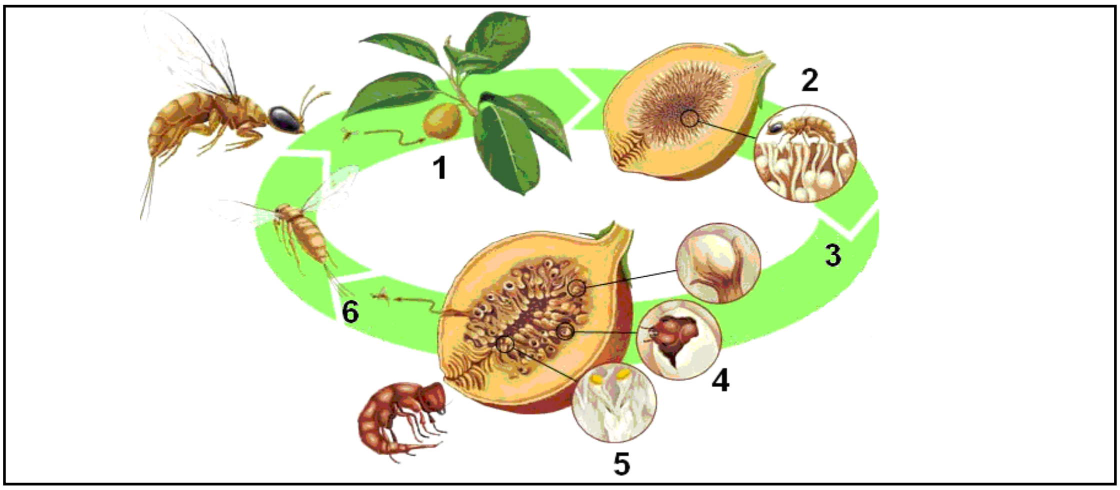


Figure 1.1 The key stages of the fig- wasp life cycle including: (1) syconium receptivity, (2) foundress oviposition, (3) larval development, (4) wasp emergence, (5) anther dehiscence, and (6) wasp dispersal and final fig ripening. Adapted from: http://www.britannica.com/EBchecked/topic-art/206044/19378/The-life-cycle-of-the-fig-wasp.

1.4.1 Morphological adaptations of pollinating fig wasps

The ostiole is the point of entry for pollinating wasps attempting to oviposit within fig flowers. Consequently, its size and structure can impose strong selection pressures on the size and shape of pollinating wasps attempting to enter. This is no more apparent than in the extreme sex-specific morphological adaptations of pollinators where female wasps exhibit flattened heads and bodies (Figure 1.2 A), large and easily detachable wings and antennal segments, and mandibular appendages covered in ventral lamellae that help pull their bodies into the inner syconial cavity (van Noort and Compton 1996). Males, in contrast, are born and die within their natal fig and, as they do not enter syconia through the ostiole, they share few morphological similarities with their sisters (Figure 1.2 B). Instead, they are apterous with vestigial eyes, antennae and tarsae and posses long genitalia to mate with females still in their galls.

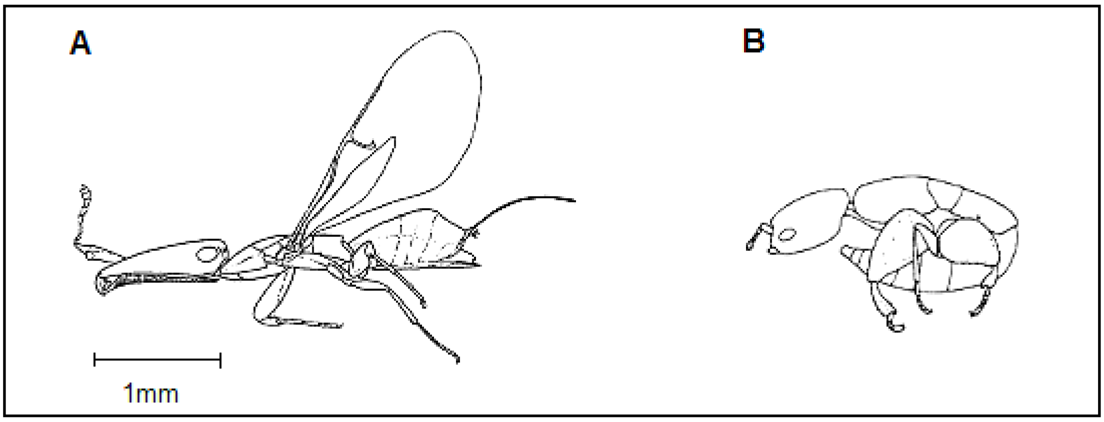


Figure 1.2 Morphological adaptations associated with (A) female (*Pleistodontes froggatti*) and (B) male (*P. imperialis*) agaonid fig wasps. Drawings from Bouček 1988 (A) and http://www.nhm.ac.uk/research-curation/research/projects/chalcidoids/agaonidae.html (B).

1.4.2 Non-pollinating fig wasps

Whilst pollinating wasps are essential for fig reproduction, another group of fig wasps also develop in fig ovules, but cheat the mutualism by failing to transfer pollen (but see Jousselin *et al.* 2001b). The majority of these non-pollinating fig wasps (NPFWs) are grouped into five different subfamilies (Epichrysomallinae, Otitesellinae, Sycoryctinae, Sycophaginae and Sycoecinae) that are exclusively associated with figs (Bouček 1988). Smaller radiations of other wasps including eurytomids and braconids are also associated with fig trees, but not exclusively so (van Achterberg and Weiblen 2000; Lotfalizadeh *et al.* 2007).

During the period from syconium pre-receptivity to post-pollination, different genera of externally ovipositing non-pollinating wasps can lay their eggs into fig ovules. Non-pollinating wasps are either herbivorous and oviposit into empty ovules inducing galls themselves (gall-formers), or they are parasitic on developing wasp larvae and oviposit into occupied ovules. The larvae of these latter wasps kill developing gall-formers and/or pollinators by parasitizing them directly (parasitoids), or pilfering their food resource (as lethal inquilines). Although the vast majority of NPFWs oviposit from the other side of the fig wall, a subset have developed morphology similar to pollinating wasps, and enter the ostiole to oviposit (van Noort and Compton 1996). As many as 30 species of NPFWs can be associated with a single fig species (Compton and Hawkins 1992; Hawkins and Compton 1992; Cook and Rasplus 2003). Although detrimental to pollinating fig wasps, recent evidence (Dunn *et al.* 2008b) suggests that NPFWs can be indirect mutualists of the fig trees by limiting pollinator wasp oviposition to a subset of ovules (see optimal foraging hypothesis).

1.4.3 The reproductive biology of Ficus

Around half of the world's 735 fig species (Moraceae) (Berg and Corner 2005) are monoecious where male and female flowers, wasps, pollen and seeds develop within a syconium. Styles of female flowers all reach the same height into the syconial cavity but vary in the lengths of their component parts. Each flower is composed of a stalk (pedicel), an ovary and a style. Flowers with short styles have long pedicels whilst those with long styles have short pedicels (Figure 1.3A). Wasp offspring developing in the ovules of short-styled ('inner') flowers do so close to the inner syconial cavity, whilst those laid in the ovules of long-styled ('outer') flowers develop closer to the syconial wall (Figure 1.3A). Explanations for the variation in style and pedicel lengths have included optimal packing (Janzen 1979a; Kjellberg *et al.* 1987; Bronstein 1992; but see Ganeshaiah *et al.* 1995) and the regulation of flower allocation to wasp and seed production (the short ovipositor hypothesis, see below).

The remaining 50% of fig species have a dioecious reproductive system where trees are either all male or all female. Male trees bear male (gall) syconia composed of short-styled female flowers and male flowers. Wasps, pollen and, in some cases, a few seeds develop. Female trees produce seed syconia composed solely of long-styled female flowers, where each style is longer than the ovipositor of the associated pollinator (Figure 1.3 B and C). Foundresses entering female syconia commit 'reproductive suicide' (Patel *et al.* 1995), being unable to oviposit into any flowers. Consequently, whilst wasps and pollen develop in male syconia, only seeds develop on female trees.

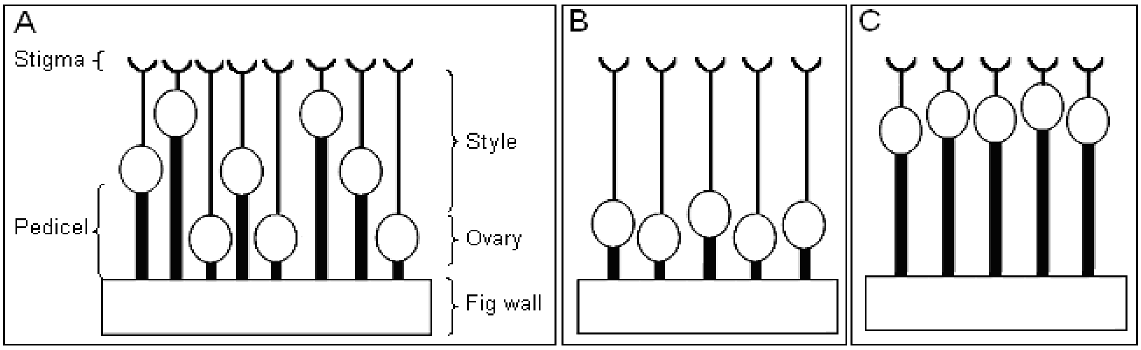


Figure 1.3 Variation in style and pedicel length in (A) monoecious syconia and (B) female (seed) and (C) male (gall) dioecious syconia. Adapted from Dunn *et al.* 2008b.

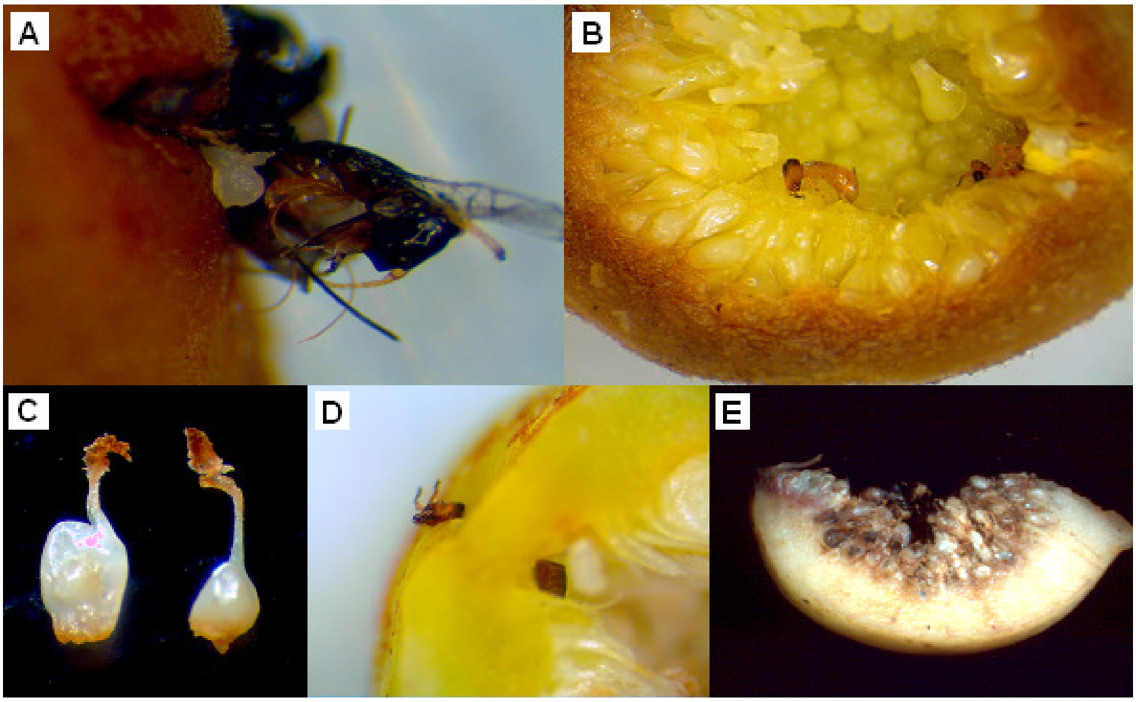


Figure 1.4 Aspects of the fig-wasp mutualism: (A) brown *P. imperialis* pollinators trapped in a *F. rubiginosa* ostiole whilst attempting to enter; (B) yellow *P. imperialis* foundresses inside a B-stage *F. rubiginosa* syconium; (C) differences in the style lengths of B-stage *F. rubiginosa* flowers; (D) *P. imperialis* female trapped whilst trying to re-emerge from a *F. rubiginosa* syconium and (E) section sliced through a *F. racemosa* syconium clearly showing pedicel length variation. Photographs taken by S. Al-Beidh.

1.4.4 Conflicts of interest in the fig/ fig-wasp mutualism

Despite being defined by their mutual benefit, reciprocal conflict is a common aspect of mutualisms (Trivers 1971; Axelrod and Hamilton 1981; Bull *et al.* 1991). In the fig/ wasp mutualism, differences in the internal floral structure associated with the fig reproductive system create separate conflicts of interest between the fig trees and their pollinating wasps.

Monoecious fig species

In monoecious species, each flower can develop into a wasp or a seed. Fig trees benefit from the production of seeds (for seed dispersal) and wasps (for pollen dispersal); however, the long-term reproductive success of the fig tree (and of future generations of fig wasps) is of little interest to each individual foundress whose fitness gains lie only in flowers that nurture her own offspring. As pollinating wasps do not receive any immediate benefits when their host tree produces seeds, they are selected to only produce wasp offspring, and to exploit the greatest number of *Ficus* ovules to do this. Similarly, as more pollinating wasps gain entry into a single syconium, more fig flowers receive pollen and the benefits accrued to the fig tree by later entering foundresses decrease. Such late entering foundresses therefore provide little additional pollination function but prevent increasing numbers of flowers from becoming seeds (Bronstein 2001). Given that pollinating wasps have life cycles many times shorter than the fig trees in which they lay their eggs, the stability of the mutualism is therefore dependent on factors that allow fig trees to control wasp oviposition to ensure that some seeds are set (Janzen 1979a; Anstett *et al.* 1997; Herre and West 1997; Anstett 2001).

In obligate mutualisms, a cheating mutant should gain a fitness advantage by accepting the benefits of the interaction but failing to provide any service in return. For this reason, such mutualisms have often been considered evolutionary 'dead ends' (Bull and Rice 1991). Consequently, in the absence of any controlling mechanisms, pollinating wasps should be under selection to oviposit in all fig ovules. This would quickly destroy the mutualistic interaction. In reality, however, wasps rarely develop in more than 70-80% of ovules and usually much less (roughly 50%: Herre 1989; Cook and Power 1996; Nefdt and Compton 1996; Dunn *et al.* 2008b). So far, four hypotheses have been put forward to explain how monoecious fig trees may limit wasp oviposition:

- **The short ovipositor hypothesis** proposed by Ramirez (1970a) suggests that style lengths in monoecious *Ficus* are either longer or shorter than the average ovipositor lengths of the associated pollinating agaonid. Given that fig wasps lay eggs next to the ovary at the base of the style (Jousselin *et al.* 2001a), an ovipositor must travel the full length of a style to successfully deposit an egg. Consequently, short-styled flowers should be accessible to the ovipositors of pollinating fig wasps whilst long-styled flowers are not. The bimodal distribution of style lengths in dioecious fig species (Weiblen *et al.* 2001) provides a clear example of the short ovipositor hypothesis in action. Female 'seed' syconia are composed entirely of long-styled female flowers with each style being longer than the ovipositor of the associated pollinator (Figure 1.3B). Consequently, no wasps develop in these syconia and all ovules, if pollinated, become seeds. The style lengths of monoecious fig species, however, show no such bimodal distribution (e.g. Bronstein 1992) (Figure 1.3A), and instead vary along a continuum from those furthest from the fig wall with short styles and long pedicels, to those closest to the fig wall with long styles and short pedicels. Moreover, evidence in some monoecious fig species has identified that the majority of styles are within reach of a pollinator's ovipositor (Bronstein 1988a,1992; Nefdt and Compton 1996; Herre 1999), suggesting that the short ovipositor hypothesis cannot be a general explanation for stability in monoecious *Ficus*.

- **The unbeatable seeds hypothesis** (West and Herre 1994) supposes that wasp eggs are prevented from developing within some ovules by as yet, unknown mechanisms. Despite this, Nefdt and Compton (1996) discovered that, in a number of fig species, experimentally increasing foundress numbers in syconia led to increasing numbers of emerging wasps until almost all flowers were occupied by wasp larvae. This invalidates the unbeatable seeds hypothesis as a stand-alone explanation for mutualism stability and to date, there is still little evidence to support its existence. As the mechanisms by which seeds are made 'unbeatable' to wasp oviposition are still unknown, this hypothesis is also a difficult one to test explicity.

- **The egg limitation hypothesis** (Nefdt and Compton 1996) suggests that too few foundresses (carrying too few eggs) gain entry into syconia to oviposit into all available flowers. Although there is evidence to support this hypothesis in four fig species (*F. burtt-davyi*, *F. thonningii* and *F. sur, F. maxima*: Nefdt and Compton 1996; Joussselin *et al.* 2004), other species (e.g. *F. aurea, F.trigonata*) have sufficient egg loads to fill all available ovules, disputing this as a general explanation for stability among monoecious fig systems (Herre 1989; Anstett *et al.* 1996a, Bronstein and Hossaert-McKey 1996).

- **The optimal foraging hypothesis** (Yu *et al.* 2004) has been the most recent proposal to explain mutualism stability among monoecious *Ficus* and represents an amalgamation of some of the earlier hypotheses. Its premise is that ovule profitability and handling time are correlated with style length. Coupled with the fact that foundresses often die before laying all of their eggs, it uses the foraging theory proposed by MacArthur and Pianka (1966) to consider how each foundress should budget her time between searching for available ovules and ovipositing into styles. The hypothesis suggests that foundresses should forage for suitable oviposition sites optimally by displaying a preference for short-styled (inner) ovules, over outer flowers closer to the fig wall for two reasons: (1) because short-styled flowers are associated with a shorter handling time (i.e. they may be easier, and quicker to oviposit into: Nefdt and Compton 1996; Yu *et al.* 2003; Shi *et al.* 2006), and/or (2) because larvae deposited into inner ovules receive some fitness benefit over those laid in outer ovules (e.g. a spatial refuge from parasitoids ovipositing externally, Dunn *et al.* 2008b). This preference for short-styled flowers will result in first entering females ovipositing into these ovules first whilst later entering foundresses will spend longer searching for preferred ovules as more eggs are laid. Outer ovules closest to the fig wall are more likely to be filled once the costs incurred by ovipositing into these ovules outweigh the time spent searching for unoccupied inner ovules. Pollinated outer ovules that fail to receive an egg therefore develop as seeds. The time spent searching for ovules means that foundresses lay fewer eggs overall than if they had not shown a preference for some ovules over others. Recent evidence supports this hypothesis with pollinators occupying the innermost ovules whilst seeds develop in ovules closest to the fig wall (West and Herre 1994; Nefdt and Compton 1996; Jousselin *et al.* 2001a; Anstett 2001; Zavodna *et al.* 2005; Dunn *et al.* 2008b).

Dioecious fig species

The conflict prevalent among monoecious species is resolved in dioecious *Ficus* where figs have 'won'. Whilst in monoecious species, wasps and seeds develop in the same syconium, in dioecious species they develop separately; wasps and pollen develop in male syconia whilst only seeds develop in female syconia. Despite the resolution of this conflict, however, a different one exists among dioecious species. Female syconia rely on the presence of agaonid foundresses to pollinate their flowers, but the style of each female flower is too long for foundresses to lay their eggs. Consequently, despite some re-emergence (Moore *et al.* 2003), many female pollinators commit 'reproductive suicide' (dying without leaving any offspring) (Patel *et al.* 1995) by entering female syconia. There should be strong selection on pollinators of dioecious species to be able to distinguish between male syconia (in which they can reproduce) and female syconia (where they cannot). There is, however, likely to be an equal but opposite selective pressure on fig trees to resemble one another as closely as possible otherwise seed figs would not be pollinated (so-called 'vicarious selection', Grafen and Godfray 1991). With the same token, whilst male and female syconia closely resemble one another before pollination (Grison-Pigé *et al.* 2001), post-pollination, it makes sense that seed syconia become attractive to frugivores whilst gall syconia do not. Consequently, after pollination, gall and seed syconia differ in size, colour, odour and nutritional content (Lambert 1992; Dumont *et al.* 2004).

1.5 Explanations for stability in other obligate pollination mutualisms

Alongside the fig/ fig–wasp interaction, three other obligate pollinator mutualisms have also been studied intensively. It is important to consider how stability has been explained in these systems in order to gain a better understanding of how it may be achieved among fig trees and their pollinators.

1.5.1 The yucca/ yucca-moth mutualism

Of the four well-known obligate pollination mutualisms, the interaction between yuccas and their pollinating moths most closely resembles that between fig trees and their wasps. Having persisted for over 40 million years (Pellmyr and Leebens-Mack 1999), yucca moths (*Tegeticula spp.*, Incurvariidae) are the sole pollen dispersers of the yucca plants (Yucca, Agavaceae) whilst the seeds of the yucca serve as food for developing moth larvae. Female moths collect pollen from yucca flowers before seeking out flowers for oviposition, often avoiding those that have already been visited (Wilson and Addicott 1998; Huth and Pellmyr 1999). Once a suitable site has been selected, females oviposit through the ovary wall to lay their eggs and pollen is then actively placed on the stigma. The process can be repeated multiple times on the same, or closely situated flowers. After each oviposition attempt, females must decide whether or not to pollinate the flower; however, depositing too little pollen and/or laying too many eggs increases the likelihood that the flowers are aborted, killing the developing larvae (Huth and Pellmyr 1999). Providing that flower abortion does not occur, moth larvae hatch a few days after oviposition and each larva feeds on a proportion of the yucca seeds (Pellmyr and Huth 1994). The larvae then exit the flower, burrow into the ground to spin a cocoon and pupate for a few weeks before emerging as adults (Bronstein and Ziv 1997). As with fig wasps, there is strong selection on female moths to lay more eggs to the ultimate ruin of the system. This conflict of interest and its apparent resolution is no better observed than between *Yucca filamentosa* and its two pollinating moths: *Tegeticula yuccasella* and *T. cassandra* (Segraves 2003). Females of *T. yuccasella* oviposit in the more commonly observed ‘deep’ ovipositions, through the ovary wall, damaging ovules in the process. If too many ovules are damaged (i.e. too many eggs are laid), *Yucca filamentosa* selectively abscises the flower precluding larval development (Marr and Pellmyr 2003). *Tegeticula cassandra*, in contrast, lays its eggs in ‘shallow’ ovipositions, at the base of *Y. filamentosa* styles. This oviposition strategy circumvents ovule damage, effectively bypassing the system in place which limits ovule overexploitation. Despite this, many *T. cassandra* larvae do not develop fully. Although larval predation is no greater when eggs are laid in shallow ovipositions (Segraves 2003), by laying their eggs in places that leave the larvae more exposed, external abiotic factors (e.g. humidity and temperature) end up prematurely killing the moth larvae making this cheating strategy, a costly one.

1.5.2 The globeflower/ globeflower-fly mutualism

Unlike other commonly cited obligate pollination mutualisms where there is a one-to-one specificity between the plant species and its obligate pollinator, this interaction involves a single species of globeflower, *Trollius europaeus*, and six species of dipteran pollinator (*Chiastocheta spp.*) (Pellmyr 1992). The flies disperse the globeflower's pollen and their larvae consume some seeds during their development. One pollinator, *C. rotundiventris*, visits young flowers, provides some pollen during oviposition and lays one egg per flower, whilst *C. dentifera* visits wilting flowers (therefore does not pollinate) and lays its entire clutch (15 eggs). Intermediate oviposition behaviours are observed in the remaining four *Chiastocheta* species (Ferdy *et al.* 2002). Female *Chiastocheta* flies lay their eggs on or between the carpels of a flower and the larvae consume some seeds as they grow. When mature, the larvae exit the flower, drop to the ground and overwinter in the soil, pupating and emerging in the spring. Unlike other obligate pollination mutualisms, *T. europaeus* is pollinated by flies of both sexes as they feed, mate and sleep within the flowers. Pollination can therefore occur in the absence of oviposition. In fact, males and non-ovipositing females pollinate flowers and impose little cost to the plant (other than nectar/ pollen removal) whereas ovipositing females reduce seed set in each globeflower. The costs incurred by ovipositing females are therefore higher than in other pollination mutualisms. As globeflowers generally only flower every second year, individual plants never receive pollen from the fly larvae that they reared (Jaeger *et al.* 2000). Moreover, globeflowers generally only produce one to three flowers per plant so flower abortion does not occur (Jaeger *et al.* 2000). Coupled with low larval mortality, it is hard to believe that this mutualism has not been overexploited. Stability is thought to be maintained through density-dependent competition among the fly populations. When only a few eggs are laid, there is little competition among larvae and the fly population size increases. At high fly densities, competition increases and the flowers release part of their seeds before the larvae are fully developed, effectively starving them (Jaeger *et al.* 2001). There is also evidence to suggest that in flowers with high *Chiastocheta* densities, increased concentrations of a C-glycosyl flavone, adonivernith, are produced which has been associated with inhibited larval growth in other species (Wiseman *et al.* 1993; Ibanez *et al.* 2009).

1.5.3 The senita/ senita-moth mutualism

Discovered in 1995, the interaction between the senita cactus (*Lophocereus schotti*) and the senita moth (*Upiga virescens*) represents one of the more recently described obligate pollination mutualisms. Female moths pollinate and oviposit into flowers and their larvae consume the senita seeds. Each senita fruit contains a single moth larva which later pupates in the branches of the cactus (Fleming and Holland 1998). Like the fig/ fig-wasp and yucca/ yucca moth systems, the senita moth is obligately dependent on the senita cactus (Holland and Fleming 1999); however, unlike other pollinator mutualisms, the senita moth is not the sole pollinator of the cactus as diurnal halictid bees can also perform this role. Despite this, as the cactus is night flowering, senita moths are the more effective pollinator (Fleming and Holland 1998). Mutualism stability has been proposed to occur by excessive flower production and selective fruit abortion to control population densities of senita moths (Holland *et al.* 2004).

Explanations for stability in three out of the four well-known obligate pollination mutualisms have been described in the literature (Addicott and Bao 1999; Csotonyi and Addicott 2001; Jaeger *et al.* 2001; Shapiro and Addicott 2003; Holland *et al.* 2004); but, despite a wealth of knowledge on how figs and their pollinating wasps interact, we still do not completely understand how this system is stabilised. This thesis investigates issues both within and across fig species to elucidate how cooperation in the fig/fig-wasp mutualism has been maintained for over 60 million years. Does variation in the distance to ovaries prevent pollinators and parasites overexploiting seeds and wasps? What roles, if any, do other insect fauna associated with fig trees have in stabilising the fig-pollinator mutualism? Does the fig reproductive system affect the selective pressures acting on the morphology of their associated wasps? I address each of these questions to elucidate how the interaction between fig trees and their agaonid pollinators has persisted for so long.

1.6 Data analysis

In the following chapters, I have often used generalised linear models (GLMs) to analyse the data. Linear regression methods where the response variables are continuous assume constant variance and normal distributed errors. However, in many cases these assumptions do not apply to real data. Instead, the data are often count or proportion-based. With count data, the response variables are integer values, and there may be many zeros in the data set. In these situations, the variance can be expected to increase with the mean. With proportion data, the response variable is bounded between 0 and 1 and the relationship between the variance and the mean can be ∩-shaped.

As an example, suppose you were looking at the number of foundresses gaining entry into *F.rubiginosa* syconia. The dependent variable (number of foundresses) is discrete because a syconium may allow 1 or 2 foundresses to gain entry but intermediate numbers (e.g. 2.5) are not possible. The distribution is also likely to be skewed (i.e. most syconia have 2 or 3 foundresses, fewer have 4 or 5, and very few have 6 or 7). In this case, it would be reasonable to assume that the dependent variable follows a Poisson distribution.

A second reason why the linear regression might be inadequate to describe a particular relationship is that the effect of the predictors on the dependent variable may not be linear in nature. For example, during the C developmental stage, the size of a syconium changes little from day to day. Its size, however, is likely to change markedly over this same time scale (24hrs) just before fig receptivity and just before wasp emergence. The relationship (or the *link*) between syconium size and day of development is therefore likely to be non-linear.

In its simplest form, a generalised linear model (GLM) is a linear model that specifies the (linear) relationship between a dependent (response) variable y, and a set of predictor (explanatory) variables, z_1 , z_2 etc., which can be continuous (leading to a regression type analysis) or categorical (leading to an ANOVA like analysis) (Crawley 2005). For example, for count data, the model might be:

model<- glm(y~z_1+z_2, family= poisson)

Each model is composed of three key parts:
(1) An error structure: Poisson errors for count data, binomial errors for proportion data.
(2) A linear predictor: the linear combination of all the explanatory variables.
(3) A link function which relates to the linear predictor (e.g. a log-link is generally used for count data, whilst a logit link is used for proportion data, Crawley 2005).

As the output of a generalised linear model does not produce an R^2 value, I use an equivalent measure which I call the proportion deviance squared (P.D.E), where:

$$\text{P.D.E} = \frac{null\ \ deviance\ \ - residual\ \ deviance}{null\ \ deviance}$$

Throughout this thesis you may notice that I sometimes state that my data were ‘overdispersed’. Overdispersion is a phenomenon that occurs occasionally with binomial and Poisson data when the actual variance of the response exceeds the GLM Poisson or binomial variance - in other words, where the actual data varies more than you would expect if the response distribution really was Poisson or binomial.

Overdispersion is apparent if, after completing a GLM, the residual deviance is larger than the residual degrees of freedom. Specifying quasi-binomial or quasi-poisson in the error structure and using F tests instead of χ^2 tests means that a more appropriate variance function is specified (Crawley 2005).

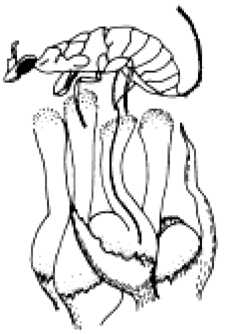
1.7 Thesis layout

The structure of this thesis does not follow the chronological order in which the research was undertaken, but rather is presented in its most logical format. In chapter 2, I consider the short ovipositor hypothesis (one of four hypotheses proposed to explain why pollinating wasps do not develop in all *Ficus* ovules), and its applicability to the three cryptic pollinator species of *Ficus rubiginosa* syconia, and to its non-pollinating wasp fauna. Chapters 3, 4 and 5 investigate in more detail organisms other than fig trees and their pollinators that may also impact upon the mutualism. Specifically, chapters 3 and 4 consider externally and internally ovipositing non-pollinating fig wasps respectively, whilst chapter 5 looks at how green tree ants may influence the system. In chapter 6, I consider how the fig trees themselves may help stabilise the mutualism by studying the morphology of their syconia and that of their associated wasps. In particular, I elucidate whether the fig reproductive system (monoecious or dioecious) has affected the observed morphology of the mutualistic partners, and whether this, in turn, has influenced the conflict over seed production that differs in the two fig reproductive systems. Finally, the findings of the thesis are summarised in chapter 7 where conclusions are drawn and guidelines for future work suggested.

The thesis has been laid out as a series of short paper-style chapters which are meant to exist as self-contained units. The intention is for any one chapter to be able to be read without reference to any other. Consequently, there is some (inevitable) overlap in descriptions across chapters such as the fig/ pollinator lifecycle and the conflict in the mutualism - aspects that are crucial to the explanation of the system and to the central question of mutualism stability.

Chapter 2

Plant versus pollinator versus parasite: the return of the short ovipositor hypothesis



Ovipositing agaonid female (Hanson and Ramirez 1995)

2.1 Abstract

Fig trees and their pollinators are involved in a tightly integrated mutualism where the reproduction of both is prevented in the absence of one or other partner. As, however, only either a wasp or a seed can develop in any one flower, the production of seeds comes at a cost to wasp development and vice versa. Whilst seed and wasp production (seed and pollen dispersal) benefit fig trees, pollinating wasps are only interested in flowers that nurture their own offspring.

Across monoecious fig species, wasps and seeds develop together within fig fruit (syconia). Wasps emerge from roughly 50% of ovules with the remaining ovules developing into seeds; however, it remains uncertain how monoecious *Ficus* prevent wasps exploiting too many flowers. The short ovipositor hypothesis (SOH) is one suggestion, proposing that pollinating wasps fail to develop in all ovules because some flowers have styles longer than the wasps' ovipositors. Evidence for the SOH is varied, being partial in some fig species but absent in others. I test for its evidence in *Ficus rubiginosa* and three of its cryptic, sympatric pollinator species (species 2, 3 and 4 of the *Pleistodontes imperialis* complex). First, I consider the morphology of each pollinator species. Although species 2 wasps have significantly longer ovipositors than those from species 3, all three species could access more than 99% of *F. rubiginosa* ovules, suggesting that the SOH is not applicable in this system. Moreover, this implies that ovipositor length and differential flower use do not provide an explanation for how multiple species co-exist within syconia.

Second, as a new twist on the SOH, I investigate its application to the externally ovipositing non-pollinating fig wasps (NPFWs) associated with *F. rubiginosa.* In general, fewer ovules were accessible to these NPFWs than to pollinators. *Sycoscapter* and *Philotrypesis* parasitoids were capable of accessing 66.8% and 91.9% of ovules, whilst *Eukobelea* and *Pseudidarnes* gall-formers could access 96.3% and 100%. These results suggest that innermost ovules may provide pollinator larvae with enemy-free space from parasitoid attack and support previous evidence for spatial segregation of pollinator and parasitoid larvae. Although the SOH in its original form is not applicable here, a parasitoid-centred SOH may contribute more to our understanding of mutualism stability by encouraging ovipositing pollinators to prefer inner ovules and avoid outer ones more accessible to parasitoids ovipositing from the other side of the fig wall.

2.2 Introduction

Given that mutualistic interactions involve individuals of different species often from highly divergent taxa, it is perhaps unsurprising that, despite providing each other with a service, partners also conflict in what is in their own best interests (e.g. Herre 1989; Herre and West 1997; Cook and Rasplus 2003). This conflict is no more apparent than between fig trees and their pollinating wasps that, despite relying completely on one another to reproduce (individual syconia provide the only egg laying sites for the wasps, and the wasps are the trees' only pollen vectors), differ considerably in their reproductive interests (Anstett *et al.* 1997; Cook and Rasplus 2003).

Female pollinating wasps enter fig fruit (syconia) during a short period of fig receptivity. Those that successfully gain entry (foundresses) lay their eggs and pollinate many of the tiny flowers that line the interior of the syconium, dying shortly afterwards (Galil and Eisikowitch 1969). Externally ovipositing non-pollinating fig wasps (NPFWs) are also associated with fig species and are highly host-specific, but effectively cheat the mutualism by laying their eggs into ovules whilst failing to disperse pollen. Some NPFWs (parasitoids and inquilines) parasitize developing wasp larvae whilst others (gall-formers) are herbivorous and oviposit into empty ovules (Compton and van Noort 1992; West and Herre 1994; Weiblen 2002). Despite variation in the timing of oviposition, the vast majority of wasp offspring emerge from their galls and disperse at the same time. After mating, female pollinators collect pollen from ripened male flowers whilst male pollinators chew holes in the wall of syconia, allowing wasps to disperse.

Monoecious species constitute roughly half of the 735 described fig species (Berg and Corner 2005), and male and female flowers develop together within syconia. Male flowers produce pollen whilst female flowers encourage the development of pollinating wasps and seeds. Crucially, pollinating wasp larvae eat the seed tissue and prevent it developing. If foundresses entering syconia were to lay an egg into every female flower, no seeds would develop and the mutualism would be unstable. Wasps, however, generally develop in only 50% of ovules (Janzen 1979b; Herre 1989; Nefdt and Compton 1996; Bronstein and Hossaert-McKey 1996; Yu *et al.* 2004) suggesting that mechanisms must be in place that limit wasp oviposition.

The short ovipositor hypothesis (SOH) was proposed to explain why pollinating wasps do not develop in all monoecious fig ovules (Ramirez 1970a). Style lengths in monoecious syconia vary along a continuum from short-styled flowers with long stems (pedicels) that develop close to the inner cavity (inner flowers), to long-styled flowers with short pedicels that develop close to the fig wall (outer flowers) (Figure 1.3A). Given that eggs are only successfully deposited if a pollinator's ovipositor can travel the full length of the style (Ramirez 1969; Janzen 1979a; Otero and Ackerman 2002; but see Zhang *et al.* 2009), the SOH suggests that a subset of flowers have style lengths longer than the ovipositors of pollinating wasps and are therefore protected from wasp exploitation. Nefdt and Compton (1996) studied 10 monoecious African fig species and concluded that in eight species, between 1 and 30% of flowers had styles that were inaccessible to pollinating wasps, whilst 40-45% of styles were out of reach to pollinators in the two remaining species. In two Neotropical species, *F. pertusa* and *F. maxima*, 18% and 17% of styles (respectively) were inaccessible (Bronstein 1988a; Jousselin *et al.* 2004). This means that observed proportions of flowers developing as seeds are often much higher than the proportion of inaccessible ovules. It also suggests that the SOH cannot be a stand-alone theory to explain how wasp oviposition is limited among *Ficus*, but it may help explain how reproduction is restricted in some fig species.

I investigate the SOH in *Ficus rubiginosa*, an Australasian fig species (section Malvanthera) that is distantly related to other lineages (e.g. Pharmocosycea, Americana, Galoglychia, Sycomorus) where the SOH has been tested (Rønsted *et al.* 2005, 2008). Originally, *Ficus rubiginosa* was thought to support a single agaonid pollinator, *Pleistodontes imperialis*. Genetic data (Haine *et al.* 2006), however, revealed that what was originally considered to be a single species is in fact four cryptic pollinator species-now termed, the '*P. imperialis* complex'. Despite the exact distribution of each species being unknown, multiple species can be found on the same tree, and sometimes within the same syconium (Haine *et al.* 2006). This supports other studies (e.g. Michaloud *et al.* 1985 (*F. ottoniifolia*); Galil and Eisikowitch 1968b, 1969 (*F. sycomorus*); Ramirez 1970b (*F. tuerckheimii*); Compton 1990; Ware and Compton 1992 (*F. lutea*)) that dispute the existence of a universal one fig: one pollinator rule (Wiebes 1963). How such coexistence among co-pollinators is achieved is a topic of considerable debate (Chesson 1991; Taper 1993; Chesson and Huntly 1997; Grover 1997).

Gause's law of competitive exclusion (Gause 1934; Hardin 1960) suggests that multiple species that compete for a limited resource cannot coexist if other ecological factors are constant. The Lotka-Volterra equation, however, states that coexistence can be maintained if intra-specific competition exceeds inter-specific competition (Armstrong and McGehee 1980). Resource partitioning (Pyke 1982) and the aggregation model (Atkinson and Shorrocks 1981) are two theories that explain how this may occur. Resource partitioning suggests that competing species may be driven by natural selection to use slightly different types of resources to facilitate coexistence

(Pyke 1982). Such resources can be temporal, spatial or morphological in nature. The aggregation model suggests that if one competitor is clustered ('aggregated') in one fraction of an available habitat or resource, the remaining empty patches can be occupied by a competing individual (Atkinson and Shorrocks 1981; Ives and May 1985; Ives 1991). The resultant clumping of closely related individuals will mean that intra-specific competition is higher than interspecific competition, thereby enabling coexistence. Both resource partitioning (Hutchinson 1959; MacArthur and Levins 1967; Levins 1968; MacArthur 1972; Pyke 1982; Kronfeld-Schor and Dayan 1999; Zhai *et al.* 2008) and the aggregation model (Shorrocks and Rosewell 1986, 1987; Hanski 1981; Comins and Hassell 1981; Atkinson 1985) have been supported in the literature with suggestions that the aggregation model may better explain coexistence among closely related competitors whilst resource partitioning is more applicable to coexistence among less closely related species (Takahaski *et al.* 2005).

In *F. rubiginosa*, as foundresses of more than one species compete for the same oviposition sites, resource partitioning or aggregation of conspecifics may facilitate coexistence. Within *Ficus rubiginosa* syconia, roughly 40% of ovules nurture pollinator larvae whilst 50% become seeds (Cook and Power 1996; Dunn *et al.* 2008b). I investigate whether the SOH can explain these observed levels of seed predation and whether variation in ovipositor length across pollinator species can contribute to morphological niche segregation (oviposition into different length styles) among sympatric *P. imperialis* species to allow coexistence.

The SOH has traditionally been considered with reference to pollinating wasps; however, not only may long-styled ovules be out of reach to pollinators ovipositing *within* the fig cavity, but long pedicel flowers that develop furthest from the syconium wall may, by a similar reasoning, be out of reach to non-pollinating fig wasps (NPFWs) ovipositing through the fig wall. I studied four NPFWs commonly found in *F. rubiginosa* syconia: the parasitoids of *P. imperialis*, *Philotrypesis* and *Sycoscapter spp.* (Cook and Power 1996; Dunn *et al.* 2008b), and two gall-forming genera, *Eukobelea* and *Pseudidarnes spp.* (Cook and Power 1996; Haine and Cook 2005). As there is little knowledge of how NPFWs oviposit into ovules (but see Compton and Robertson 1988; Peng *et al.* 2005), I also recorded the oviposition paths of NPFWs killed during oviposition and the relative proportion of ovules accessible to wasps of each genus.

2.3 Materials and methods

2.3.1 Study species

Ficus rubiginosa has a native range that extends along most of the eastern coastline of Australia (Dixon *et al.* 2001). It grows in both rainforests and on granite outcrops (Fairley and Moore 2000), and is host to four cryptic pollinator species that exhibit significant mitochondrial DNA divergence (9-17%, Haine *et al.* 2006). Three of the four species (species 1, 3 and 4) are brown/ black whilst the other is yellow (species 2). At present, we can only reliably identify the yellow species without genetic data.

2.3.2 Ovules accessible to pollinating wasps

I collected 98 receptive syconia containing at least one live ovipositing foundress from seven trees in Townsville, Australia (19° 15' 0 S, 146° 48' 0 E). Receptive syconia containing foundresses were often distinguished by the presence of wings protruding from the ostiole (Khadari *et al.* 1995; Anstett *et al.* 1996b). Collected syconia were halved with a blade to confirm that foundresses inside were alive and at least one was actively ovipositing. Any empty syconia or any containing only dead foundresses were discarded to ensure that style lengths represented the actual distances encountered by ovipositing females.

I measured the maximum length and width of each collected syconium and then stored them individually in 40% ethanol to preserve the ovules and prevent the styles from drying out. To achieve a large and representative sample of the variation in ovule and style lengths across receptive *F. rubiginosa* syconia, I measured a random sample of 25% of style lengths from each syconium. To do this, syconia were sliced into eighths using a razor blade and, working from left to right for each segment, every fourth ovule was removed using forceps and placed in a watch glass of water to prevent desiccation. This was repeated for each eighth. Style lengths were measured from where they joined the ovary to the top of the stigma (Figure 2.1A (a)) and were recorded to the nearest 0.03mm using a dissecting microscope at 40x magnification.

I recorded the head length and width, and hind femur and tibia lengths for each of 145 foundresses from collected syconia. The ovipositor was dissected from the body of each wasp so that its total length (first and second valvulae) could be measured. I also calculated a relative measure of ovipositor length (ovipositor length/ femur length) to account for variation in individual body size. Field observations of pollinating fig wasps have confirmed that during oviposition, the entire length of the first and second valvulae are inserted into the style (Nefdt and Compton 1996). Consequently, total ovipositor length is the most accurate measure of the oviposition distance accessible to each foundress. I carried out wasp dissections at 40x magnification under a dissecting microscope using fine forceps and stored each wasp individually in 100% ethanol to prevent DNA degradation.

2.3.3 Identification of Pleistodontes imperialis species

To determine whether ovipositor lengths of *P. imperialis* species varied significantly, I first identified foundresses to species by DNA barcoding. I kept wasp heads for reference and carried out DNA extractions on the remaining bodies using a Chelex extraction as detailed in West *et al.* (1998). I amplified 444bp fragments of mitochondrial cytocrome b using primers CB1 (5’-TAT GTA CTA CCA TGA GGA CAA ATA TC-3’) and CB2 (5’-ATT ACA CCT CCT AAT TTA TTA GGA AT-3’). Polymerase Chain Reactions (PCR) were performed on a Techne Touchgene Gradient machine with three minutes at 94°C, followed by 30 cycles of 15 seconds at 95°C, 15 seconds at 45°C and 30 seconds at 72°C, and a final elongation step of 10 minutes at 72°C. For each PCR product, 2µl was electrophoresed through a 1% agarose gel to determine amplification size, and 18µl of PCR product was then sent to Macrogen for purification and sequencing using the same CB1primer as in the original PCR. A total of 110 successful sequences, along with 33 *P. imperialis* cytochrome b sequences collected from a BLAST search were edited with FinchTV (version 1.4.0, http://www.geospiza.com/finchtv) and aligned in Bioedit (version 7.0.0, Hall 1999). Final classification of individuals to species was made using a distance matrix of aligned sequences and plotting all sequences on a DNAdistance neighbour phylogenetic tree in Treeview 1.6.6 (Page 1996). Members identified to the same species exhibited 0-7% divergence whilst those from other species showed 10-15% divergence (Haine *et al.* 2006).

2.3.4 Ovules accessible to non-pollinating fig wasps

2.3.4.1 Investigating the oviposition paths of NPFWs

Seven non-pollinating fig wasps (NPFWs) from three genera (*Philotrypesis*, *Eukobelea* and *Pseudidarnes*) were collected whilst ovipositing by crushing them with a finger onto the syconium. To observe the path of the ovipositor, successive wafer-thin slices were cut longitudinally through the syconium using a razor blade until the ovipositor was revealed. The wasp and its syconium were then stored in diluted ethanol (40%) and used to assess ovule accessibility (see below).

2.3.4.2 Measuring oviposition distances encountered by NPFWs

A total of 125 non-pollinating fig wasps were sampled during oviposition on *F. rubiginosa* syconia. Each wasp and its syconium were stored in 40% ethanol. To assess the proportion of ovules accessible to each NPFW, individual syconia were halved using a razor blade and where entire ovules (from the pedicel to the top of the ovary) were visible, minimum (fig wall to where ovary joins the pedicle, Figure 2.1B (b)), and maximum (fig wall to the top of the ovary, Figure 2.1B(c)) oviposition distances for one half of each syconium were recorded. Roughly 15 ovules per syconium were suitable for measurement using this method. Minimum and maximum oviposition distances were recorded as, to my knowledge, it is not known where within an ovule (i.e. top or bottom) these NPFWs oviposit in order to successfully deposit an egg. I measured the head length and width, hind femur and tibia lengths and total ovipositor length of each ovipositing NPFW under a dissecting microscope at 40x magnification.

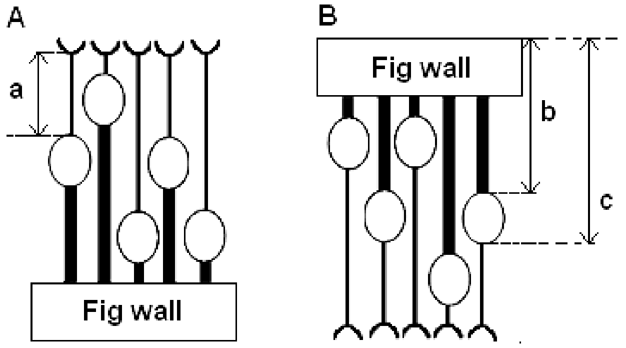


Figure 2.1 Measurements of a) style length and, b) minimum (fig wall+pedicel), and c) maximum (fig wall+pedicel+ovary) oviposition distances with respect to pollinators (A), and externally ovipositing NPFWs (B).

2.3.5 Data analysis

For each wasp, I considered the proportion of ovaries ‘accessible’ to be those with oviposition distances shorter than, or equal to, the total length of the ovipositor. I performed ANCOVA models on each of ovipositor length, head length, hind tibia length and relative ovipositor length (ovipositor/ femur length) (response variables) among *P. imperialis* species (categorical explanatory variable), including fig volume and site as a covariate and random effect respectively, to observe whether *P. imperialis* species were morphologically different. I also used an ANOVA to investigate whether the proportion of accessible ovules varied significantly between pollinator species, using individual syconia as a random effect in the analysis. All models were simplified by backward elimination of terms, using a χ^2 test to check the validity of these removals (Crawley 2005).

2.4 Results

2.4.1 Ovules accessible to pollinating wasps

I successfully identified foundresses from 71/98 syconia to one of three *P. imperialis* species: species 2 (n=44), 3 (n=59) and 4 (n=7). Most syconia (80.3%) were occupied by a single foundress, 12.7% by two foundresses and 7% by more than two (see Table 2.1). Ninety percent of syconia contained a single species of *P. imperialis* with the remaining 10% containing two species. It is worth noting that these foundress numbers are likely to be slight underestimates as some syconia left on the tree would inevitably receive more foundresses (Khadari *et al.* 1995). Moreover, whilst <10% of figs had two species of *P. imperialis*, this is largely due to the fact that 80% of syconia contained a single wasp. In figs with two or more wasps, 50% (7/14 syconia) had two species of pollinators.

Table 2.1 Relative numbers (percentage) of syconia containing 1, 2 or more than 2 foundresses, and the associated number of *P. imperialis* species.

	Number of species		
Number of foundresses	**1**	**2**	**3**
1	57 (80.28)	NA	NA
2	4 (5.63)	5 (7.04)	NA
>2	3 (4.23)	2 (2.872)	0 (0.00)

Species 2 (yellow) individuals had significantly longer heads, tibias and ovipositors than species 3 foundresses (ovipositor length: *P*=0.05, Adjusted R^2=0.05, $F_{5, 97}$=2.11; head length: *P*<0.001, Adjusted R^2=0.11, $F_{2, 106}$=7.85; tibia length: *P* <0.01, Adjusted R^2=0.05, $F_{2, 98}$=3.54). Despite this, relative ovipositor length (*P*=0.30, Adjusted R^2=4.87E-03, $F_{2, 103}$=1.20), and ovule accessibility (*P*=0.63, $F_{2, 291}$=0.46, Adjusted R^2= -0.0037) were not significantly different across species, with all three species capable of accessing a minimum of 99.4% of *F. rubiginosa* ovules (Figure 2.2).

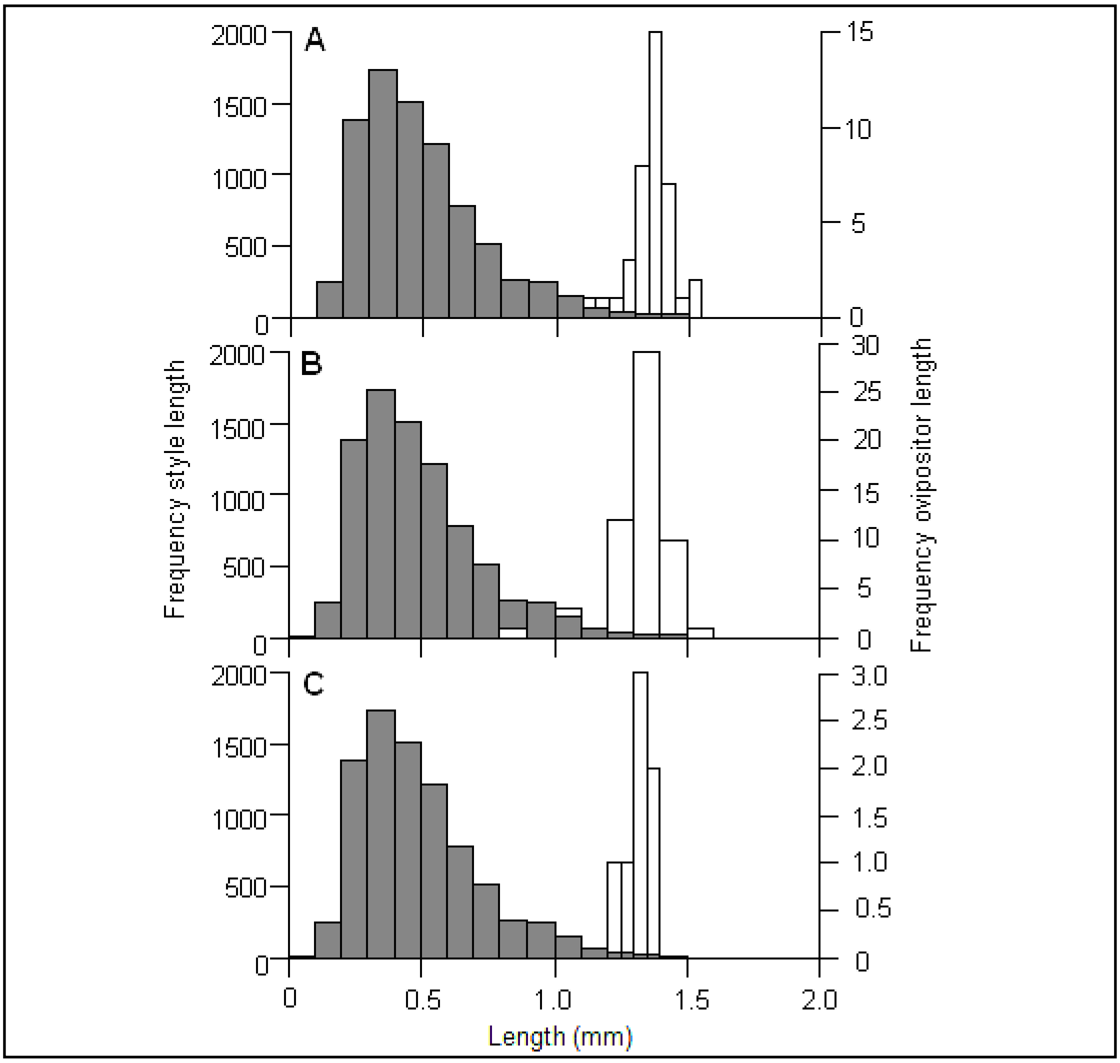


Figure 2.2 Style length distribution (grey) for *F. rubiginosa* ovules from 98 syconia, and ovipositor (white) distributions for (A) species 2, (B) species 3 and (C) species 4 *P. imperialis* foundresses.

2.4.2 Ovules accessible to non-pollinating fig wasps.

2.4.2.1 Investigating the paths non-pollinating wasp ovipositors take during oviposition.

For seven NPFWs (*Philotrypesis* (n=3), *Pseudidarnes* (n=2)*, Eukobelea* (n=2)) crushed during oviposition, the path of the ovipositor represented the shortest route from the fig wall to the focal ovule (Figure 2.3). Unfortunately, the ovipositors of *Sycoscapter* wasps collected during oviposition did not remain within syconia and the paths of their ovipositors could not be traced.

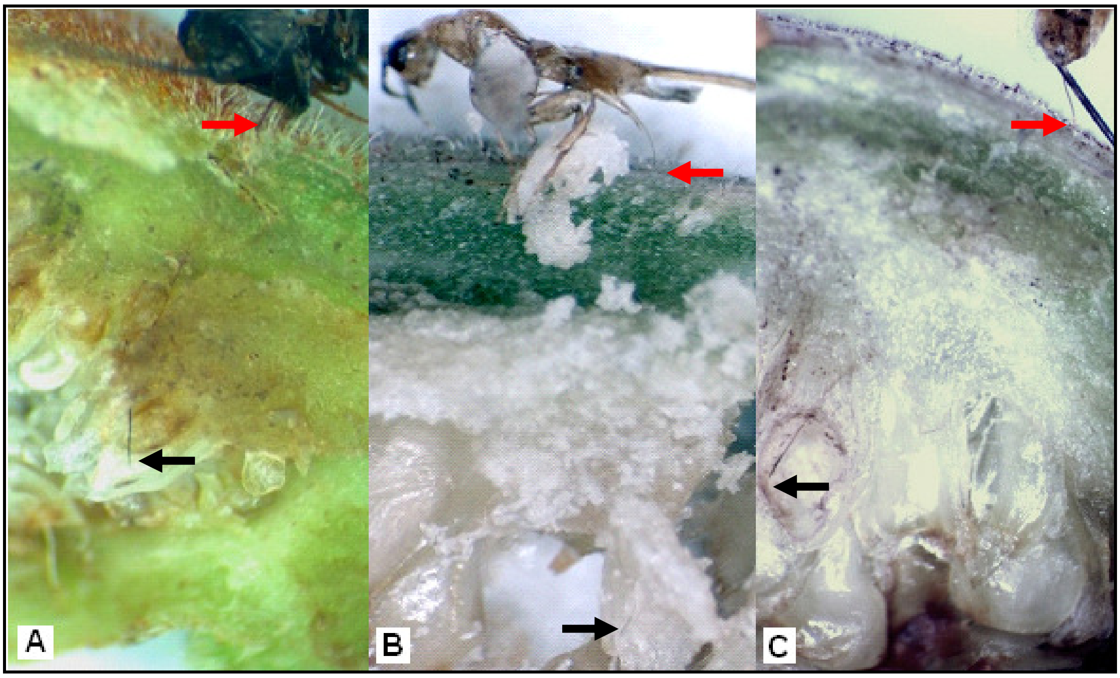


Figure 2.3 Oviposition pathways for (A) *Pseudidarnes*, (B) *Philotrypesis*, and (C) *Eukobelea* NPFWs. Arrows indicate the start (red) and end (black) points of each ovipositor. Photographs taken by S. Al-Beidh.

2.4.2.2 Measurement of oviposition distances encountered by NPFWs

Philotrypesis and *Sycoscapter* NPFWs (parasitoids of *P. imperialis*) attack syconia relatively late in their development when pollinator eggs have already been laid (Table 2.2). In contrast, wasps from the two gall-making genera attack earlier; *Eukobelea* attacking syconia 0.90cm in diameter whilst *Pseudidarnes minerva* attack earlier still (Table 2.2). As both genera are gall-formers, they often attack syconia even before they have been pollinated. On average, *Sycoscapter* wasps could access 66.8% of ovules whilst *Philotrypesis*, with longer ovipositors, could reach 91.9% of ovules. *Eukobelea* wasps were capable of accessing 96.3% of ovules whilst *Pseudidarnes* wasps had ovipositors that could reach all ovules (Figure 2.4).

Table 2.2 Numbers (N) and average ovipositor lengths of NPFWs collected during oviposition from different figs, trees and study sites and the average size of the *F. rubiginosa* syconia they attack.

Wasp	N	Ovipositor length (S.E.) (mm)	Fig diameter (S.E.)(mm)	Trophic status	Site	Tree	Figs
Pseudidarnes	6	3.00 (0.13)	6.21 (0.32)	Gall-former	1	2	5
Eukobelea	5	3.57 (0.12)	9.02 (0.31)	Gall-former	1	1	5
Sycoscapter	42	3.32 (0.10)	12.11 (0.25)	Parasitoid	3	6	40
Philotrypesis	72	4.40 (0.08)	12.55 (0.17)	Parasitoid	4	6	68

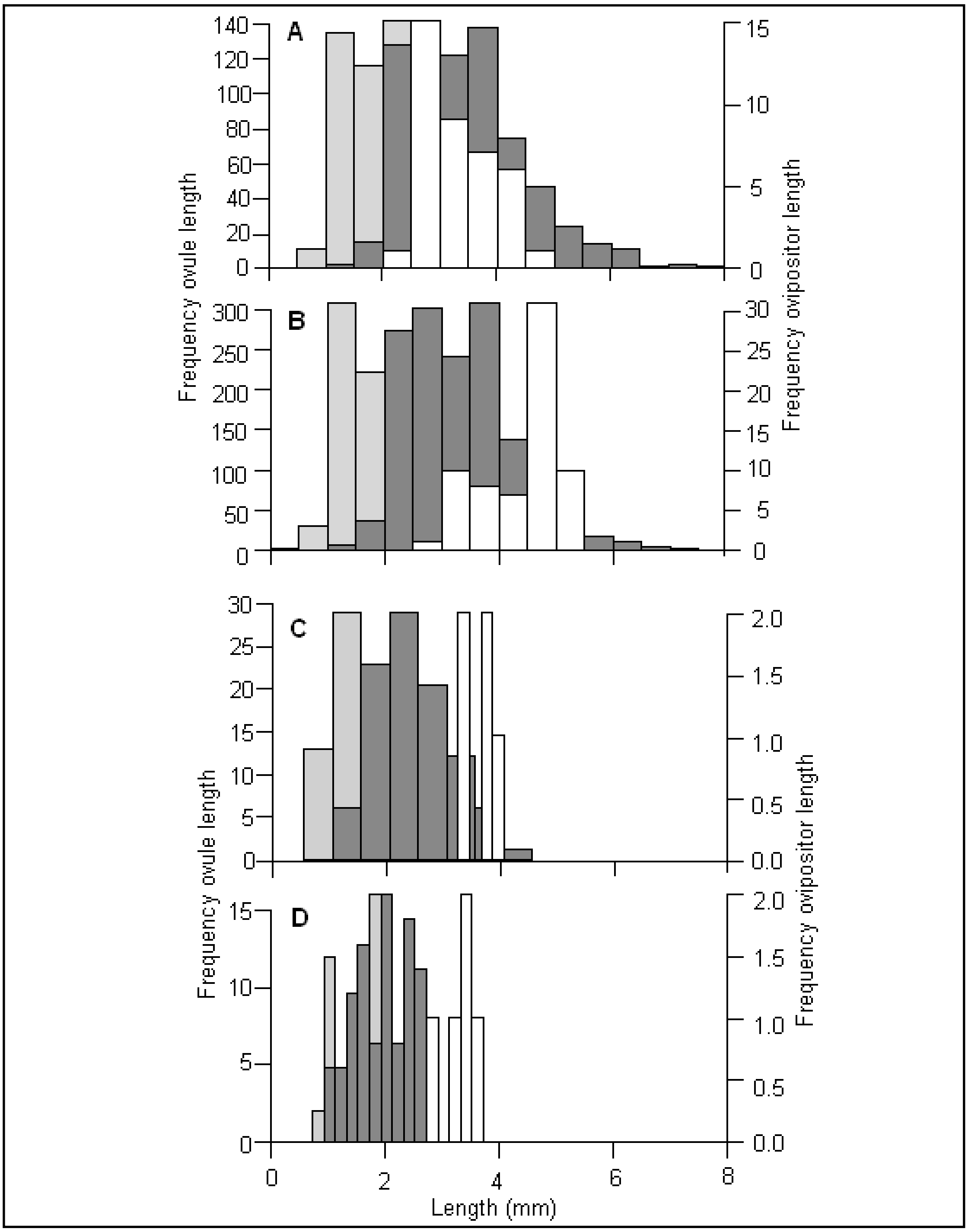


Figure 2.4 Minimum (light grey) and maximum (dark grey) oviposition distances to *F. rubiginosa* ovules for (A) *Sycoscapter*, (B) *Philotrypesis*, (C) *Eukobelea* and (D) *Pseudidarnes* ovipositors (white).

2.5 Discussion

Mutualism dynamics with coexisting pollinator species

Three *P. imperialis* species (species 2, 3 and 4) were identified among the wasps sampled from *F. rubiginosa* syconia and all three species could access over 99% of ovules. Moreover, although the criteria for syconium collection (only those containing live foundresses) may have underestimated the total numbers of foundresses entering syconia, 90% contained only a single pollinator species. Of the remaining 10% that contained different species, in the majority of cases (85.7%) at least one of these foundresses was a yellow (species 2) pollinator. This high incidence of syconia where dark (species 3 and 4) and light (species 2) coloured *P. imperialis* coexist may prove significant because although these results show no evidence for niche separation on the basis of morphology, temporal niche separation is, as yet, an unstudied possibility. Evidence from *Ficus sycomorus*, which has both dark and pale pollinator species, suggests that *Ceratosolen arabicus*, the yellow pollinator, has enlarged eyes and is nocturnal, whilst *C. galili* is black and flies during the day (Galil and Eisikowitch 1974; Compton *et al.* 1991; Warren *et al.* 2010). That yellow pollinators are night-dispersing and brown pollinators, day-dispersing, has also been described in other fig species (Harrison 2003). Further work should test for such differences in the dispersal patterns of the *Pleistodontes imperialis* species.

The NPFW short ovipositor hypothesis

Dunn and others (2008b) proposed that parasitoid ovipositor length may play a role in the stability of the fig/ fig-wasp mutualism. The authors suggested that inner flowers may provide an area of enemy-free space for pollinator wasp larvae if these flowers were out of reach to parasitoid wasps ovipositing externally. Data from *F. rubiginosa* identified that the risk of parasitism by *Sycoscapter* or *Philotrypesis* wasps ranged from 0% in the innermost flowers to 80% closer to the fig wall; however, neither parasitoid ovipositor length nor ovule accessibility at the time of oviposition was measured. Moreover, the placement of wasp eggs has been investigated for very few fig wasp species (Cook and Rasplus 2003).

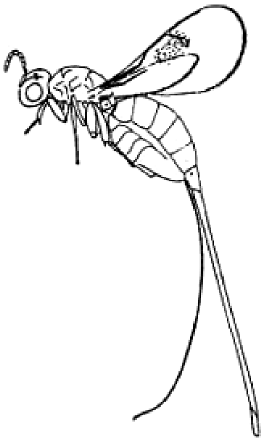
I successfully traced the ovipositors of seven individuals from three NPFW genera. In each case, the ovipositor was traced directly into an ovule below its entrance point, suggesting that on at least some occasions, NPFWs oviposit via the shortest possible route. This is a crucial point as it validates the measurement of ovule accessibility among NPFWs assuming oviposition via a direct route. I also assumed that NPFWs insert the full length of their ovipositors into the syconium wall to successfully deposit an egg. Although this is essentially true for externally ovipositing wasps with short ovipositors (personal observation), for wasps possessing very long ovipositors (such as *Sycoscapter* and *Philotrypesis*) oviposition assumes a ‘tip-toe’ posture, with the ovipositor positioned between the forelegs (Gardiner 1966; Ramirez 1986), and with part of it often remaining above the fig wall. The results for the parasitoid species in particular are therefore likely to over-estimate ovule accessibility, which is probably more restricted.

The proportions of ovules available to NPFW varied between gall-formers and parasitoids. The two gall-forming genera, *Eukobelea* and *Pseudidarnes*, do not rely on already occupied ovules in which to oviposit and therefore attack syconia earlier in their development, often even before pollinator entry (personal observation). These wasps were capable of accessing over 94% of ovules. In comparison, two parasitoid NPFW genera (*Sycoscapter* and *Philotrypesis*), despite often having longer ovipositors, were able to access fewer ovules (on average 66.8 and 91.9% respectively) because they rely on developing host larvae and therefore must oviposit into syconia at a later stage of development where distances from the fig wall to focal ovules have increased (Compton and Nefdt 1990). This suggests that parasitoid NPFWs are unable to access the innermost ovules and supports evidence from Dunn *et al.* (2008b) that considers the spatial stratification of gall occupants.

In conclusion, the short ovipositor hypothesis was originally proposed as an explanation for mutualism stability by considering whether some flowers were inaccessible to ovipositing foundresses. As over 99% of styles were accessible to foundresses in *F. rubiginosa*, the pollinator SOH cannot explain seed predation levels (roughly 50% of flowers, Cook and Power 1996; Dunn *et al.* 2008b) in this fig species. Given that all three *P. imperialis* species could access the vast majority of fig flowers, morphological niche segregation via oviposition length also seems unlikely. Nevertheless, by applying the SOH to parasitic NPFWs, I show that parasitoid wasps ovipositing externally are unable to reach all ovules. This may, in turn, encourage pollinators to prefer short-styled flowers where larvae are at less risk from parasitism. This foundress 'oviposition profile' (Yu *et al.* 2004) indirectly impacts on seed set as ovules least preferred by foundresses, if pollinated, become seeds. Similar work in other fig/ wasp partnerships from different lineages that considers the applicability of the short ovipositor hypothesis with respect to NPFWs should be carried out to determine whether this may contribute more generally to stability within the fig/ fig-wasp mutualism.

Chapter 3

Do the fig wasp parasitoids of *Ficus racemosa* condemn themselves to life imprisonment?



Sycoscapter sp. female (Hymenoptera: Sycoryctinae) (Boucek 1988)

3.1 Abstract

Fig trees and their pollinating wasps constitute a well-known obligate mutualism, where each partner relies on the other for reproduction. This mutualism is exploited by other wasps that also reproduce within fig fruit (syconia). Many of these wasps are parasitoids of developing wasp larvae, whilst others are herbivorous, galling fig ovules themselves. Male pollinating wasps have three crucial functions: (1) to mate with female pollinators, (2) to release them from their galls and (3) to chew exit tunnels in the wall of the syconium to enable them to escape. If they fail in the second task, individual females can remain trapped within their galls, even if mated. Failure in the third task often results in female pollinators being unable to exit their natal syconium. Parasitic fig wasps often have jaws unsuitable for biting through the syconium wall and therefore also rely on the holes chewed by male pollinating wasps in order to disperse.

I investigated the natural proportion of syconia that fail to exit (‘unexited’ syconia) in *Ficus racemosa*, and whether failure to emerge is a result of too few pollinator males. I also discuss whether low pollinator male number is caused by low foundress number and/or high rates of parasitism. Results indicate that 4% of *F. racemosa* syconia contain wasps that fail to disperse. The probability of an exit hole being made increases with the number of pollinator males, and with fewer than ten males in a syconium, it is unlikely that any wasps will emerge. Significantly higher proportions of non-pollinating fig wasps and lower proportions of male and female pollinating wasps were found in unexited syconia compared to syconia where wasps exited normally. Unexited syconia, however, did not have significantly higher numbers of *Platyneura agraensis* - the parasitoid of pollinators, suggesting that low foundress number is likely to be the primary reason for wasps failing to exit. Without an alternative release mechanism, wasps in these syconia will die. I discuss the possibility that dispersal through holes produced by other insect fauna may sometimes provide an alternative means of escape for these trapped wasps.

3.2 Introduction

Pollinating fig wasps (Chalcidoidea, Agaonidae) and their fig trees (Ficus, Moraceae) are a classic example of an obligate mutualism. The fig trees provide the pollinating wasps with oviposition sites in which to lay their eggs whilst the wasps are the sole dispersers of a fig tree's pollen. Egg laden foundresses gain entry into receptive (B- stage, Galil and Eisikowitch 1968a) fruit (syconia) through a pore called the ostiole. Once inside, females lay their eggs into flowers and transfer pollen carried from their natal syconium onto receptive stigmas. They die within the syconial cavity and their offspring develop during the coming weeks (C-stage). Male pollinating wasps are the first to emerge from their galls. They are wingless and practically blind and their entire lives are spent within their natal fig. Despite this, they have three roles that are crucial to the fig/ wasp life cycle. Once they have emerged from their own galls (D-stage), male pollinators bite holes in the galls containing female pollinators and mate with them through these openings. The holes are later enlarged so that females can emerge from their galls (Zammit and Schwarz 2000). Finally, the males chew exit tunnels through the syconium wall to facilitate wasp dispersal. Female pollinators collect pollen (actively or passively) that has ripened in male flowers and disperse through these tunnels in search of new receptive syconia. After wasp dispersal (E-stage), the fig ripens fully, attracting birds, bats and monkeys which disperse its seeds (Janzen 1979a).

As is the case in most mutualisms, the fig-pollinator system is subject to attack by parasites (Bronstein 1991; Yu 2001): a suite of non-pollinating fig wasps (NPFWs) that have generally been regarded as costly to both mutualists and exploit the system (Kerdelhué and Rasplus 1996; West *et al.* 1996; but see Dunn *et al.* 2008b). Like the pollinators, NPFWs use the flowers within syconia to reproduce. Some NPFWs have herbivorous larvae that directly consume the tissue of the syconium, whereas others reduce the number of emerging wasps by parasitizing developing larvae (Kerdelhué and Rasplus, 1996; West *et al.* 1996; Weiblen, 2002; Cook and Rasplus 2003; Dunn *et al.* 2008b). Parasites of pollinators destroy a single pollinator larva in order to successfully mature, whether they are inquilines usurping the developing pollinator from its gall, or parasitoids that kill the pollinators directly (Tzeng *et al.* 2008). Like female pollinators, the jaws of many NPFWs are unsuited to chew through the fig wall (Cook *et al.* 1997; Bean and Cook 2001, but see Compton *et al.* 1994), and the holes produced by male pollinators are often necessary to enable them to disperse. Consequently, pollinator females and many NPFWs rely on pollinator males to achieve reproductive success.

When mating takes place between the offspring of one, or a few mothers, and where daughters disperse but sons do not, a female-biased sex ratio is favoured (Hamilton 1967). Pollinating fig wasps provide a good example of sex ratio theory and lay strongly female-biased sex ratios (Hardy 1994). In single foundress syconia, an ovipositing female should lay just enough sons to 1) mate with all of her daughters and 2) bite exit holes in their galls and the syconium wall to ensure their release. As the number of ovipositing foundresses within a syconium increases, each female must lay more sons because there is increasing competition between unrelated males for access to females (so-called 'Local Mate Competition', Hamilton 1967). Consequently, where foundress numbers are low, ovipositing females must 'weigh up' the cost of producing too many unnecessary males with the possibility of laying too few and having no dispersing offspring. Moreover, where rates of parasitism are high, the probability that pollinator males are killed increases. Given the dependence of female pollinators and many NPFWs on male pollinators, insufficient numbers of males means that all wasps fail to disperse to a new host and therefore achieve zero reproductive success.

In *Ficus racemosa*, syconia can reach the post-floral (E) stage without wasps exiting, despite wasps developing in many ovules (personal observation). This scenario has, to my knowledge, failed to be clearly acknowledged in the literature with the exception of a brief mention in *F. craterostoma* where such syconia have been termed 'coffin figs' (Greeff 2002). Unexited *F. racemosa* syconia appear red and swollen with no exit holes and, if opened by hand, release many hundreds of wasps. Whilst some NPFWs can exit syconia themselves (Cook and Power 1996; Hill 1967; West *et al.* 1996; Peng *et al.* 2005), members of the Sycophaginae and Sycoryctinae cannot. The NPFW community of *F. racemosa* is composed of wasps from only these two taxa and they are therefore unable to emerge from syconia unaided. For these 'unexited' syconia, all wasps are therefore likely to suffer death without dispersal. The tree itself also incurs a cost, as pollen from these fails to be dispersed; however, providing that wasps in at least some syconia on the tree exit normally, this cost is probably small.

I consider (1) whether failure to produce an exit tunnel in *F.racemosa* syconia is explained solely by the presence of too few pollinator males, and (2) whether low male count is a consequence of a) developmental mortality from parasite attack and/or b) low foundress number (i.e. too few males laid by foundresses). I estimated the natural frequency of fruit on *F. racemosa* trees where the wasps fail to emerge, and experimentally manipulated male numbers to determine how many males are necessary for wasp emergence. I also measured the naturally occurring variance in the pollinator sex ratio and the relative abundance of NPFWs in syconia from which wasps dispersed successfully and those in which all wasps were 'imprisoned.'

3.3 Materials and methods

3.3.1 Study species

Ficus racemosa (section Sycomorus) is a monoecious fig species, where male and female flowers are produced within the same syconium (Cook and Rasplus 2003). It favours swampy soil in moist valleys (Yang *et al.* 2000) or along rivers and is widely distributed, occurring from India, through south-east Asia to northern Australia (Yang *et al.* 2000). Unlike many monoecious figs that produce their syconia along branches, *F.racemosa* produces fruit that hang in clusters at the end of thin stems (racemes) that protrude from its trunk. *Ficus racemosa* is pollinated by *Ceratosolen fusciceps*. Five species of non-pollinating fig wasps (NPFWs): *Apocrypta westwoodi*, *A. sp 2.* (Chalcidoidea: Sycoryctinae), *Platyneura testacea, P. mayri* and *P. agraensis* (Chalcidoidea: Sycophaginae), are known to be associated with *F. racemosa* in China (Wang and Zheng 2008) and India (Proffit *et al.* 2007). Recent evidence suggests that *P. testacea* and *P. mayri* are gall-formers, *A. westwoodi* and *A. sp. 2* are their parasitoids and *Platyneura agraensis* is the parasitoid of *C. fusciceps* (Wang and Zheng 2008). Work for this study was carried out in Townsville, Australia (19° 15' 0 S, 146° 48' 0 E) during May - August 2007 and September - December 2008.

3.3.2 Estimation of natural rates of unexiting syconia

Between September - December 2008, I measured the maximum widths of 87 C-stage syconia using callipers (Table 3.1). Syconium size was recorded daily until wasp emergence, or until syconia dropped from their racemes, providing an estimate of the natural occurrence of syconia where wasps fail to emerge.

Table 3.1 Numbers of C-stage syconia from different trees and study sites, followed through development.

Study site	Tree	Total syconia
Aplins wier	AW1	10
Aplins wier	AW2	4
Aplins wier	AW3	14
Cranbrook Park	1	8
Palmetum	P1	7
Riverside gardens	R3	10
Riverside gardens	R1	14
University	U1	8
University	U2	4
University	U3	8

3.3.3 Comparison of wasp composition between D and E-stage syconia

Between May - August 2007, I collected 28 E-stage syconia without exit holes, and 17 D-stage syconia where the wasps had not yet emerged (Table 3.2). Once collected, each syconium was placed individually into plastic collecting pots topped with muslin and left in the laboratory under natural conditions for 48 hours. No wasps emerged from any of the E-stage syconia (henceforth 'unexited syconia'), whilst wasps from all D-stage syconia dispersed successfully ('exited' syconia). Individual syconia and their associated wasps were stored together in 80% ethanol. I measured the maximum length and width of syconia to the nearest 0.05mm using digital callipers and halved each syconium using a razor blade. All wasps that had successfully emerged from their syconium and all those loose within the lumen plus any still in their galls were separated into three groups: (i) gall-formers (*Platyneura mayri* and *P. testacea*), (ii) parasitoids of pollinators (*P. agraensis*) and (iii) parasitoids of gall-formers (*Apocrypta westwoodi* and *A. sp. 2*). Wasps were identified using a binocular microscope at 20x magnification. Congeneric male NPFWs could not be identified to species by eye and were therefore assigned to the same proportions as females. The pollinator sex ratio was calculated as the number of male pollinators divided by the numbers of female pollinators for each syconium. The rate of parasitism was calculated as the proportion of NPFWs out of all wasps within a syconium.

Table 3.2 Numbers of D (exited) and E (unexited) stage syconia collected.

Study site	Tree	D-stage	E-stage	Total syconia
Aplins Weir	AW1	0	2	2
Aplins Weir	AW2	1	1	2
Aplins Weir	AW3	1	0	1
Aplins Weir	AW4	1	5	6
Cranbrook park	1	1	0	1
Pallerenda	P1	0	2	2
Palmetum	P8	1	2	3
Riverside Gardens	R16	0	4	4
Riverside Gardens	R18	2	6	8
Riverside Gardens	R2	0	2	2
Riverside Gardens	R3	6	2	8
Riverside Gardens	R4	2	0	2
Riverside Gardens	R8	2	2	4

3.3.4 Manipulating numbers of male pollinators in syconia

Between October and December 2008, I collected early D-stage syconia whenever I encountered fruit at this stage of development. In the laboratory, each syconium was halved using a blade and only those where wasps had begun emerging from their galls but male pollinators had not yet begun chewing exit holes (N = 26) were retained (Table 3.3). Female wasps that had emerged from their galls were allowed to disperse whilst (wingless) male pollinators were kept within syconia. For each syconium, I gently removed all visible male pollinators from one half and placed them in the other half using tweezers. Each half was then re-sealed between two matching Petri dishes using masking tape. The masking tape was pierced with holes large enough to allow air to enter the Petri dishes but small enough to prevent wasp escape. Sealed dishes were left for 24 hours to allow sufficient time for male pollinators to chew exit holes. I then recorded the number of exit holes and male pollinators in each experimental half.

Table 3.3 Number of syconia used from different trees and study sites in the experimental manipulation of male pollinator number.

Study site	Tree	Total syconia
Aplins wier	AW1	2
Riverside gardens	R3	12
University	U3	1
Cranbrook park	1	10
Cranbrook park	2	1

3.3.5 Data analysis

Data were analysed using R statistical software (R Development Core Team 2005). To compare wasp compositions between syconia where wasps exited normally with those where they did not, generalised linear models (GLMs) with binomial errors were constructed. The binary response variable was whether (or not) wasps exited their natal syconium, whilst the numbers and proportions of pollinators and NPFWs (out of all wasps) were included as explanatory variables to investigate their influence on wasp emergence. I controlled for unmeasured variance that could be attributed to environmental factors between study sites, trees and syconia by including them as random effects in maximal models. Initial models were simplified to minimum adequate models by step-wise deletion using χ^2 tests to assess the significance of the deleted terms (Crawley 2004). Where models exhibited overdispersion, a quasi-binomial error structure was specified and models were simplified using F tests. Final models were achieved when further removal of terms significantly increased the Akaike Information Criterion value (Crawley 2004).

3.4 Results

3.4.1 Estimation of natural rates of unexiting syconia

Eighty-seven C-stage syconia were followed daily until wasps exited or the syconia dropped from their racemes. Seventy-five of these developed to maturity. Of these, wasps exited normally from 72 syconia (96%) whilst the remainder (4%) failed to produce exit holes.

3.4.2 Comparison of wasp composition between D and E-stage syconia

Syconia where wasps failed to emerge had significantly fewer and lower proportions of pollinators and lower pollinator sex ratios than syconia where wasps dispersed normally (Figure 3.1, Table 3.4). The lowest number of male pollinators in unexited syconia was zero, compared with a minimum of 11 in syconia where wasps had successfully emerged. Neither syconium size nor the numbers of NPFWs influenced whether wasps emerged successfully (Table 3.5); however, proportions of all NPFWs were significantly higher in unexited compared with exited syconia. Proportions of individual NPFW groups were not significantly different among syconia.

Table 3.4 Average numbers (S.E.) of wasps in D-stage syconia where wasps emerged normally (exited syconia) and E-stage syconia where they did not (unexited syconia).

Total wasps	**Exited**	**Unexited**
Total pollinators	176.00 (39.24)	18.61 (3.36)
- Male pollinators	67.76 (14.37)	4.18 (1.02)
- Female pollinators	108.23 (28.24)	14.43 (2.53)
Total NPFWs	251.29 (27.17)	209.40 (33.35)
- Gall-formers (*P. mayri & P. testacea*)	94.03 (19.99)	91.17 (14.90)
- *P. agraensis* parasitoids	22.56 (8.18)	3.69 (2.37)
- *Apocrypta* parasitoids	134.71 (23.42)	114.53 (19.60)

Table 3.5 The results of 12 GLMs identifying the influence of pollinating and NFPW numbers and proportions (out of all wasps) on whether or not wasps emerged from syconia (P.D.E=proportion deviance explained).

Variable	***P***	***t***	**D.F.**	**P.D.E.**
Number of male pollinators	<0.001	-4.25	37	0.75
Proportion male pollinators	<0.01	-3.65	37	0.50
Number of female pollinators	<0.05	-2.32	37	0.42
Proportion female pollinators	<0.05	-2.38	37	0.17
Pollinator sex ratio	<0.05	-2.02	35	0.14
Syconium volume	NS (0.17)	-1.39	37	0.04
Number of NPFWs	NS (0.59)	-0.54	37	6.36E-03
-gall-formers (*P. mayri & P. testacea*)	NS (0.52)	-0.64	37	9.14E-03
-*P. agraensis* parasitoids	NS (0.24)	-1.19	37	0.04
-*Apocrypta* parasitoids	NS (0.99)	-0.01	37	0.01
Proportion NPFWs	<0.005	3.22	37	0.33
-gall-formers (*P. mayri & P. testacea*)	NS (0.68)	0.41	37	3.48E-03
-*P. agraensis* parasitoids	NS (0.50)	0.68	37	0.01
-*Apocrypta* parasitoids	NS (0.23)	-1.23	37	0.03

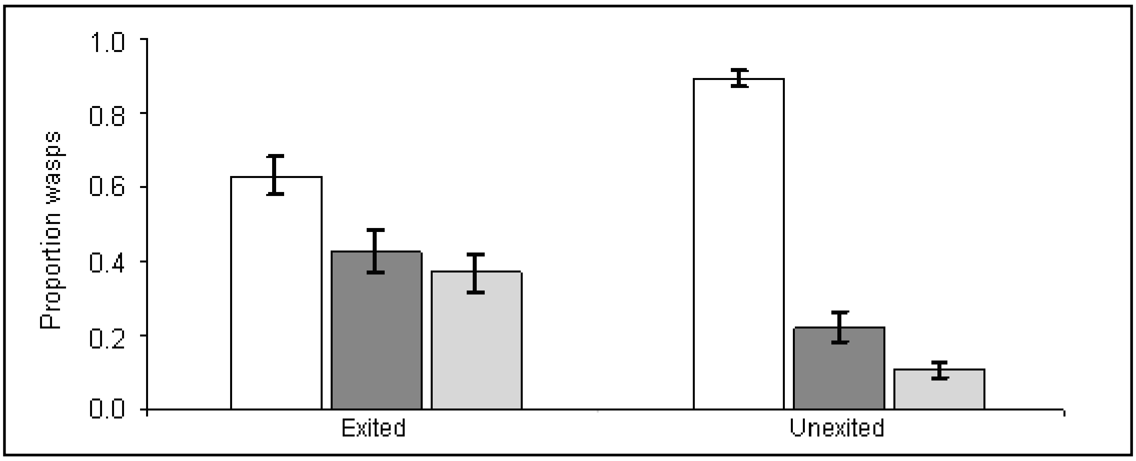


Figure 3.1 The proportion of all NPFWs (white), pollinator sex ratio (dark grey) and the proportion of pollinators (light grey) out of all wasps for exited and unexited syconia. Bars represent means± 1 S.E.

3.4.3 Male experiment

Using the male counts from each experiment, syconium halves where wasps had successfully emerged had significantly more male pollinators than those with no exit holes (P<0.01, z =2.679, proportion deviance explained=0.81, D.F. =51) (Figure 3.2). Moreover, although male pollinators began chewing multiple holes, after some time, they would collectively focus on only one or two of these. The result was that a maximum of two exit holes were produced even when there were close to 150 males in a syconium half (Figure 3.2A).

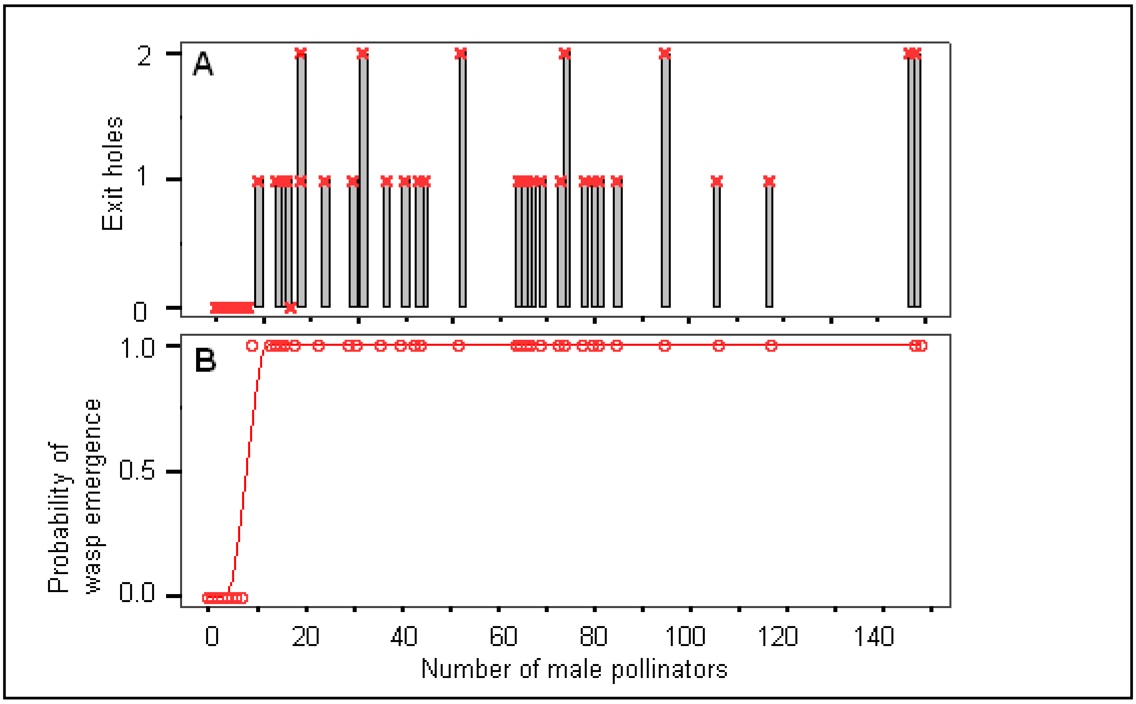


Figure 3.2 The number of emergence holes produced (A) and the likelihood of wasp emergence (B) as a function of male pollinator number.

3.5 Discussion

Syconia where wasps failed to exit had fewer and lower proportions of male pollinators than syconia where wasps exited normally. With less than about ten males, wasps are unlikely to emerge from these 'coffin figs' (Greeff 2002) as the probability of an exit hole being produced, decreases (Figure 3.2). Low numbers and proportions of males could be a consequence of (1) low foundress number (Herre 1985, 1987; Kinoshita *et al*. 1998) and/or (2) increased mortality through parasitism. As mature syconia were immersed in a solution of ethanol before they were dissected, foundress remains could not be distinguished from other female pollinator bodies within the cavity so their numbers could not be ascertained. A lower pollinator sex ratio coupled with reduced numbers of both male and female pollinator wasps in unexited syconia, however, suggests that foundress number may be an important factor in wasps failing to disperse from syconia.

When a foundress mother oviposits her offspring into syconia where she is the only occupant, she lays fewer males than if she were in a syconium where other females are also ovipositing because in the latter scenario, there is increased competition for mates (Local Mate Competition; Hamilton 1967). Unexited syconia may therefore reflect the situation where fewer foundresses have gained entry and consequently fewer males have been laid. As male pollinators cooperate to chew a single exit hole (personal observation), if too few males are laid then no wasps will emerge and the foundress mother(s) gains zero reproductive success.

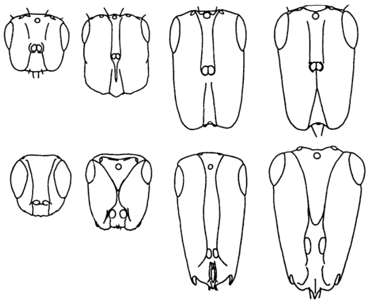
The second possibility for low male count is mortality through parasitism. Despite the proportion of all NPFWs being significantly higher in unexited syconia, neither the numbers nor the proportions of *P. agraensis* (the parasitoid of the pollinators), were significantly different across syconia suggesting that pollinating wasps were not more heavily parasitised in syconia where wasps did not emerge.

Although male pollinating wasps are crucial in the production of exit holes in syconia, some wasps may be released from their natal syconium by other means. Seed dispersers partially eating syconia (Handley *et al.* 1991; Kalko *et al.* 1996) and ripened fruit falling to the floor and breaking open may occasionally provide trapped wasps with some possibility of escape. Other insect fauna resident on *F. racemosa* (Yang *et al.* 2003) may also provide a more consistent avenue for wasp dispersal. Within the Lepidoptera, members of the Crambidae, Oecophoridae and Pyralidae families are known to feed internally on syconia. Moth and beetle larvae will often tunnel through syconia late in their development (Basset *et al.* 1997), leaving large holes in the syconium that could serve as enlarged exit holes for trapped wasps. *Ficus racemosa*, in particular, appears to have many more Lepidopteran larvae in its syconia than other fig species within the same geographical area (e.g. *F. rubiginosa*, *F. microcarpa*, *F. benjamina*, personal observation). The identity of these species, however and the effects of their larvae on the fig trees and their associated wasps have received almost no study (but see Sugiura and Yamazaki 2004). Despite this, recent work on *F. racemosa* in Townsville has identified three moth species whose larvae often temporarily inhabit its figs: *Autoba dispar* (Noctuidae, Acontiinae), *Cirrhochrista sp.* (Pyralidae, Pyraustinae), and *Tirathaba sp.* (Pyralidae, Galleriinae) (Lin 2007). At least one pyralid moth species (*Pachybotys spissalis*) is currently considered a predator of fig wasp larvae (Saguira and Yamazaki 2004), suggesting that some of these inhabitants may represent a double-edged sword to trapped fig wasps. Nonetheless, I have observed many wasps escaping through the holes made by these insects, and this does seem to provide some means of escape for wasps that would otherwise remain imprisoned.

To summarise, unexited *Ficus racemosa* syconia are most likely to be explained by low numbers of foundresses entering syconia and laying too few males to produce dispersal holes. The situation appears universally detrimental, leaving all developing larvae and the foundress pollinators with zero reproductive success and the fig with pollen that does not get dispersed. Entrance holes produced by other insects tunnelling through syconia may provide fig wasps with a viable means of escape on some occasions but this needs further study. More work is needed to assess their full impact on the fate of fig wasps trapped inside these syconia and how likely they are to act as indirect mutualists of the system.

Chapter 4

The impact of the internally ovipositing fig wasp, *Philocaenus barbarus* (Hymenoptera: Sycoecinae), on wasp and seed production in *Ficus burkei*



Morphological coevolution between pairs of unrelated pollinating (top) and internally ovipositing non-pollinating (bottom) fig wasps that enter syconia to reproduce (Adapted from van Noort and Compton 1996).

4.1 Abstract

Fig trees and their pollinating wasps are partners in a 60 million year old obligate mutualism; however, the interaction is usurped by non-pollinating fig wasps (NPFWs) that use *Ficus* ovules to reproduce. Although the vast majority of NPFWs oviposit from outside the fig wall, a subset enters syconia at the same time as the associated pollinators. These internally ovipositing non-pollinating fig wasps (INPFWs) and their impacts on the fig-pollinator system have been little studied. I investigate the influence of an internally ovipositing non-pollinating wasp, *Philocaenus barbarus*, on seed and pollinator wasp production in the African, *Ficus burkei*. Results indicate that *P. barbarus* offspring did not occur in the absence of the pollinator wasp, *Elisabethiella stuckenbergi*, and that its galls clustered at the fruit's centre. In their absence, *E. stuckenbergi* offspring occupied longer flowers than in syconia shared with *P. barbarus*. Whether parasitising pollinator larvae or competing for favoured inner ovules, the presence of *P. barbarus* had a negative impact on the male function of *F. burkei* (pollen dispersal), but did not affect its female function (seed production). I suggest the need for further work on this system to determine the trophic status of *P. barbarus*, and to better assess its impact on the pollinating wasps of *F. burkei*. Moreover, increasing studies on the impact of INPFWs are necessary to assess their overall influence on the fig/ fig-wasp mutualism.

4.2 Introduction

Fig trees (*Ficus*, Moraceae) and their agaonid pollinators (Chalcidoidea, Agaonidae) represent a 60 million year old obligate pollination mutualism (Janzen, 1979a; Weiblen, 2002; Cook and Rasplus, 2003; Herre *et al.* 2008). The wasps pollinate the fig trees and in return, the flowers within fig fruit (syconia) act as nurseries for developing fig wasp larvae. The system is obligate as neither fig tree nor pollinator wasp can reproduce in the absence of the other partner. The life cycle begins when female agaonid wasps enter receptive fig fruit (syconia) through a narrow slit called the ostiole. Coevolution in the morphologies of fig ostioles and their internally ovipositing wasps has resulted in females showing extreme adaptations for ostiole entry such as flattened heads and bodies, and mandibles covered in multiple rows of backward pointing teeth (van Noort and Compton 1996). Females that have successfully entered syconia transfer pollen onto the stigmatic surfaces of some flowers whilst also laying eggs down their styles. They lay as many eggs as possible before dying within the syconium. Weeks later, their offspring emerge and mate, and winged females collect pollen from their natal syconia before dispersing.

Alongside pollinating wasps, a suite of non-pollinating fig wasps (NPFWs) can also oviposit into fig ovules but generally provide no benefit to a fig's male function. Whilst the majority of NPFWs oviposit into *Ficus* ovules from outside the fig wall using telescopic ovipositors (Kerdelhué and Rasplus 1996), a minority enter syconia similarly to pollinating wasps and oviposit internally. With the exception of *Ceratosolen galili* in *F. sycomorus* (Compton *et al.* 1991) and *Eupristina sp.* in *F. altissima* (Peng *et al.* 2008), internally non-pollinating fig wasps (INPFWs) do not belong to the family Agaonidae and instead generally derive from the subfamilies Sycoecinae, Sycophaginae and Otitesellinae. Like pollinating agaonids, they possess morphological adaptations for ease of entry through the ostiole (van Noort and Compton 1996), and often enter syconia at the same time as pollinators (Galil *et al.* 1970).

Pollination by fig wasps can occur actively or passively. Where fig trees are passively pollinated, syconia have many male flowers and produce large quantities of pollen (Kjellberg *et al.* 2001). Once wasps have emerged from their galls, pollen is scattered throughout the syconium and is distributed on the bodies of the wasps where it is carried (passively) to new receptive syconia. Actively pollinated syconia in contrast, have a lower anther to ovule ratio (Kjellberg *et al.* 2001), produce far less pollen and have pollinators with specialised morphological adaptations (coxal combs and pollen pockets) involved in the collection and storing of pollen. Although INPFWs do not actively pollinate fig flowers (Jousselin *et al.* 2001a; Zhang *et al.* 2008b), where they are associated with passively pollinated fig species, they can often pollinate as well as (Zhang *et al.* 2008b), or even better than (Jousselin *et al.* 2001a) the 'legitimate' pollinator. Despite this, INPFWs still generally rely on the presence of the agaonid pollinator for their offspring to develop (Zhang *et al.* 2008a, b; but see Kerdelhué and Rasplus 1996).

Half of all known fig species are monoecious where male and female flowers and wasps and seeds develop together within syconia. Within these syconia, female flowers are variable in length (Figure 1.3A). Short flowers have ovules that develop close to the fig wall (outer flowers) whilst the ovules of long flowers develop close to the inner syconium cavity (inner flowers) (Ganeshaiah *et al.* 1995, 1999; Jousselin *et al.* 2001a, 2004). Ovipositing agaonid females favour inner flowers to lay their eggs over those closer to the fig wall (Nefdt and Compton 1996; Jousselin *et al.* 2001b; Weiblen 2002; Yu *et al.* 2004; Yao *et al.* 2005; Dunn *et al.* 2008b; Herre *et al.* 2008). Flowers that are pollinated but fail to receive a wasp egg (often outer flowers) develop into seeds. Monoecious fig species therefore often show stratification of gall occupants with fig wasps developing in long and intermediate length (inner) ovules whilst seeds develop in shorter, outer ovules. To my knowledge, no study has yet investigated the ovule lengths occupied by INPFWs. Because they enter receptive syconia at a similar time to pollinating agaonids (Cook and Rasplus, 2003; Zhang *et al.* 2008a, b), INPFWs may use the inner flowers preferred by pollinators and therefore compete directly with them for oviposition sites. Despite increasing studies on the impact of externally ovipositing NPFWs on the fig-pollinator mutualism (West *et al.* 1996; Kerdelhué and Rasplus 2006; Dunn *et al.* 2008b), studies on NPFWs that enter syconia to oviposit are still relatively rare, and their impact on the fig-pollinator system is even less well studied (but see Jousselin *et al.* 2001b; Zhang *et al.* 2008a, b).

The purpose of this paper is to measure the potential impact of INPFWs on the fig-pollinator interaction in the African fig, *Ficus burkei*. I measure differences in the morphology between the INPFWs and the associated agaonid pollinator, and compare the lengths of pollinator occupied flowers with those containing INPFWs, externally ovipositing parasitic NPFWs and seeds. I also measure the relative abundance of, and test for the influence of INPFWs on pollinator and seed development.

4.3 Materials and methods

4.3.1 Study species

Ficus burkei is evergreen or semi-deciduous and grows up to eight metres in height. It grows in savannah woodland and wet or dry forests, and is planted as a shade tree in sub-urban areas. It is a relatively widespread species, found from South Africa to Ethiopia and Senegal (Berg and Wiebes 1992). *Ficus burkei* is actively pollinated by *Elisabethiella stuckenbergi* Grandi (van Noort and Compton 1996); however, *Alfonsiella brongersmai* Wiebes and *A. longiscapa* Joseph also pollinate and oviposit among its flowers. A further 25 species of fig wasps are associated with *F. burkei* (Bouček *et al.* 1981). Two of these, *Crossogaster odorans* Wiebes and *Philocaenus barbarus* Grandi, are INPFWs. In the samples collected for this study, *E. stuckenbergi* was the only pollinator, and *P. barbarus* the only INPFW present. As *F. burkei* is actively pollinated (van Noort and Compton 1996), *P. barbarus*, having neither coxal combs nor pollen pockets, plays no significant role in pollination (Vovlas *et al.* 1998).

4.3.2 Collection of syconia and data recording

During January and February 2007, 28 early D-stage syconia (prior to wasp emergence; Galil and Eisikowitch 1968a) from four *F. burkei* trees in Grahamstown, South Africa were collected by JMC and DD. Immediately after collection, syconia were stored in a plastic container filled with 80% ethanol. In the laboratory, each syconium was carefully divided into eighths using a razor blade. For 18 syconia, each female flower was carefully removed with fine forceps and placed into a watch glass containing 100% ethanol for categorisation and measurement. The remaining 10 syconia were also sliced into eighths; however, only a quarter of all flowers were used for further analysis. To do this randomly, flowers were chosen by systematically removing every fourth flower, starting from one end of a syconium segment and then working towards the opposite end (see Dunn *et al.* 2008b for details). Actual and estimated counts of seeds and wasps using this method are unlikely to differ significantly (Kerdelhué *et al.* 1997). The length of each selected flower was measured to the nearest 0.01 mm using an eyepiece graticule mounted on a binocular microscope. Flower length was determined as the total flower length (pedicel + ovary or wasp gall) (Figure 1.3A) excluding what remained of the style. After measurement, each flower was assigned to one of four categories: (1) *Seeds*: pollinated flowers that had developed into seeds, (2) *Pollinator*: galls containing pollinator wasps (*E. stuckenbergi*), (3) *Parasite*: galls containing externally ovipositing NPFW species similar in size to pollinators and classed as parasitoids or inquilines (*Philotrypesis parca* and *Sycoryctes/ Sycoscapter spp.*), or (4) *Internally ovipositing non-agaonid*: galls containing the INPFW, *P. barbarus*. Not all syconia contained wasps from all categories (Table 1). My aim was to identify any spatial stratification of seeds, pollinators, externally ovipositing NPFWs and INPFWs, therefore I did not include galls containing large galling wasps or their parasites (Otiteselline, Epichrysomalline or Eurytomid wasps) in the data set.

I measured the total body length and the ovipositor length to the nearest 0.01 mm, for each *E. stuckenbergi* and *P. barbarus* female. Total body length was determined as the dorsal distance from between the mandibles to where the ovipositor joins the abdomen. The total length of the functional ovipositor (first and second valvulae) was measured after its removal from the abdomen (Nefdt and Compton 1996). Measurements were taken using an eyepiece graticule and microscope at 20x magnification. Syconium length and width were measured to the nearest 0.05mm using callipers, to enable syconium volume ($\frac{4}{3}\pi r^3$) to be estimated. Any wasps loose in the lumen (mainly male *E. stuckenbergi*) were counted.

4.3.3 Data analyses

The data from all syconia were pooled and then analysed using R statistical software (version 2.3.1.; R Development Core Team 2005). For tests involving counts, data from those syconia from which a quarter of flowers were sampled were quadrupled (Kerdelhué *et al.* 1997). To confirm the accuracy of this method, I created two sets of generalised linear models prior to the final analyses; one using only counts from whole syconia (N = 18), and the other using counts from all 28 syconia. In each case, the same general trends were apparent so I present results from the pooled dataset (N = 28 syconia). In all models 'site' was a random factor removing unmeasured variance that could be attributed to differences between localities (West and Herre 1994; Cook and Power 1996; Dunn *et al.* 2008a, b). Syconium volume was included in all models as a covariate. Where appropriate, models were simplified by backwards elimination by the removal of non-significant terms followed by a χ^2 test (where data were not overdispersed), or an F test (where overdispersion was present) to ensure that such removals did not significantly increase the residual error of the model (Crawley 2005).

4.4 Results

4.4.1 Spatial stratification of wasps

A generalised linear model identified that INPFWs (*P. barbarus*), pollinators (*E. stuckenbergi*), externally ovipositing non-pollinating wasps (NPFWs), and seeds were significantly spatially stratified within syconia ($P < 0.0001$, D.F. = 2717, proportion deviance explained = 0.10). In all instances, NPFWs occupied ovules closest to the outer wall of the syconium. *Philocaenus barbarus* offspring were not present in syconia that did not also contain *E. stuckenbergi* (Tables 4.1 and 4.2), and where they were present, ovules containing *P. barbarus* were longer than those containing *E. stuckenbergi* (Figure 4.1). Moreover, flowers containing *E. stuckenbergi* were shorter in syconia where *P. barbarus* was present compared to syconia where they were absent ($P < 0.0001$, D.F. =1337, proportion deviance explained = 0.10).

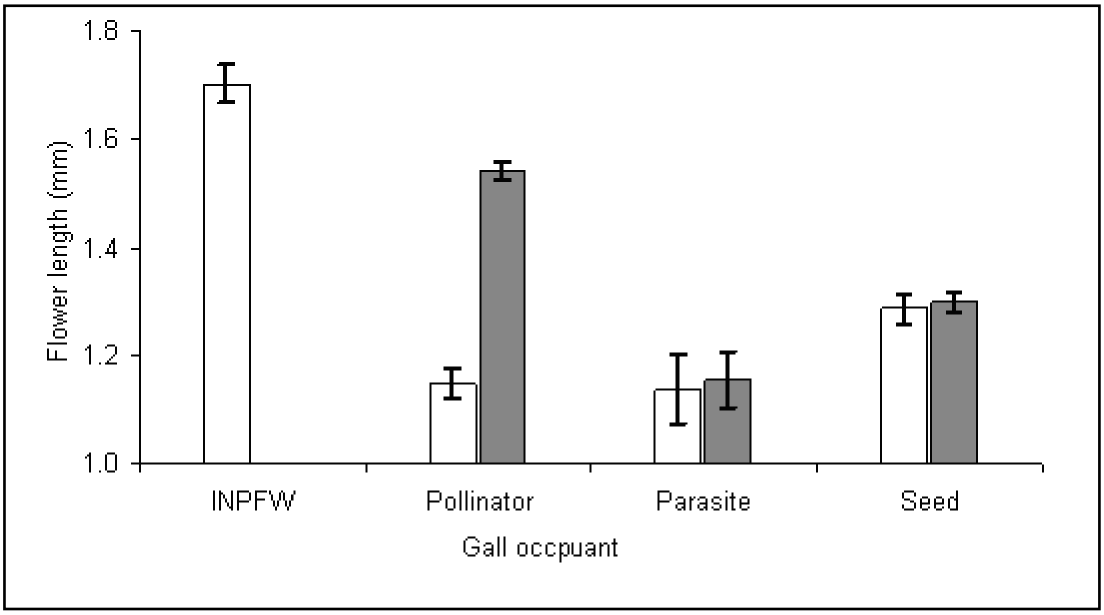


Figure 4.1 Mean (± S.E.) flower lengths for galls containing INPFWs (*P. barbarus*), pollinators (*E. stuckenbergi*), externally ovipositing parasites (*Philotrypesis parca* or *Sycoryctes/ Sycoscapter* spp.) and seeds. Coloured bars represent presence (white) or absence (grey) of *P. barbarus*.

4.4.2 Does P. barbarus influence pollinator and seed production?

I included syconia containing pollinators and/or INPFWs in a Freeman-Halton extension of the Fisher exact probability test for a 2x3 contingency table (Table 4.1). Results indicated that the observed numbers of syconia containing INPFWs and pollinators were significantly different to what would be expected if the wasps were not influenced by (i.e. were independent of) one another's presence (p<0.0001).

Table 4.1 Contingency table for the observed (and expected) number of syconia containing INPFWs and pollinators, with and without *P. barbarus*.

Syconia	Just INPFW	Just Pollinator	Both	**Total**
Without *P.barbarus*	0 (0)	21 (15.8)	0 (5.25)	**21**
With *P. barbarus*	0 (0)	0 (5.25)	7 (1.75)	**7**
Total	**0**	**21**	**7**	**28**

I also investigated in greater detail, the direction of the effect *P. barbarus* had on pollinator and seed production within syconia by constructing three generalised linear models with poisson (count data) and binomial (proportional data) error structures. The dependent variable for each model was: (1) the number of pollinator wasps per syconium, (2) the proportion of pollinator wasps out of all wasps in a syconium and 3) the number of seeds in each syconium. For each model, the presence/absence of *P. barbarus*, and site and syconium volume were used as the explanatory factor and as covariates respectively. Where models exhibited overdispersion, quasi-poisson and quasi-binomial error structures were used.

Syconia containing both *P. barbarus* and *E. stuckenbergi* had fewer wasps overall and fewer numbers and proportions of pollinators than syconia in which *P. barbarus* was absent (Figure 4.2). There was no difference in either seed production or total numbers of externally ovipositing NPFWs between syconia that contained *P. barbarus* and those that did not (Table 4.3).

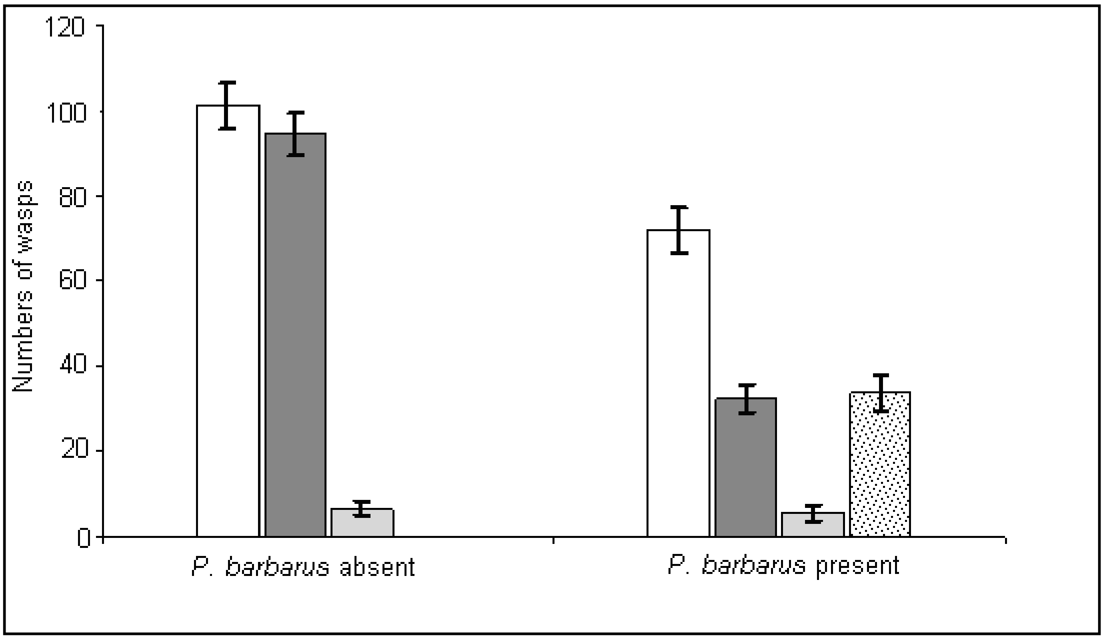


Figure 4.2 Numbers (± S.E.) of all wasps (white), pollinators (dark grey), externally ovipositing NPFWs (light grey) and internally ovipositing NPFWs (dotted) when *P. barbarus* was present or absent among syconia.

Table 4.2 Summary of wasps found in 28 *Ficus burkei* syconia. Each row represents possible combinations of pollinating wasps, externally ovipositing NPFWs and INPFWs in a syconium (I.e. row one represents the four syconia that only contained *E. stuckenbergi*. $\bar{x}$ ES for the first row represents the mean number of *E. stuckenbergi* in syconia containing only these wasps).

Wasp	Trophic status	Syconia (%)	$\bar{x}$ ES (s.e)	$\bar{x}$ PB (s.e)	$\bar{x}$ Para (s.e)
E.stuckenbergi (ES)	Pollinator	4(14.28)	103.75 (17.86)	-	-
P. barbarus (PB)	INPFW	0(0)	-	-	-
Philotrypesis/ Sycoscapter/ Sycoryctes (Para)	Externally ovipositing NPFW	0(0)	-	-	-
ES+PB		0(0)	-	-	-
ES+Para		17(60.71)	92.41(9.69)	-	8.18(3.63)
PB+Para		0(0)	-	-	-
ES+PB+Para		7(25)	32.43(4.38)	33.86(5.60)	5.71(2.41)
Seeds		28(100)	79.04(16.94)	8.46(7.92)	6.39(3.34)

Table 4.3 Results of generalised linear models illustrating the influence of *P. barbarus* presence *(p)* and absence *(a)* on wasp and seed numbers (N) and proportions (P) within syconia. (Para = *Philotrypesis/ Sycoscapter/ Sycoryctes* wasps, P.D.E. = proportion deviance explained, $\bar{x}_p$ = mean number/ proportion of wasps in syconia containing *P. barbarus*).

Variable	$\bar{x}_p$ (s.e)	$\bar{x}_a$ (s.e)	*P*	*t*	D.F.	P.D.E
Total wasps (N)	72 (5.32)	101.19 (5.35)	<0.01	-3.01	26	0.32
Pollinators (N)	32.43 (3.31)	94.57 (5.49)	<0.001	-7.04	26	0.71
Pollinators (P)	0.22 (0.02)	0.50 (0.03)	<0.001	-5.68	26	0.58
Para (N)	5.71 (1.82)	6.62 (1.59)	NS (0.84)	0.2	23	0.42
Seeds (N)	46.71 (7.53)	57.24 (6.68)	NS (0.44)	-0.79	34	0.25

Elisabethiella stuckenbergi females were smaller (mean body length ± s.e = 1.30 ± 0.004 mm) than female *P. barbarus* (mean body length ± s.e = 1.41 ± 0.007 mm; $F_{1, 956} = 138.14$, $P < 0.0001$). The result of an ANCOVA including body size as a covariate and species as a factor, however, found that *P. barbarus* females had shorter ovipositors than *E. stuckenbergi* females ($F_{1, 878} = 2672.41$, $P < 0.001$) (Figure 4.3).

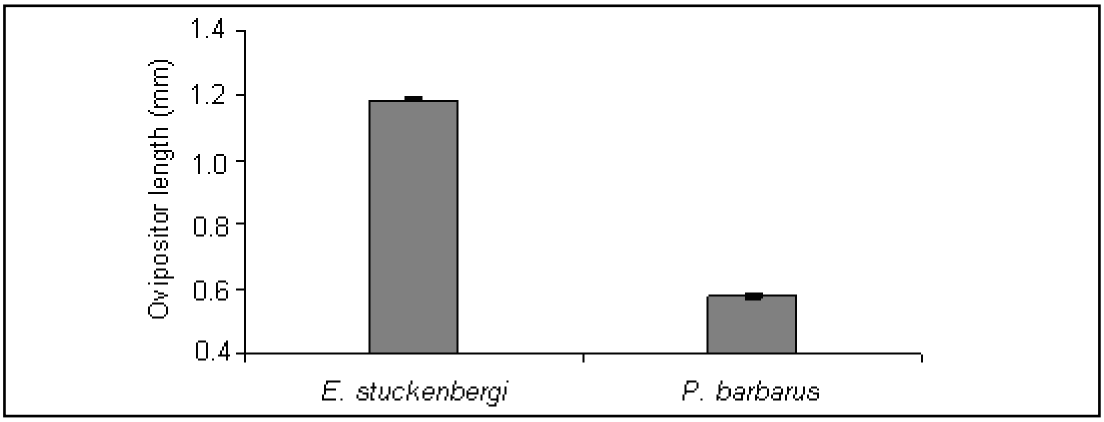


Figure 4.3 Mean (± S.E.) ovipositor lengths of female pollinators (*E. stuckenbergi*) and INPFWs (*P. barbarus*).

4.5 Discussion

Syconia containing *P. barbarus* had significantly fewer pollinating wasps and no significant difference in either the numbers of seeds or the numbers of externally ovipositing NPFWs. The pollinator, *E. stuckenbergi*, occupied shorter ovules closer to the fig wall when *P. barbarus* was present compared with syconia where it was absent. None of the collected syconia contained only *P. barbarus* wasps suggesting that it may rely on *E. stuckenbergi* for its offspring to successfully develop. It is possible that these results do not reflect a real pattern but are simply the result of a limited sample size; however, I consider this unlikely because similar patterns have been recently reported in other INPFW species (e.g. Zhang *et al.* 2008a, b). Consequently, I have assumed that *P. barbarus* is dependent on *E. stuckenbergi* entering *F. burkei* syconia, and that only syconia where both species co-habit, or syconia containing only *E. stuckenbergi*, develop fully. Given these assumptions, I propose two explanations for the results: 1) that *Philocaenus barbarus* is a gall-former but only enters syconia after entry by *E. stuckenbergi*, or 2) that it is the parasitoid of *E. stuckenbergi* larvae.

As a gall-former, the reasons *P. barbarus* may first require entry by *E. stuckenbergi* females may be two-fold: a) entry by the smaller *E. stuckenbergi* may help widen the ostiole to facilitate entry by *P. barbarus*, or b) only syconia where at least some flowers have been pollinated do not abort, and/or only these syconia nurture *P. barbarus* larvae. In *F. curtipes*, the INPFWs *Diaziella yangi* and *Lipothymus sp.* do not enter syconia if it has not been entered by the associated agaonid, *Eupristina sp.* (Zhang *et al.* 2008b). If, however, the head of a female *E. sp.* is allowed to enter the ostiole and is then immediately removed, *D. yangi* and *L. sp.* will enter syconia immediately (although their offspring will fail to develop). This suggests that entry may be dependent on the presence of a chemical pheromone around the ostiole, or through the physical enlarging of the ostiole by the agaonid (Zhang *et al.* 2008b). In *F. burkei*, a gall-forming *P. barbarus* may enter syconia at roughly the same time, or after pollinator entry. If entry by *P. barbarus* females occurs before pollination, there is a risk that an *E. stuckenbergi* female may not enter and a) the syconium may then abort (Marussich and Machado 2007, but see Bronstein 1991; West *et al.* 1996), or b) not all *P. barbarus* offspring will develop fully (Herre and West 1997; Jousselin *et al.* 2003b). Given that seed set was unaffected by the presence of *P. barbarus*, it is unlikely that *P. barbarus* enters syconia after *E. stuckenbergi* as I would expect more (not fewer) emerging wasps, and fewer seeds among syconia where both species co-habited syconia compared to those where only *E. stuckenbergi* entered. If, however, *P.barbarus* enters syconia at roughly the same time as *E. stuckenbergi*, there may be increased pseudo-interference (i.e. decreased search efficiency as a result of increased wasp density) which could delay the oviposition rate of females. Moreover, being larger in total body size, *P. barbarus* may physically interfere with *E. stuckenbergi* oviposition, reducing the number of eggs each pollinator lays and the consequent number of offspring that emerge from each syconium (Ramirez 1970a; Gibernau *et al.* 1996; Moore and Greeff 2003).

An alternative explanation for the results is that *P. barbarus* is not a gall-former, but a parasite (parasitoid or inquiline) of *E. stuckenbergi*. The lack of an increase in seed number in syconia containing *P. barbarus*, coupled with a failure to find syconia containing only this INPFW provides good evidence that *P. barbarus* may rely on galls containing pollinator larvae in order to reproduce. That *E. stuckenbergi* offspring were located in longer-styled flowers when they co-habited syconia with *P. barbarus* may represent those flowers that occupy enemy-free space from *P. barbarus* ovipositors. Recent work by Dunn and others (2008b) identified that externally ovipositing parasitoids can be indirect mutualists of the fig/ wasp system by encouraging foundresses to show a preference for inner flowers where their offspring occupy a spatial refuge from parasite attack. The mutualistic impact of NPFWs on the system arises from a pollinator's relative avoidance of outer flowers, facilitating seed development. Like NPFWs, INPFWs may also affect the value of ovules to ovipositing pollinators. If *P. barbarus* wasps are parasites of *E. stuckenbergi,* ovipositing pollinators may avoid the innermost ovules to reduce the risk of parasitism. Through the avoidance of outer ovules (from parasitism by externally ovipositing NPFWs) and inner ovules (from parasitism by INPFWs), intermediate-length flowers may have the lowest overall likelihood of larval parasitism among pollinators. The positioning of *Elisabethiella stuckenbergi* larvae in intermediate length ovules may therefore reflect 1) an active avoidance of inner (and outer) ovules by ovipositing foundresses, or 2) the resultant positions of unparasitised larvae that occupy a refuge from their parasites that are unable to reach them. These results alone cannot differentiate between the two possibilities.

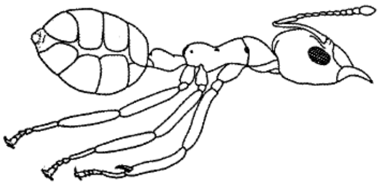
Further experiments are necessary to elucidate the full impact of *P. barbarus* on *E. stuckenbergi* females. Introducing recently emerged *P. barbarus* into receptive syconia and immediately bagging them to prevent further entry would identify whether or not *P. barbarus* can develop in *F. burkei* ovules in the absence of *E. stuckenbergi*. If syconia abort, the introduction of *E. stuckenbergi* females with their ovipositors removed would elucidate whether *P. barbarus* is simply a gall-former or a parasite of *E. stuckenbergi* larvae. Field observations on the order of entry of *E. stuckenbergi* and *P.*

barbarus females, along with their oviposition behaviour in halved syconia would clearly help to establish whether *P. barbarus* uses its larger size to physically interfere with *E. stuckenbergi* during oviposition. Finally, the apparent competition for the same subset of ovules suggests that *P. barbarus* also displays a preference for short-styled flowers. This may be because a) short-styled flowers are easier to oviposit into (Verkerke 1986, 1988), b) short-styled ovules are the only ones accessible as *P. barbarus* wasps posses shorter ovipositors than *E. stuckenbergi* (i.e. it reflects a morphological constraint and could potentially provide one of the few real examples of the short ovipositor hypothesis (Ramirez 1970a)), and/ or c) because *P. barbarus* offspring are also parasitised by externally ovipositing NPFWs and offspring laid in short-styled flowers are at lower risk from parasite attack (Dunn *et al.* 2008b).

In conclusion, the constant rate of seed production regardless of the presence of *P. barbarus* suggests that this INPFW imposes no seed production costs on *F. burkei* syconia and that its presence does not lead to any increase in pollination services over and above that provided by the ‘legitimate’ pollinator. This concurs with some other INPFW species (Zhang *et al.* 2009) but is contrary to others (Jousselin *et al.* 2001b). Although I have established that *P. barbarus* has a negative effect on *E. stuckenbergi* pollinators, further work is needed to determine its trophic status and to clarify the full extent of its impact on the system.

Chapter 5

Invasion of the green body snatchers: how *Oecophylla smaragdina* reduces parasitism in *Ficus racemosa*



Oecophylla sp. worker (Weber 1946)

5.1 Abstract

Figs and fig wasps form a classic mutualistic system, but this is also influenced by interactions with other species. Fig wasps are often predated upon by ant workers resident on fig trees, but predatory ants may also have positive effects on the fig/wasp mutualism as they can discourage externally ovipositing non-pollinating fig wasps (NPFWs) from stopping on the surface of the fig fruit (syconia) to lay eggs. I tested whether patrolling by the green tree ant, *Oecophylla smaragdina*, decreases the proportions of non-pollinating gall-formers and parasitoids emerging from *Ficus racemosa* syconia.

Syconia treated with Tanglefoot to exclude ants nurtured increased proportions of NPFWs and reduced proportions of pollinators. Survey data also highlighted a significant negative correlation between ant density and the proportion of emerging parasitoids. This negative influence of *O. smaragdina* on numbers of developing NPFWs suggests that they may be considered indirect mutualists of the fig/ pollinator system by reducing parasitism in *F. racemosa* syconia. Both the ubiquity of ants in the tropics and their presence on fig trees suggests that the dynamics of fig/ fig wasp interactions may often be influenced by these third-party mutualists.

5.2 Introduction

The fig/ wasp mutualism represents over 60 million years of stability (Rønsted *et al.* 2005) between the pollinating fig wasps; a fig tree's sole pollen disperser, and the flowers within fig fruits (syconia), which are the only places where wasp offspring can develop. The life cycle begins on fig trees where young buds develop into larger syconia which are receptive to pollinating wasps. Each syconium is completely enclosed with the exception of the ostiole - a tiny hole which opens briefly to enable wasp access. Female pollinators that enter syconia pollinate and oviposit among the flowers. They die inside the syconium and their offspring develop by galling fig ovules. Non-pollinating fig wasps (NPFWs) also lay their eggs into fig flowers; however, most species do so from outside the syconium and do not contribute to pollination. Some NPFWs will oviposit in empty ovules which they gall themselves (gall-formers), whilst others (inquilines or parasitoids) oviposit into occupied ovules. Weeks later, once fully developed, wasps emerge from their galls and mate, and male pollinators tunnel holes through the fig wall, facilitating wasp dispersal.

Fig trees are home not only to fig wasps, but also to arboreal ant species that, as foraging opportunists, are attracted to the wasps (Bronstein 1988b; Compton and Robertson 1988, 1991; Dejean *et al.* 1997; Schatz and Hossaert-McKey 2003; Schatz *et al.* 2006). During syconium receptivity and wasp emergence, fig wasps are abundant outside syconia and are vulnerable to predation by patrolling ants (Bronstein 1988b; Compton and Robertson 1988; Schatz and Hossaert-McKey 2003; Schatz *et al.* 2008). There is growing evidence, however, that predatory ants reduce parasitism within syconia by discouraging externally ovipositing non-pollinating fig wasps from prolonged oviposition (Schatz *et al.* 2006). Cushman and others (1998) studied the African *F. sur* where the presence of a homopteran attracted ants which, in turn, reduced the numbers of gall-formers and parasitoids emerging from syconia. Another study investigated the effects of ants on the numbers of NPFWs landing on *F. condensa* (in Brunei), *F. carica* (France) and *F. racemosa* (India) (Shatz *et al.* 2006). Whilst the former study involved a homopteran-tending ant species and an African fig species, the latter only investigated the numbers of parasites observed on the surface of syconia rather than their actual numbers developing within the fig ovules. This paper investigates whether densities of the non-tending predatory ant species, *Oecophylla smaragdina*, on *F.racemosa* syconia 1) vary with syconial development under natural conditions to coincide with periods of wasp abundance, and/or 2) influence the proportions of pollinating and non-pollinating fig wasps emerging from syconia.

5.3 Materials and methods

Observations and experiments were performed on *Ficus racemosa* trees between September and December 2008 at five sites around Townsville, Australia (19° 15' 0 S, 146° 48' 0 E). *Ficus racemosa*, also known as the cluster fig, is so-called as its fruit grow in bunches from thin branches (racemes) that protrude from the trunk and primary branches of the tree. It is pollinated by *Ceratosolen fusciceps*; however, its flowers are also home to five species of non-pollinating fig wasps (NPFWs) that oviposit through the fig wall from outside the syconium: *Platyneura mayri, P. testacea, P. agraensis*, *Apocrypta westwoodi* and an undescribed *A. sp.* 2. *Platyneura testacea* and *P. mayri* are gall-formers whilst *Apocrypta sp. 2* is a parasitoid of *P. testacea* (Wang and Zheng 2008). These three species oviposit into ovules before syconia have been entered by pollinators (Kerdelhué *et al.* 2000; Weiblen 2002, Wang and Zheng 2008). *Apocrypta westwoodi* and *P. agraensis* oviposit last, doing so into occupied ovules after syconia have been pollinated. *Apocrypta westwoodi* is considered the parasitoid of *P. mayri* whilst *P. agraensis* parasitizes *C. fusciceps* larvae (Wang and Zheng 2008).

The dominant and most prevalent ant species resident on the trees used in this study was the green tree ant, *Oecophylla smaragdina*, also commonly known as the Asian weaver ant. Workers use silk extracted from their larvae to weave together leaves that form their characteristic tree top nests (Hölldobler and Wilson 1990). With a diet composed mainly of small insects (Way and Khoo 1992; Offenberg *et al.* 2004), and as efficient ambush predators, weaver ants are aggressive deterrents to any intruder and are often used as biocontrol agents of food crop pests (Way and Khoo 1991; Peng and Christian 2004; Van Mele *et al.* 2002).

5.3.1 Do numbers of O. smaragdina vary with syconium development?

I recorded i) the maximum diameter of each syconium, ii) the number of *O. smaragdina* workers observed during 10 seconds and iii) the number of days since the first observation of a focal syconium, for each of 194 receptive (pre-pollination) syconia (average diameter=1.50cm ±0.04) on 10 *F. racemosa* trees. Daily visits were made to each syconium between 0600-1200h until syconia were at wasp dispersal stage (D-stage). Records of the day of observation enabled fruit of the same developmental stage to be grouped together. Fully developed syconia were collected just before wasp emergence and were used in the survey data below. Where wasp emergence occurred before syconia had been harvested, measurements were continued until syconia dropped from the trees and allowed ant densities to be observed post-emergence.

5.3.2 Does O. smaragdina influence proportions of emerging fig wasps?

a) Natural conditions (survey data):

Of the original 194 syconia, only 69 developed to wasp dispersal stage (D stage, Galil and Eisikowitch 1968a). The remainder suffered premature abortion (N=59), were invaded by moth larvae which precluded further development (Bronstein 1988b) (N=2), or were picked off by passers-by (N=37).

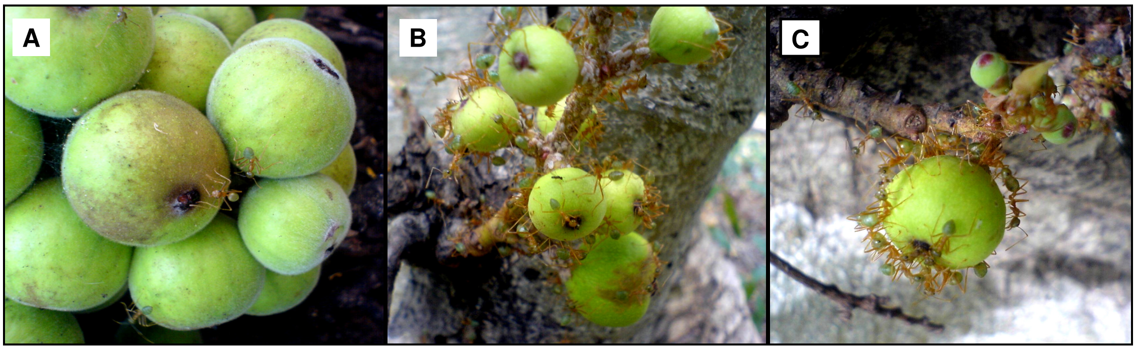


Figure 5.1 Variation in ant abundance in weakly (A) and more heavily (B, C) ant patrolled *F. racemosa* syconia. Photographs taken by S. Al-Beidh.

b) Ant exclusion experiments

For ant exclusion trials, I selected 21 syconia from four trees that were not included in the previous data set. Closely situated pairs of pre-receptive syconia were identified and surrounding non-experimental fruit were removed. One syconium from each selected pair (N=10) was left to develop naturally (control), whilst the other (N=11) was treated with Tanglefoot. Tanglefoot is a sticky, non-toxic substance that is effective at excluding ants (and other walking predators) on trees (Stephenson 1982; Smiley 1986; Mackay 1991; Bishop and Bristow 2003; Reithel and Billick 2006). For each ant-excluded syconium, Tanglefoot was applied onto a collar of card that had been taped around the raceme attaching the focal syconium. Reapplication of Tanglefoot was made as and when necessary and daily visits ensured that ants were successfully excluded from treated syconia for the duration of the experiment.

5.3.3 Collection and classification

Syconia from both survey data and ant exclusion trials were harvested just before wasp dispersal and their maximum diameters were recorded before placing them in mesh topped containers to collect emerging wasps (typically 24-48 hours). Wasps were then killed in 80% ethanol and syconia were dissected to collect any wasps still trapped in galls. All wasps were identified and classified into one of four groups: i) pollinators (*Ceratosolen fuscipeps*), ii) gall-formers (*P. mayri* and *P. testacea*), iii) parasitoids of pollinators (*P. agraensis*), and iv) parasitoids of gall-formers (*A. westwoodi* and *A. sp. 2*). Male *Platyneura* wasps could not be separated into the above groups by eye and were assigned according to the relative abundance of females.

5.3.4 Data analysis

Data were analysed using R statistical software (version 2.3.1; R Development Core Team 2005). Generalised linear models with binomial error structures were used to investigate the effect of *Oecophylla smaragdina* workers a) under natural conditions and b) using ant exclusion trials. The proportions of emerging wasps within syconia were defined as the response variables whilst ant density and ant presence/ absence were used as the explanatory variables for natural and ant exclusion trials respectively. Site and fig volume ($\frac{4}{3}\pi r^3$) were included as covariates in all models to control for differences across sites and syconia, respectively.

5.4 Results

5.4.1 Do ant numbers vary with syconium development?

Numbers of *O.smaragdina* workers varied significantly with syconium development, displaying a bimodal pattern in ant abundance (Figure 5.2A). Peak numbers of ant workers were observed on days 3-9 and 31-38 (P<0.05 and P<0.0001 respectively, proportion deviance explained =0.52, D.F. =3292) with densities on days 31-38 being significantly higher than those on days 3-9 (P<0.001, proportion deviance explained =0.52, D.F. =3292). Syconium diameter was also correlated with ant density, with syconia 1.3cm and 3.6cm in diameter associated with peak ant abundance (p<0.0001, proportion deviance explained =0.01, D.F. =3305) (Figure 5.2B).

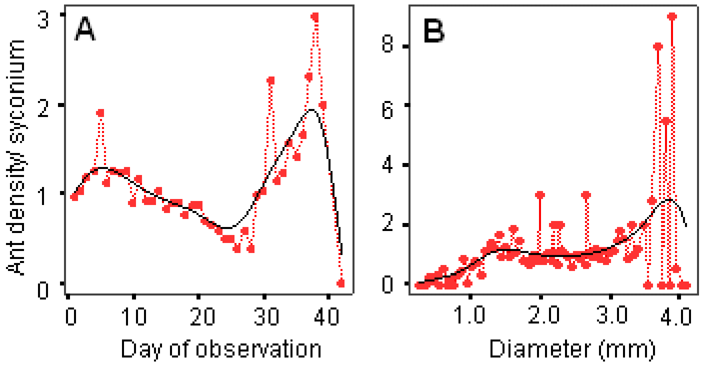


Figure 5.2 Variation in ant density with day of observation (A) and syconium size (B).

5.4.2 Does O. smaragdina influence the proportion of emerging fig wasps?

a) Natural conditions (survey data)

Ant density was negatively correlated with the proportions of emerging parasites (*P. agraensis* and *Apocrypta spp.*), but did not significantly influence either the proportions of gall-formers (*P. mayri and P. testacea*) or pollinating wasps emerging from syconia. The pollinator sex ratio, however, was positively associated with ant density (Table 5.1).

Table 5.1 The effect of ant density on emerging proportions of pollinating and non-pollinating fig wasps (P.D.E = proportion deviance explained).

Wasps	***P***	**D.F.**	**P.D.E**
P. agraensis	<0.01	52	0.28
Apocrypta spp.	<0.001	64	0.67
P. mayri & P. testacea	NS (0.57)	60	0.47
Pollinators	NS (0.08)	61	0.62
Pollinator sex ratio	<0.05	59	0.32

b) Ant exclusion experiments

The proportions of all emerging NPFWs were significantly higher in ant absent (Tanglefoot-treated) than ant present (control) syconia (Figure 5.3). In contrast, the proportion of emerging pollinating wasps was significantly lower among syconia where ants had been excluded compared with controls. The pollinator sex ratio was not significantly different between treatments (Table 5.2).

Table 5.2 The effect of ant absence/ presence on emerging proportions of pollinating and non-pollinating fig wasps (P.D.E =proportion deviance explained).

Wasps	***P***	**D.F.**	**P.D.E**
P. agraensis	<0.01	19	0.35
Apocrypta spp.	<0.05	11	0.75
P. mayri & P. testacea	<0.001	12	0.92
Pollinators	<0.0005	16	0.90
Pollinator sex ratio	NS (0.69)	14	0.01

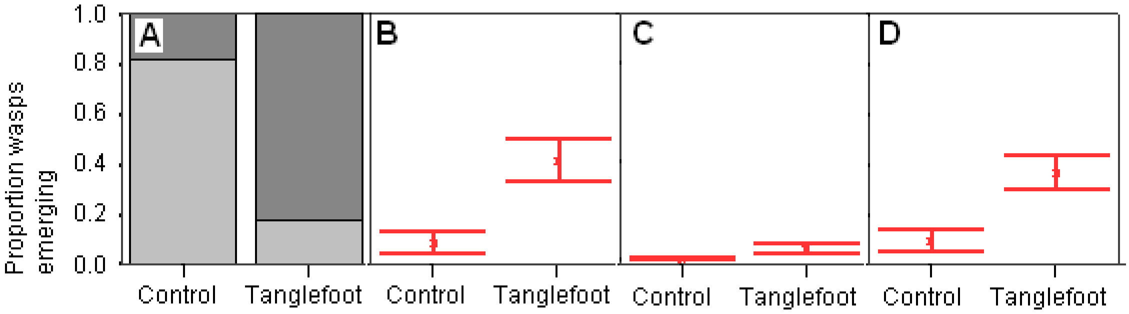


Figure 5.3 The influence of ant presence (controls) and absence (Tanglefoot) on the proportion of emerging pollinators (light grey) and all NPFWs (dark grey) (A), and their impact on individual NPFW groups; (B) gall-formers (*P.mayri* and *P. testacea*), (C) parasitoids of pollinators (*P.agraensis*) and (D) parasitoids of gall-formers (*Apocrypta westwoodi* and *A. sp.2*) emerging from syconia. Error bars represent means ± 1 S.E.

5.5 Discussion

The number of *O. smaragdina* workers varied significantly with both the day of observation and syconium size. Syconia 1.3cm and 3.6cm in diameter, and observation days 3-9 and 31-38 were associated with peak ant abundance. These diameters fall into *F. racemosa* species averages for receptive and pre-emergence stages (Zhang *et al.* 2006) and the observation days associated with peak ant densities (days 3-9 and 31-38) are also likely to correlate with these developmental stages. Significantly more ants were recorded in the late (days 31-38) compared with the early (days 3-9) days of observation which is likely to reflect prey availability as hundreds more wasps emerge from syconia compared with the (relatively) few foundresses that enter them during receptivity.

Recent evidence suggests that ants are able to predict when wasp entry and emergence are imminent using the fig volatiles emitted by syconia immediately preceding periods of peak wasp activity (Ranganathan and Borges 2009). Such ‘eavesdropping’ of fig volatiles by non-intended receivers has been documented among *Oecophylla smaragdina* workers on *F. racemosa* syconia where, in a series of choice tests, workers responded positively to volatiles from receptive and wasp dispersal stage syconia, but were indifferent to volatiles from pre-receptive and interfloral stages (Ranganathan and Borges 2009). This implies that ant workers are able to ‘sense’ imminent prey availability, a suggestion reiterated by field observations where workers were observed in ambush positions (i.e. facing the ostiole) just prior to wasp entry (receptive phase) and emergence (dispersal phase).

In addition to peak ant densities coinciding with periods of peak wasp abundance; using a combination of survey data (natural conditions) and ant exclusion experiments, results also suggested that *O. smaragdina* workers significantly influenced the proportions of fig wasps emerging from syconia. Ant-excluded syconia nurtured fewer pollinators and more NPFWs than ant patrolled syconia; however, even before syconia were harvested, the impact of Tanglefoot application on numbers of ovipositing NPFWs was clearly visible in the field. Ant excluded syconia acted as hotspots for ovipositing NPFWs whilst closely situated controls were actively avoided. The landing behaviour of pollinating and non-pollinating fig wasps was also influenced by ant presence, with wasps hovering above ant patrolled syconia, apparently hesitating, before landing (see also Schatz *et al.* 2008) whilst no such behaviour was observed surrounding ant absent syconia.

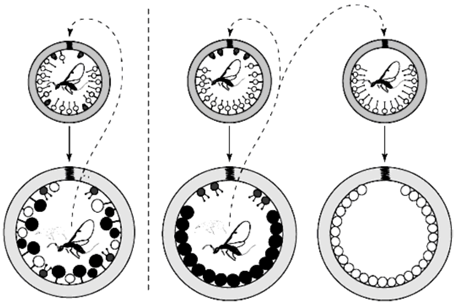
Under natural conditions, ant densities were negatively correlated with the proportion of emerging parasitoids, but they did not influence the proportions of emerging pollinators or gall-formers. Pollinating wasps enter ostioles in a matter of seconds and, as they lay their eggs within the relative sanctuary of the inner lumen, the impact of externally patrolling ants is unlikely to be high. Parasites (gall-formers and parasitoids), in contrast, oviposit externally and spend significantly more time on the surface of syconia. Parasitoids must also oviposit into already occupied ovules and do so later than most herbivorous gall-formers. Consequently, as ant numbers are elevated during syconium receptivity, NPFWs ovipositing post-pollination (predominantly parasitoids) may be more prone to predation than those ovipositing before pollinator entry (gall-formers). Moreover, *P. agraensis*, the parasitoid of the pollinator, has a relatively longer ovipositor (ovipositor / femur length) than the other fig wasp species associated with *F. racemosa* (*Apocrypta spp.* average =8.35±0.10, *P. mayri/ P. testacea* average =13.68±0.16; *P. agraensis* average =25.13±0.00, *C. fusciceps* average =6.04±1.03, S. Al-Beidh unpublished data). It is likely to be especially vulnerable to predation by patrolling ants as it takes longer to insert, and retract its ovipositor from ovules than do the other non-pollinating species equipped with shorter ovipositors (personal observation).

Interestingly, the pollinator sex ratio was positively associated with ant density under natural conditions, becoming less female-biased with increasing ant presence. This could suggest three things 1) that more foundresses gain entry into more heavily ant patrolled syconia (more ovipositing foundresses lay less female-biased sex ratios than syconia entered by a single foundress: Frank 1985; Herre 1985, 1987; Kinoshita *et al.* 1998; Pereira and Prado 2006), 2) that the same number of foundresses gain entry to heavily ant patrolled syconia but they all lay less female-biased sex ratios, or 3) that ovipositing NPFWs on heavily ant patrolled syconia parasitize relatively more female pollinator occupied ovules than those ovipositing on syconia with fewer ant patrols. As neither of the first two options seem likely, I turn my attention to the third possibility. In monoecious fig species like *F. racemosa*, style lengths are continuously distributed. Wasps laid in short-styled flowers (inner ovules) develop closer to the inner cavity whilst those laid in long-styled flowers (outer ovules) develop closer to the outer wall (Figure 1.3A). Inner ovules are therefore less accessible to the ovipositors of externally ovipositing NPFWs and are favoured by ovipositing foundresses (Dunn *et al.* 2008b; Chapter 2: this thesis). As fig wasps lay strongly female biased sex ratios, the loss of a son is relatively more ‘costly’ than the loss of a daughter. Moreover, as males are crucially important in mating and releasing females from their syconium, syconia with too few males nurture wasps that are condemned to death without dispersal (Chapter 3: this thesis). Foundresses should therefore position their sons in ‘higher value’ inner ovules where they are less likely to be parasitized. Non-pollinators ovipositing among heavily ant patrolled syconia may be under pressure to lay their eggs quickly due to the risk of predation. Consequently, they may save time by ovipositing into ovules closest to the fig wall and in so doing, will parasitise female pollinator offspring more often than males. That female pollinators place their sons and daughters in different positions within syconia is an idea that has been little studied in the literature (but see Zavodna *et al.* 2005). Sex-specific positioning of offspring based purely on the basis of style length has received even less attention and may provide a fruitful avenue for further research. Having said this, ant exclusion trials failed to highlight an effect of ant presence/absence on the pollinator sex ratio. This may be because where controls and Tanglefoot syconia were in close proximity to one another, NPFWs avoided syconia with ants altogether in favour of those where ants were absent. Consequently, NPFWs would not have needed to lay their eggs as quickly as those ovipositing among heavily ant patrolled syconia. Alternatively, it could suggest that the result from the survey data was driven by an unmeasured variable rather than the influence of ants per se. Further studies are needed to identify which is the case.

In conclusion, numbers of *O. smaragdina* workers followed a bimodal pattern, peaking during periods of syconium receptivity and wasp emergence. Ant presence negatively influenced the proportions of emerging NPFW parasitoids and in so doing, positively influenced proportions of emerging pollinators. *Oecophylla smaragdina* workers can therefore be considered indirect mutualists of both *F. racemosa* trees (by increasing their pollen dispersing function), and pollinating fig wasps (by reducing parasitism among their larvae). Given that ants are present on many fig species, further work is needed to elucidate whether they can be considered more generally as third-party mutualists across multiple fig/ wasp systems.

Chapter 6

Fig breeding system influences the trajectory of fig/ wasp morphological coevolution



The differences in the relative allocation of wasps and seeds in monoecious (left) and dioecious gall (centre) and seed (right) syconia. (Adapted from Cook and Rasplus 2003)

6.1 Abstract

Despite fig trees and their pollinating wasps being obligate mutualists that rely on one another to reproduce, their reproductive interests are not identical. I focus on the conflict of interest over the development of seeds inherent among monoecious *Ficus* but resolved in dioecious species. Whilst controlling for phylogeny, I incorporate 87 fig species in analyses to observe whether fig trees, their pollinating wasps and their associated parasitoids exhibit differences in their morphology that are correlated with the fig reproductive system.

Across all species, fig species with larger syconia had more flowers, bigger galls, larger pollinators, parasitoids with longer ovipositors and thicker fig walls than species with smaller syconia. Some aspects of fig and wasp morphology were, however, also influenced by fig breeding system (monoecious versus dioecious). Pollinating wasps of larger monoecious syconia had 1) more elongate heads and 2) longer ovipositors than those associated with smaller syconia. These effects were not significant among pollinators of dioecious fig species because a) the ostioles of dioecious syconia are easier to penetrate and b) because pollinators of dioecious fig species do not need longer ovipositors as all styles in male syconia are short. Moreover, larger dioecious, but not monoecious fig species had 1) disproportionately thick fig walls to minimise parasitism of pollinator larvae (as parasitoids provide no apparent mutualistic function in dioecious species), and 2) parasitoids with disproportionately long ovipositors in order to access their hosts in these deeper ovary layers. I discuss in detail how these results relate to the conflict over the relative allocation of fig flowers to wasp and seed production that differs with the fig reproductive system.

6.2 Introduction

Natural selection, the process which ‘selects’ against traits that are detrimental to an individual’s reproductive success leaving only those that are beneficial (Darwin 1859), is responsible for most of the adaptations that we can observe in organisms today. Every currently expressed trait represents the outcome of a trade-off between the costs and benefits incurred during its development. Where these costs and benefits have been reciprocally influenced by two or more interacting species, the trait is said to have coevolved, and the process is termed ‘coevolution’ (Janzen 1980; Futuyma and Slatkin 1983).

The interaction between *Heliconius* butterflies and their *Passiflora* host plants is a classic example of coevolution (Ehrlich and Raven 1964). *Passiflora* species contain both toxic alkaloids and cyanogenic glycosides which can deter even the most specialised of herbivores (Hay-Roe and Nation 2007). One group that has, however, overcome this defence are the larvae of *Heliconius* butterflies that are laid on the leaves of the passionflower vines. The toxins in the leaves are sequestered in the bodies of the larvae, making them unpalatable to predatory birds and lizards. To reduce herbivory, a number of *Passiflora* species have hooked hairs called trichomes that impale caterpillars (MacDougal 1994), however, some herbivores (*Heliconius charithonia* and *Dione moneta*) have developed mechanisms to avoid getting speared (Benson *et al.* 1975; MacDougal 1994; Cardoso 2008). Other, less direct anti-herbivore tactics rely on the fact that competition on leaves causes cannibalism among larvae. Egg mimicry, where leaves are dotted with yellow spots, and leaf shrivelling, where leaves mimic larval infestation, are two mechanisms employed by some passionflowers to discourage oviposition by female heliconids (Hochberg and Van Baalen 1998; Ulmer and MacDougal 2004). Such measures and counter measures between the passionflower hosts (to limit oviposition) and their associated *Heliconius* butterflies (to counteract these defences) have escalated into a coevolutionary arms race that has been well documented in the literature (Gilbert 1971, 1975; Smiley 1978; Mugrabi-Oliveira and Moreira 1996; Engler *et al.* 2000). Despite a plethora of academic papers describing coevolutionary arms races between interacting species (Anderson and May 1982, Minchella 1985; Hochberg *et al.* 1992; Forbes 1993; Lafferty 1993; Sorci *et al.* 1997), however, far fewer studies have explored mutualistic interactions in such detail.

Fig trees (*Ficus*, Moraceae) and their pollinators (Agaonidae, Chalcidoidea) represent an extreme type of mutualism where neither partner can reproduce in the absence of the other. The mutualism is obligate as fig trees provide the only egg-laying sites for the pollinating fig wasps, whilst the wasps are the sole dispersers of a fig tree's pollen. As each fig species is pollinated by species-specific wasps, partners display extreme specialisations associated with the interaction. Coupled with the fact that there are over 700 pair-wise interactions between fig trees and their associated wasps (Berg 1989), the fig/ fig-wasp mutualism provides evolutionary biologists with an excellent system for coevolutionary study.

The fig-wasp lifecycle begins during a brief receptive period early on in the development of each inflorescence (syconium) when volatile chemicals are emitted from a narrow tunnel (the ostiole) located at its apex. The chemicals attract pro-ovigenic female pollinators that are laden with pollen from their natal fig (Grison-Pigé *et al.* 2002; Ware *et al.* 1993). The females possess extreme morphological adaptations in order to gain entry into inflorescences (Figure 1.2) and once inside, these 'foundress' wasps pollinate and oviposit among the female flowers for the rest of their brief lives (Galil and Eisikowitch 1969). Pollinated ovules that have not received an egg become seeds whilst those containing an egg nurture the developing wasp larvae. Fully developed wasps emerge from their galls a few weeks later and mate. Female pollinators collect pollen from mature male flowers, and male pollinators chew holes through the syconium wall to enable the mated females to disperse in search of new receptive syconia.

Despite this reproductive dependence, fig trees and their pollinators have conflicting reproductive interests. Each pollinator larvae consumes the endosperm of a potential seed as it develops (Galil and Eisikowitch 1968a, b). Consequently, for monoecious species, where wasps and seeds develop within the same inflorescence, the development of each wasp reduces seed set by one. As fig trees benefit from the development of both seeds and wasps whilst wasps have no short-term interest in seed production, the scene is set for a coevolutionary arms race between partners; monoecious fig trees must limit wasp entry to allow some seeds to develop, whilst pollinators attempt to maximise their lifetime reproductive success by ovipositing in as many flowers as possible (Yu *et al.* 2004).

Although this conflict is rife among monoecious species, it is absent in dioecious *Ficus* where the development of wasps and seeds is kept separate through the existence of male and female trees. Foundresses entering gall syconia on male trees are able to oviposit in most, if not all ovules, whereas those entering seed syconia on female trees are exempt from ovipositing in any as style lengths are too long to be accessed by their ovipositors (e.g. Weiblen *et al.* 2001). Consequently, seed syconia nurture the development of seeds only, whilst gall syconia are effectively fig wasp nurseries.

Alongside pollinating fig wasps, the offspring of externally ovipositing non-pollinating wasps (NPFWs) also consume the contents of potential seeds. Whilst some NPFWs oviposit in empty ovules (gall-formers) costing a fig tree one potential seed for every egg laid, other NPFWs (parasitoids and inquilines) oviposit in occupied ovules killing the developing larvae. Each parasitic larva signals a two-fold loss: that of the pollinating wasp and the seed that it, in turn, had consumed. Until recently, parasitoids of pollinators were thought to be solely detrimental to fig trees, being responsible for their reduced pollen-dispersing and seed-dispersing functions. Evidence from three monoecious fig species, however, suggests that NPFW parasites, in particular, parasitoids of pollinators, can be indirect mutualists of monoecious fig trees by encouraging pollinator foundresses to oviposit in inner ovules where their offspring are at a reduced risk from parasite attack (Dunn *et al.* 2008b; Chapter 2: this thesis). This increases the likelihood that outer ovules do not receive a pollinator egg, and if pollinated, develop as seeds. In dioecious *Ficus*, where wasps and seeds develop in separate syconia, parasitoids appear to serve no benefit to the trees or their pollinators.

To date, coevolutionary studies involving fig trees and their associated wasps have identified that larger fruited Neotropical monoecious species have more flowers, produce more seeds and more wasps, and allow more foundresses entry into their ostioles (Herre 1989; Herre and West 1997). A combination of increased foundress number and more virulent nematodes (Herre 1996), however, means that the next generation of wasps are less efficient pollen dispersers than species with smaller syconia due to their less female-biased sex ratios (Herre 1985, 1989, 1996; Herre *et al.* 2008). This, coupled with the fact that larger monoecious fruit also have a higher probability of fungal infection (Michalaides and Morgan 1994) suggests that bigger is not always better. Few studies have done the same level of investigative work among dioecious fig species (but see Patel and Hossaert-McKey 2000; Weiblen 2000) although it has been shown that pollinators of dioecious fig species have shorter ovipositors than those of monoecious species (Wiebes 1994; Weiblen 2004). Moreover, the rate of parasitism and the search efficiencies of parasites are lower in dioecious species given that seed figs produce no pollinators but NPFWs do not discriminate (the so-called ‘parasitoid-sink hypothesis’, Weiblen *et al.* 2001). Consequently, whilst there has been some investigation into coevolution between fig trees and their wasps both within (monoecious: Herre 1989; dioecious: Weiblen 2001) and across fig reproductive systems (Wiebes 1979; Jousselin *et al.* 2003a; Weiblen 2004), few studies have specifically compared the morphology of the two fig reproductive systems to investigate whether the aforementioned conflict (or lack of) over the relative production of wasps and seeds may directly impose selection pressures on fig and wasp morphology (but see Harrison and Yamamura 2003).

6.2.1 Predictions of morphological coevolution

Syconia produced by different fig species can display considerable size variation from some the size of peas to others as large as peaches (Cook and Rasplus 2003; Herre *et al.* 2008, Figure 6.1). Across both monoecious and dioecious *Ficus*, larger syconia are likely to have more flowers than smaller ones (Herre and West 1997). After receiving an egg, these flowers are likely to develop into larger galled ovules which nurture bigger wasps (Herre 1989). Despite these similarities, the presence or absence of the wasp/ seed trade-off in monoecious and dioecious species respectively may mean that different selective pressures act on fig trees and their pollinators as a consequence of the fig reproductive system. In monoecious *Ficus*, style lengths are continuously distributed (Figure 1.3A) and despite an oviposition site preference for inner flowers over outer ones that develop closer to the fig wall (Dunn *et al.* 2008b), later entering females may have to lay their eggs into less favoured (outer) ovules (Yu *et al.* 2004). Once oviposition sites become limiting, females with longer ovipositors may be at an advantage over those with shorter ovipositors as an ability to access the remaining ovules, despite the increased risk of larval parasitism (Dunn *et al.* 2008b), should be preferable to an inability to oviposit in any of the remaining ovules. The combination of increasing foundress number (Herre 1989; Cook (unpublished)) and wasp fecundity (Nefdt and Compton 1996) associated with larger syconia and bigger pollinators respectively, is likely to mean that competition for ovules increases in larger fig species (J. M. Cook pers. comm.), despite the increased flower number. This would suggest that longer (and perhaps disproportionately long) ovipositors among pollinators would be advantageous. These longer ovipositors would mean that pollinator larvae in larger syconia are likely to be distributed in a wider range of ovary layers. Parasitoids of these pollinator larvae would, in turn, be selected to have longer ovipositors to access their hosts in these deeper ovary layers. As parasitoids often only lay a few eggs in multiple syconia, rather than all their eggs at once (Compton *et al.* 2009), competition for oviposition sites is expected to be weaker among parasitoids than among their internally ovipositing hosts. Consequently, selection for disproportionately long ovipositors is likely to be relatively weak.

In contrast, the style lengths among dioecious gall syconia are uniformly short (Figure 1.3 B, C). Although larger syconia may have longer styles, they are likely to still be accessible to pollinators without strong selection on increased ovipositor length.

As all pollinators develop in a single ovary layer at a roughly uniform distance from the fig wall, similarly weak selection on increased ovipositor length in parasites is expected. Based on this logic, I can make predictions based on what I expect all fig species to share in common, and those that may be different as a consequence of the fig reproductive system:

1) *Similarities*: across both monoecious and dioecious *Ficus*, I expect species with large syconia to have i) more flowers, ii) larger galls and iii) larger pollinators than fig species with smaller syconia.

2) *Differences*: In monoecious, but not dioecious *Ficus*, I expect larger syconia to have i) pollinating wasps with longer ovipositors, and ii) parasitoids equipped with longer ovipositors to access their hosts.

6.3 Materials and methods

6.3.1 Data collection

I collected data from 87 *Ficus* species across six subgenera either by dissections from preserved specimens (17 monoecious species) or from the published literature (42 monoecious, 28 dioecious species) (Table 6.1). All syconium data refer to ripe (D-stage) syconia prior to wasp emergence. For dioecious species, all values refer to gall syconia. For specimens dissected by myself, species were sometimes home to more than one species of agaonid pollinator (*F. rubiginosa*, *F. pleurocarpa*, *F. crassipes* and *F. obliqua*). In such instances, as the *average* size of the pollinators entering syconia was the variable of interest, pollinator morphology refers to an average across all pollinator species associated with a particular fig species. Multiple pollinating species have also been recorded from other species present in my data set; however, morphological data for all pollinating species was not always available. Parasitoids (of pollinators) are represented by two closely related genera, *Sycoryctes* and *Sycoscapter* (Tzeng *et al.* 2008), which are the most widespread of all fig-wasp parasitoids on *Ficus* (Bouček 1988, 1993; Lopez-Vaamonde *et al.* 2001). Their impact, although originally considered in a purely negative light, has recently gained importance as Dunn *et al.* (2008b) identified that parasitoids of pollinators (including *Sycoscapter spp.*) act as indirect mutualists of the system by encouraging ovipositing foundresses to deposit their eggs in ovules furthest from the fig wall where the risk of larval parasitism is lowest. By including parasitoids in the analyses, we can determine whether, like fig trees and their pollinators, they too display coevolved morphological characters associated with their hosts, making their impact on the system more general than was at first considered.

Table 6.1 *Ficus* species, their taxonomic placing based on morphology following Berg and Corner (2005), reproductive system (dioecious (D)/ monoecious (M)), source of data (literature (L)/ dissected specimen (S)), number of syconia dissected, and literature source for fig and/ or wasp morphology (Abdurahiman and Joseph 1975 (10); Balakrishnan Nair *et al.* 1981(4); Bronstein 1992 (19); Herre and West 1997 (11); Herre 1989 (16); Hill 1969 (21); Kathuria *et al.* 1995 (7); Kieffer 1912 (17); Kjellberg *et al.* 2001 (appendix) (5); Nair *et al.* 1981 (15); van Noort and Compton 1996 (1); Waterston 1921 (14); Weiblen 2004 (3);Wiebes 1963(8), 1964 (2), 1965 (20), 1979 (13), 1980 (12), 1982 (9), 1992 (18), 1994 (6).

Ficus	**Subgenus**	**Section**	**Reproductive system**	**Source**	**Syconia dissected**	**Literature**
abutilifolia	Urostigma	Galoglychia	M	L	1	1,5
adenosperma	Sycomorus	Adenosperma	D	L		2,3
albipila	Pharmacosycea	Oreosycea	M	L		3
altissima	Urostigma	Urostigma	M	L	3	4, 5
asperifolia	Sycidium	Sycidium	D	L		5
aurata	Ficus	Eriosycea	D	L	4	5
auriculata	Sycomorus	Sycomorus	D	L		3,6
benghalensis	Urostigma	Urostigma	M	L		7
benjamina	Urostigma	Urostigma	M	S	10	
bernaysii	Sycomorus	Sycocarpus	D	L		3,8
binendikii	Urostigma	Urostigma	M	L		5,9
botryocarpa	Sycomorus	Sycocarpus	D	L		3,8
burkei	Urostigma	Galoglychia	M	S	29	
burt davyii	Urostigma	Galoglychia	M	S	14	
callosa	Pharmacosycea	Oreosycea	M	L	2	5,10
citrifolia	Urostigma	America	M	L	55	5,11
conocephalifolia	Sycidium	Sycidium	D	L		2,3
conosociata	Urostigma	Urostigma	M	L		5,9
copiosa	Sycidium	Sycidium	D	L		3,12
crassipes	Urostigma	Malvanthera	M	S	4	5
craterostoma	Urostigma	Galoglychia	M	L	1	1,5
deltoidea	Ficus	Ficus	D	L	3	5
destruens	Urostigma	Malvanthera	M	S	10	
drupacea	Urostigma	Urostigma	M	L		7
edelfeltii	Pharmacosycea	Oreosycea	M	L		3,13
erecta	Ficus	Ficus	D	L		5
fistulosa	Sycomorus	Sycocarpus	D	L		5,14
glumosa	Urostigma	Galoglychia	M	L	1	1,5
grossularioides	Ficus	Ficus	D	L	3	3,6
hesperidiiformis	Urostigma	Malvanthera	M	S	2	
hirta	Ficus	Eriosycea	D	L		5,15
hispidoides	Sycomorus	Sycocarpus	D	L		3,8
ingens	Urostigma	Urostigma	M	L	1	5,12
insipida	Pharmacosycea	Pharmacosycea	M	L		3
itoana	Sycomorus	Papuasyce	D	L		3,8
lateriflora	Sycidium	Sycidium	D	L	5	5
liliputania	Urostigma	Malvanthera	M	S	2	
lutea	Urostigma	Galoglychia	M	L	1	1,5
macrophylla	Urostigma	Malvanthera	M	S	7	
maxima	Pharmacosycea	Pharmacosycea	M	L	4	5
microcarpa	Urostigma	Urostigma	M	S	7	
microdictya	Sycomorus	Papuasyce	M	L		3
mollior	Sycomorus	Adenosperma	D	L	1	5,12
natalensis leprieurii	Urostigma	Galoglychia	M	L	1	1,5
nodosa	Sycomorus	Sycomorus	D	L		3,12
nymphaefolia	Urostigma	Americana	M	L	65	5,11,16
obliqua	Urostigma	Malvanthera	M	S	6	
obtusifolia	Urostigma	Americana	M	L	34	5,11,16
odoardi	Synoecia	Rhizocladus	D	L		3
ottonifolia	Urostigma	Galoglychia	M	L		7
ovata	Urostigma	Galoglychia	M	L		17
paraensis	Urostigma	Americana	M	L	22	5,16
parietalis	Sycidium	Sycidium	D	L	6	5
pellucidopunctata	Urostigma	Urostigma	M	L		3,18
perforata	Urostigma	Americana	M	L		16
pertusa	Urostigma	Americana	M	L		11,16,19
platypoda	Urostigma	Malvanthera	M	S	2	
pleurocarpa	Urostigma	Malvanthera	M	S	9	
prasinicarpa	Urostigma	Urostigma	M	L		3
prolixa	Urostigma	Urostigma	M	L	4	5,6
pumila	Synoecia	Rhizocladus	D	L		5
racemosa	Sycomorus	Sycomorus	M	L	33	3,5
reflexa	Urostigma	Galoglychia	M	L	1	1,5

Ficus	Subgenus	Section	Reproductive system	Source	Syconia dissected	Literature
rubiginosa	Urostigma	Malvanthera	M	S	12	
rumphii	Urostigma	Urostigma	M	L		5
sagittifolia	Urostigma	Galoglychia	M	L	1	1,5
septic	Sycomorus	Sycocarpus	D	L	15	3,5,6
stuhlmannii	Urostigma	Galoglychia	M	L	1	1,5
subgelderi	Urostigma	Urostigma	M	L		5
sundaica	Urostigma	Urostigma	M	L		5,20
superb	Urostigma	Urostigma	M	L	1	3
sur	Sycomorus	Sycomorus	M	L	2	3,5
sycomorus	Sycomorus	Sycomorus	M	L	1	5,7
tesselata	Urostigma	Galoglychia	M	L	1	1,5
tinctoria	Sycidium	Paleomorphe	D	L		3,21
trachypison	Sycidium	Sycidium	D	L		3
trichopoda	Urostigma	Galoglychia	M	L	1	1,5
triradiata	Urostigma	Malvanthera	M	S	1	5
uncinata	Sycomorus	Sycocarpus	D	L	1	5,8
variegata	Sycomorus	Sycomorus	D	L		3
virens	Urostigma	Urostigma	M	S	7	
virgata	Sycidium	Paleomorphe	D	L	30	3,5,21
wassa	Sycidium	Sycidium	D	L		3,8
watkinsiana	Urostigma	Malvanthera	M	S	17	
xylophylla	Urostigma	Urostigma	M	L	3	5,18
xylosycia	Urostigma	Malvanthera	M	S	1	
yoponensis	Pharmacosycea	Pharmacosycea	M	L		3,19

6.3.2 Dissecting preserved specimens

I dissected 140 syconia from 17 *Ficus* species (Table 6.1) that had been collected by myself or JMC on previous field trips, and had been stored in 80% ethanol. For all species, syconia were sliced into eighths, and ovules were selected randomly using a ‘one in four’ approach. This involved removing every fourth ovule for each segment using forceps and transferring selected ovules to a watch glass of 80% ethanol for later analyses. For *F. burkei*, 14 syconia were dissected in this way but due to low numbers of parasitoids, all ovules (rather than just one in four) for an additional 18 syconia supplemented the data. Where multiple syconia from one fig species were available (Table 6.3), measurements were combined across syconia to create a mean value for each variable and for each fig species. Alongside the variables already mentioned in section 6.2.1, the effects of other morphological characters were also investigated. In total, I collected data on seven variables:

(a) *Syconium volume (mm)*: approximated to that of a sphere ($\frac{4}{3}\pi r^3$), unless the volume of a cone ($\frac{1}{3}\pi r^2 l$) provided a better approximation (*F. crassipes* and *F. pleurocarpa*); (b) *Flower number*: total number of female flowers per syconium; (c) *Gall volume (mm)*: approximated to the volume of a sphere; (d) *Pollinator body length (mm)*: ventral distance from the clypeal margin to the point at which the ovipositor sheath joins the abdomen; (e) *Ovipositor length (mm)*: total (first and second valvulae) and relative ovipositor length (total length/ thorax length) for pollinating wasps and their parasitoids;

(f) *Syconium wall thickness (mm)*: total and relative (total thickness/ syconium radius) thickness of the syconium wall; (g) *Pollinator head shape*: ($\frac{head\ \ width}{head\ \ length}$), where head width was measured as the maximum distance across the eyes, and head length represented the distance from the back of the head to the clypeus. A value of one represents a square-shaped head, whilst measures of less than one represent heads that are increasingly elongate.

All traits with the exception of fig size (diameter) and fig wall thickness were recorded to the nearest 0.024 mm using an eyepiece graticule attached to a binocular microscope. Fig size and wall thickness were measured to the nearest 0.05mm using digital callipers. Average wall thickness was calculated from 24 measures of the syconium wall taken at three points (at each end and in the middle) for each eighth.

6.3.3 Creating a phylogenetic tree using molecular data

Phylogenetic non-independence is the term used to describe the situation where closely related species are more likely to show morphological similarities than those more distantly related (Freckleton 2009). Consequently, analyses involving morphology across multiple species can be confounded when phylogeny is not considered. Moreover, it is best that the phylogeny is inferred from data that are independent of the variables under consideration. I therefore used DNA sequences of the internal transcribed spacer (ITS) from GenBank to create a molecular phylogeny for the 87 fig species. Three fig species (*F. maxima*, *F. insipida* and *F. yoponensis*) from the section Pharmocosycea were chosen as outgroups following overwhelming evidence for this as the basal group of *Ficus* (Wieblen 2000; Jousselin *et al.* 2003a). I pre-aligned the sequences in Clustal X2 (version 2.0.11) (gap opening parameter =10, gap extension =5, delay divergent sequences = 40%) and manually adjusted the resulting alignment in Bioedit (version 7.0.9.0). I used MrBayes (version 3.1.2) to create phylogenies using Bayesian analysis, running the chains for 10^6 generations and sampling every 100 generations. The first 2,500 trees were discarded as ‘burnin’ and a consensus tree (Figure 6.3) of the remaining 7,500 trees was created and imported into R (version 2.9.1) (R Development Core Team 2005) for analyses with morphological data.

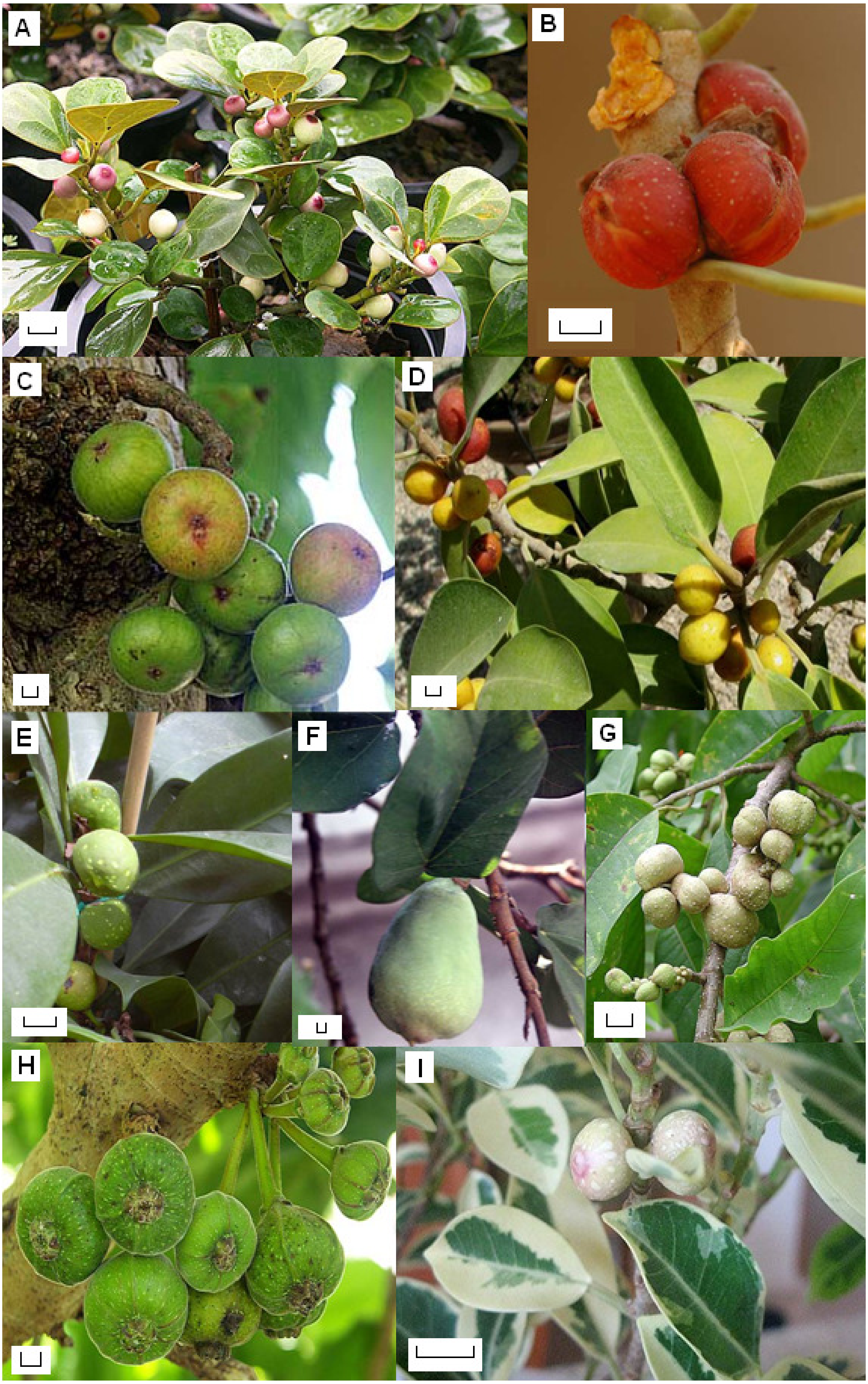


Figure 6.1 The variation in syconium size across nine fig species: (A) *Ficus deltoidea*, (B) *F. benghalensis*, (C) *F. racemosa*, (D) *F.rubiginosa*, (E) *F. macrophylla*, (F) *F. pumila*, (G) *F. lutea*, (H) *F. auriculata* and (I) *F. microcarpa*. Scale bar = 5mm. Adapted from Toptropicals (2009).

6.3.4 Morphological data analysis

I log transformed all morphological variables except those involving ratios (relative ovipositor lengths, pollinator head ratio and relative fig wall thickness) which remained untransformed. I then used phylogenetic generalised linear models (also known as phylogenetic GLS, Grafen 1989, 1992; Gage and Freckelton 2003) to analyse the phylogenetic and morphological data. Once a tree including those fig species associated with the variable of interest had been constructed, a function (pglmEstLambda) used maximum likelihood to estimate λ, a measure of how strongly correlations between traits are influenced by phylogeny (Pagel 1999; Freckleton *et al.* 2002). A value of $\lambda = 0$ represents a standard linear model with no shared phylogenetic history, and $\lambda = 1$ indicates data structured according to a Brownian model of trait evolution (i.e. where the variance in the trait values increase over time). I then specified the morphological variable of interest (e.g. flower number/ fig wall thickness etc.) as the response variable, and fig volume as the continuous explanatory variable.

6.4 Results

Across all species (monoecious and dioecious), larger syconia had significantly more (female) flowers, larger galls, larger pollinating wasps, externally ovipositing parasitoids with longer ovipositors and thicker syconial walls compared to species with smaller syconia (Table 6.2). Larger monoecious (but not dioecious) syconia had pollinating wasps with longer ovipositors and more elongate heads than those from smaller syconia. Pollinators from neither monoecious nor dioecious fig species had ovipositors that were longer than expected for their size alone. In contrast, dioecious fig species had parasitoids with disproportionately long ovipositors and disproportionately thick fig walls, something not shared with monoecious fig species (Table 6.2).

Table 6.2 Results of morphological data analysis using phylogenetic generalised linear models for monoecious (M) and dioecious (D) fig species.

Effect	**N**	***P***	**F**	**R^2**	**λ**
Flower number (M)	43	<0.0001	107.22	0.72	6.61E-05
(D)	16	<0.0001	17.75	0.53	6.61E-05
Gall size (M)	21	<0.0001	60.22	0.75	0.40
(D)	18	<0.0001	17.24	0.49	6.61E-05
Pollinator size (M)	32	<0.0001	32.40	0.50	0.84
(D)	21	<0.01	8.34	0.31	6.61E-05
Pollinator OL (M)	33	<0.0001	26.10	0.44	0.07
(D)	16	NS (0.19)	1.92	0.06	6.61E-05
Relative pollinator OL (M)	11	NS(0.40)	0.77	-0.02	6.61E-05
(D)	18	NS (0.43)	0.66	-0.02	1.40E-02
Pollinator head shape (M)	29	<0.005	13.17	0.30	0.37
(D)	21	NS (0.06)	4.12	0.14	6.61E-05
Parasitoid OL (M)	12	<0.005	14.49	0.55	6.61E-05
(D)	12	<0.05	5.47	0.29	6.61E-05
Relative parasitoid OL(M)	6	NS (0.10)	4.41	0.40	6.61E-05
(D)	14	<0.05	5.49	0.26	6.61E-05
Fig wall thickness (M)	24	<0.0001	87.49	0.79	6.61E-05
(D)	16	<0.0005	28.12	0.67	9.90E-01
Relative fig wall thickness (M)	24	NS (0.06)	4.21	0.12	6.61E-05
(D)	17	<0.0001	94.53	0.85	6.61E-05

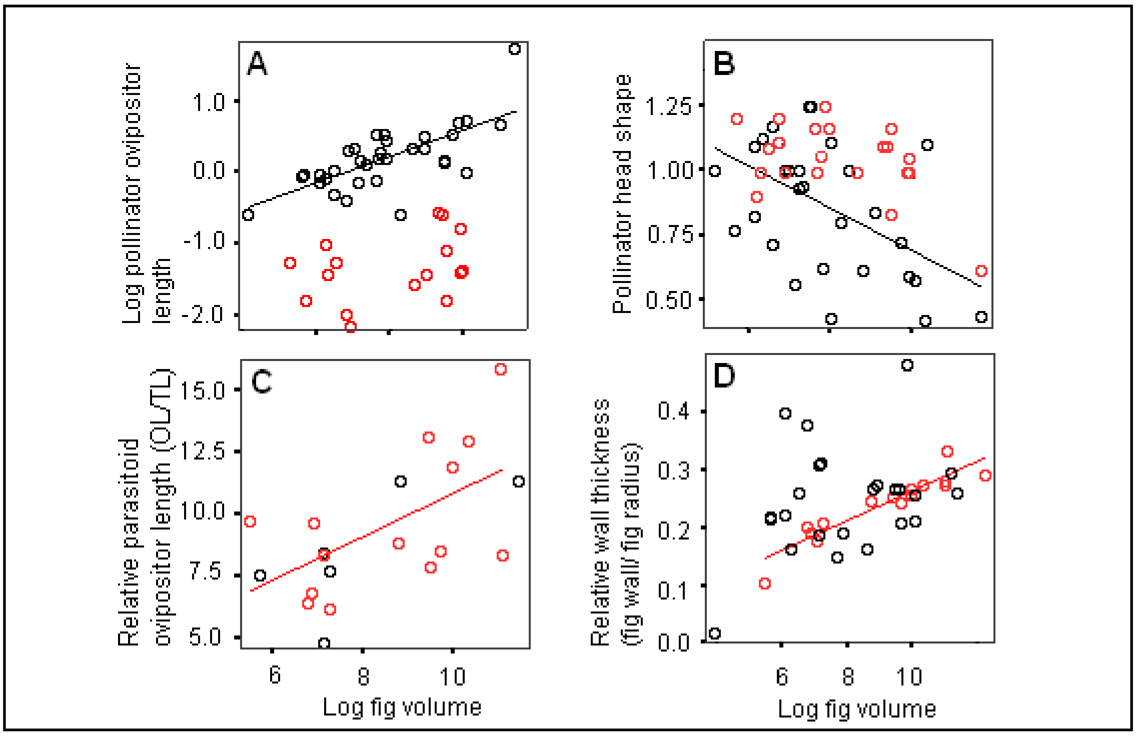


Figure 6.2 Relationships between (log) fig volume and (A) pollinator ovipositor length, (B) pollinator head shape (C) relative parasitoid (*Sycoscapter/ Sycoryctes spp.*) ovipositor length and (D) relative fig wall thickness in monoecious (black) and dioecious (red) fig species. The relationships are not significant in (A) and (B) for dioecious species, nor in (C) and (D) for monoecious species.

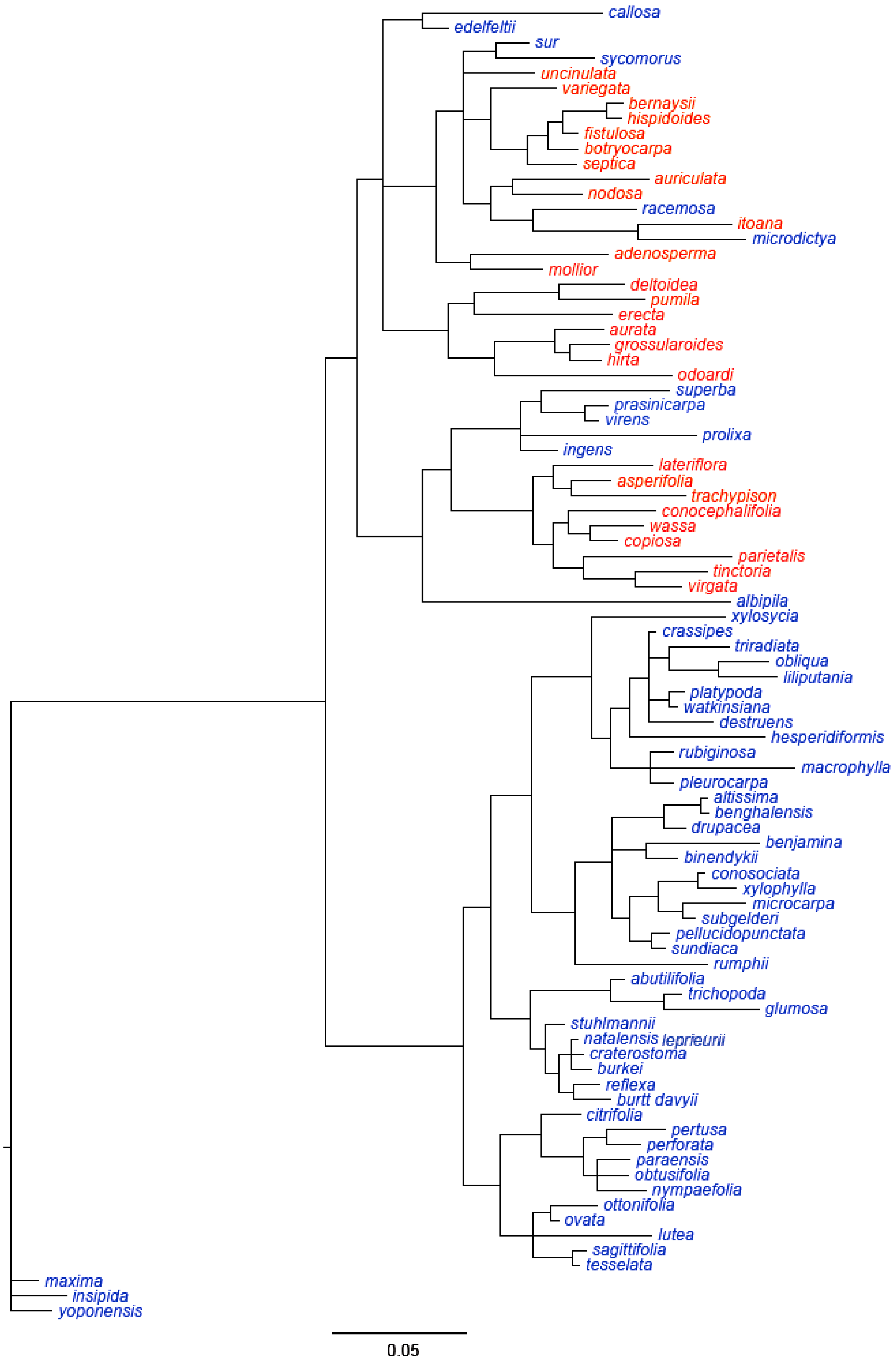


Figure 6.3 The consensus tree of 7500 ITS trees for 87 monoecious (blue) and dioecious (red) fig species.

Table 6.3 Morphological fig and wasp data for 87 fig species (D =dioecious, M=monoecious, OL= ovipositor length, BL=body length, Poll. = pollinator, *Syco*. =*Sycoscapter/ Sycoryctes* wasps).

FICUS	Reproductive system	Fig volume (mm3)	Female flowers	Fig wall thickness (mm)	Relative wall thickness	Gall volume (mm3)	Poll. BL (mm)	Poll. head shape	Poll. OL (mm)	Poll. OL/BL	*Syco.* OL (mm)	*Syco* BL (mm)	*Syco.* OL/BL (mm)
deltoidea	D	179.59	94										
trachypison	D	197.89		0.4	0.10	0.46			0.29				
virgata	D	314.79	116	1.5	0.21	0.16	0.85	1.20	0.17	0.20	2.15	1.30	1.65
adenosperma	D	562.21		1.4			1.20	1.00	0.24	0.20			
wassa	D	698.81		1.2	0.19	0.39	1.50	1.09	0.29	0.19			
tinctoria gibbosa	D	904.78	100	1.2	0.20	0.28	1.25	1.20	0.14	0.11	2.10	1.22	1.72
aurata	D	904.78	968				1.60	1.11				1.63	
grossularioides	D	1022.65	826	1.2	0.19	0.78	1.30	1.00	0.12	0.09			
parietalis	D	1150.35	153								3.77	1.80	2.09
bernaysii	D	1288.25			0.18	0.61	1.40		0.19	0.14	8.23	2.00	4.12
asperifolia	D	1767.15	1000										
hirta	D	2144.66	400				1.30	1.17					
mollior	D	2284.31	427			0.34	1.40	1.00					
uncinata	D	2572.44	445				2.70	1.06			5.49		
erecta	D	2806.16	190				1.40	1.25			7.16		
lateriflora	D	2806.16	1170										
fistulosa	D	3053.63	800				1.45	1.17					
conocephalifolia	D	5964.12		3.9	0.24	1.60	2.70	1.00	0.21	0.08	10.63		
nodosa	D	11383.56		4.5	0.26	0.88	1.60	1.10	0.58	0.36			
copiosa	D	12862.75		5.4	0.27	0.86	1.80	1.10	0.56	0.31			
septica	D	14080.69	248	2.9	0.25	0.57	2.00	0.83	0.17	0.09	7.43	2.47	3.01
variegata	D	14137.17	1800	4.0	0.27	0.51	1.40	1.17	0.34	0.24			
itoana	D	21092.16		8.5	0.33	1.38	2.15	1.00	0.46	0.21			
hispidoides	D	21594.48		6.8	0.27	1.10	2.30	1.00	0.25	0.11			
botryocarpa	D	22449.3		3.7	0.25	0.46	1.50	1.05	0.26	0.17			
odoardi	D	23031.53		4.7	0.27	0.88			0.24				
auriculata	D	65406.25		7.0	0.28	0.90	2.00	0.91	0.37	0.19	10.93		
pumila	D	127831.73	5500				2.50	0.63					
prasinicarpa	M	65.45		0.1	0.04	0.18			0.56				
binendikii	M	179.59					1.20	1.00					
prolixa	M	268.08	217				1.40						
reflexa	M	268.08	182										
liliputania	M	294.01		0.9	0.22		1.57	0.76	0.94	0.6			
microcarpa	M	309.4	236	0.9	0.21	0.08	1.21	1.20	0.97	0.8	7.23		
perforata	M	394.57	200										
burt davyii	M	468	190	1.8	0.38	0.24	0.96	1.09	0.96	1.00			
obliqua	M	475.04	343	1.1	0.22	0.35	1.30	0.82	0.86	0.66			
craterostoma	M	523.6	408								5.91		
benjamina	M	558.88	467	0.86	0.17	0.30	1.07	1.13	0.92	0.86			
pertusa	M	689.33	300						1.03				
superba	M	696.91	513	1.4	0.25	0.45	1.00	0.71	0.74	0.74			
conosociata	M	696.91					1.80	1.17					

Ficus	Reproductive system	Fig volume (mm3)	Female flowers	Fig wall thickness	Relative wall thickness	Gall volume (mm3)	Pollinator BL (mm)	Poll. head shape	Poll. OL (mm)	Poll. OL/BL	*Syco.* OL (mm)	*Syco.* BL (mm)	*Syco.* OL/BL (mm)
virens	M	944.41	345	2.16	0.36	0.30	1.25	1.00	0.68	0.54	9.13	2.23	4.09
benghalensis	M	1022.65	400				1.80	1.00	1.37	0.76			
albipila	M	1022.65							0.67				
glumosa	M	1022.65	252								1.93	1.25	1.54
subgelderi	M	1150.35	311										
altissima	M	1288.25	816				1.90	1.00			3.26		
destruens	M	1327.5	338	2.01	0.30	0.46	1.79	0.93	0.87	0.49			
burkei	M	1416.4	172	2.07	0.30	0.52	1.30	0.93	1.19	0.92			
xylosycia	M	1499.21				0.33	2.85	0.56	1.41	0.49			
rumphii	M	1515.11	400								3.41	1.60	2.13
pellucidopunctata	M	1646.31		1.30	0.19	0.82	1.20	1.25	1.12	0.93			
ingens	M	1767.15	384				1.40	1.25					
trichopoda	M	1767.15	722										
citrifolia	M	1875.31	325								3.24	2.61	1.24
microdictya	M	2144.66		3.10	0.26	1.12			0.89				
abutilifolia	M	2144.66	869						1.71				
rubiginosa	M	2305.87	541	1.29	0.16	0.66	1.84	0.62	1.20	0.65			
yoponensis	M	2352.07	359						1.33				
sundaica	M	2438.64					1.40						
paraensis	M	2711.05	413										
macrophylla	M	2750.84	434	1.64	0.19	0.97	2.91	0.42	1.69	0.58	5.74		
edelfeltii	M	2806.16		4.10	0.26	1.02	1.60	1.11	1.20	0.75			
lutea	M	2806.16	626						1.57				
natalensis leprieurii	M	2806.16	179										
stuhlmannii	M	3315.23	257										
maxima	M	3591.36	445				2.44	0.8					
callosa	M	4188.79	510				1.90						
drupacea	M	4188.79					1.60	1.00	0.55	0.34			
platypoda	M	5874.55		1.90	0.17		1.99	0.61	1.39	0.70	4.17		
tesselata	M	5964.12	806								3.54		
triradiata	M	7938.24	225	3.30	0.27		1.78	0.83	1.39	0.78	2.73		
ottonifolia	M	8181.23							1.67				
sagittifolia	M	8181.23	740								5.08		
nymphaefolia	M	11742.1	675								6.04	2.77	2.18
obtusifolia	M	12507.66	976										
sur	M	14137.17	1938	3.90	0.26	2.27			1.17				
insipida	M	14137.17	1230	7.50	0.28	1.35	2.44	0.72	1.14				
crassipes	M	17201.04	678	3.85	0.21		2.95	0.59	1.70	0.58	3.33	1.23	2.71
watkinsiana	M	20023.18	1048	7.42	0.45	1.23	2.62	0.57	2.01	0.77			
pleurocarpa	M	25180.73	1295	5.19	0.25	1.93	3.25	0.42	2.05	0.63			
racemosa	M	25881.94	1300	3.90	0.21	1.20			0.99				
xylophylla	M	27093.1	1738				2.1	1.10			5.26	2.25	2.34
ovata	M	27611.65					2.00						
sycomorus	M	65449.85	2623						1.95				
hesperidiiformis	M	94975.63		7.20	0.26	4.06	3.92	0.43	5.60	1.43			

6.5 Discussion

The principal findings of this study were that larger syconia of both monoecious and dioecious *Ficus* had significantly more (female) flowers, larger galls, thicker syconial walls, larger pollinating wasps and externally ovipositing parasitoids with longer ovipositors. Bigger galls can support the development of larger pollinating wasps and given that across monoecious fig species, pollinator size and fecundity are strongly correlated (Nefdt and Compton 1996), more flowers are necessary to increase the likelihood that at least some seeds are set. In dioecious species, despite the absence of a trade-off in the development of seeds and wasps, increasing flower number is also advantageous. Gall syconia with more flowers can nurture more pollinators which, in turn, equates to increased pollen dispersal ability for the fig tree. These advantages may leave us wondering why any fig species have small syconia, however, large syconium size has its own drawbacks such as the increased proportion of non-pollen dispersing males associated with higher foundress numbers (Harrison and Yamamura 2003), and an increased necessity to transpire to prevent wasps from fatally overheating (Patiño *et al.* 1994).

Although there were several common trends in fig and wasp morphology across monoecious and dioecious *Ficus*, there were also interesting differences between the two fig reproductive systems. Across monoecious species, pollinating wasps associated with larger syconia had longer ovipositors than species with smaller syconia. This was not true among dioecious fig species. Moreover, pollinators of dioecious fig species generally have shorter ovipositors than those of monoecious species (Ramirez 1980; Weiblen 2004). As monoecious fig trees must limit wasp oviposition to promote seed set, style lengths are likely to scale positively with seed (and therefore syconium) size, such that foundresses associated with larger syconia are likely to need longer ovipositors to be able to successfully oviposit into ovules. In dioecious species, a separation in the development of wasps and seeds to gall and seed figs respectively has resulted in gall figs having short styles that can be easily accessed by foundresses. Although bigger gall figs are likely to have more foundresses that in turn, are likely to suffer increased competition for oviposition sites, having an ovipositor longer than these styles is unlikely to provide any additional benefit (Weiblen 2004). Selection for longer ovipositor lengths among pollinators is therefore unlikely to be strong. In contrast, strong positive selection on ovipositor length would be expected for females that enter seed syconia where the vast majority of styles are too long to be accessed by pollinating females. Having said this, given that no male flowers (and therefore no pollen) develop in seed figs, any pollinator with an ovipositor long enough to reach even a few ovules would have offspring that would be unable to reproduce themselves (having no pollen to collect on emergence from their natal syconium and limiting the chances that their larvae develop successfully (Jousselin *et al.* 2003b)). Interestingly, neither reproductive system was associated with pollinators with ovipositors longer than expected for their size alone suggesting that a longer ovipositor is likely to be associated with a significant energetic cost (Ganeshaiah *et al.* 1995; Quicke *et al.* 1999; Yu *et al.* 2004).

Parasitoids of larger dioecious, but not monoecious fig species had disproportionately long ovipositors. Although this result was based on relatively few species (Table 6.2), the trend is likely to be a real one given that larger dioecious, but not monoecious syconia, had walls that were disproportionately thick. Thicker syconial walls help fig trees reduce the number of pollinating wasps being parasitised. In monoecious *Ficus*, having syconial walls too thick is, however, also suboptimal. Recent evidence from monoecious fig species (Dunn *et al.* 2008b) indicates that first entering foundresses select short-styled ovules first, whilst later entering foundresses are forced to oviposit in increasingly longer-styled ovules, closer to the fig wall. If, for a given monoecious fig species, syconial walls become thicker over time (and assuming that parasitoids do not develop longer ovipositors quickly enough to respond), parasitoids would be able to reach fewer ovules. Consequently, foundresses that had no preference for any flowers would lay more offspring than those that wasted time searching for short-styled flowers. Over time, the preference to oviposit into inner ovules would erode, decreasing seed set accordingly. At the other extreme, syconial walls too thin would allow externally ovipositing parasites access to more ovules, precluding pollinator wasp development in these flowers. This would benefit neither fig tree (reduced pollen dispersal) nor pollinator wasp (parasitism). Selection should therefore favour monoecious syconia that allow parasitoids access to some, but not all ovules, encouraging foundresses to prefer some flowers over others and allowing seeds to develop in those least favoured flowers.

As parasitoids provide no apparent mutualistic benefit to dioecious fig trees, a thicker syconial wall presents no such disadvantage and will only serve to further reduce the incidence of parasitism among pollinator larvae. The disproportionately thick syconial wall coupled with the suggested costs of having an ever longer ovipositor (Ganeshaiah *et al.* 1995; Quicke *et al.* 1999; Yu *et al.* 2004; but see Sivinski *et al.* 2001) and the fact that parasitoids appear not to differentiate between gall and seed figs, (despite their hosts only being present within male figs, Weiblen *et al.* 2000) may help explain why rates of parasitism are significantly lower in dioecious compared to monoecious fig species (Weiblen *et al.* 2001).

Fig wall thickness has a strong impact not only on parasite ovipositor length, but also on pollinator head shape. Pollinators associated with larger monoecious species had more elongate heads than those from smaller syconia. The same correlation was observed among dioecious species although the latter result was (marginally) non-significant. Thicker fig walls equate to a longer ostiolar passage, and longer heads complete with longer mandibles and more teeth may prove beneficial in ratcheting individuals into the syconial cavity. Previous evidence (van Noort and Compton 1996) has identified a strong association between internally ovipositing wasp head shape and syconium size in monoecious figs; pollinating and non-pollinating fig wasps from two independent lineages (Agaoninae and Sycoecinae respectively) have more square-shaped heads when associated with smaller fruited fig species, whilst those associated with larger syconia are equipped with heads that are more elongate (van Noort and Compton 1996). Dioecious fig trees do not need to limit pollinator wasp entry to the extent that monoecious species do given the lack of a trade-off between wasp and seed development. Moreover, re-emergence of foundresses after pollination is much more likely in dioecious than monoecious species (Moore *et al.* 2003; Hu *et al.*, in press). Taken together, these results suggest that the ostioles of dioecious fig species may be more 'porous' than those of monoecious syconia.

Other factors are also likely to influence fig wall thickness. Larger syconia, unlike smaller ones, use evapotranspiration to ensure that the fig interior is sufficiently cool to prevent pollinating wasps from overheating within their galls (Patiño *et al.* 1994). Consequently, there must be an upper limit to syconium wall thickness to ensure that temperatures within syconia remain viable for the developing wasps. Fig trees must also 'consider' the impact of wall thickness on both seed-dispersing agents and non-hymenopteran seed predators. Thicker syconial walls, although perhaps less favoured by seed-dispersing frugivores (McKey and Kaufmann 1989; West *et al.*1996), may help dissuade burrowing staphylinid beetles (Nadel *et al.* 1992), moths and weevils (Bronstein 1988b) from attacking ripening syconia.

Despite different conflicts of interest between wasps and either monoecious or dioecious *Ficus*, the results of this study suggest that some patterns of morphological coevolution are common to both reproductive systems. The fig reproductive system, however, does affect selection on some aspects of pollinator and parasitoid morphology. Stronger selective pressures on the head shape and ovipositor length of pollinators associated with monoecious fig species reflect the conflict of interest in the relative production of wasps and seeds that is absent among dioecious species. Nevertheless, disproportionately thickened fig walls in dioecious fig species select for extra-long ovipositors of parasitoids that attack wasp larvae from the other side of the fig wall.

Although the results of comparative analyses cannot dissociate cause and consequence to ‘prove’ reciprocal adaptation, they are nevertheless useful as additional sources of information, especially if the results are replicated in other data sets. Comparative studies that consider the coevolutionary selective pressures acting in monoecious and dioecious fig species are lacking in the literature, and more work is needed to expand our knowledge in this area. In particular, analyses involving multiple morphological measures can help elucidate the factors that are under strong selection between mutualistic partners, and those in turn, may help us understand how stability in the system is being maintained.

Chapter 7

General conclusions

7.1 Introduction

At first glance, mutualisms can appear as altruistic and therefore costly interactions between unrelated partners. They are, however, perhaps better conceptualised as examples of reciprocal parasitism and mutual exploitation (Nowak *et al.* 1994; Leight and Rowell 1995; Doebeli and Knowlton 1998). Given the selfishness of natural selection, a cheating mutualistic partner that receives the benefit of the interaction, but provides little service in return, is at an advantage over one that reciprocates. Mutualistic interactions are therefore rife with conflict as each partner attempts to exploit the other (e.g. Poulin and Grutter 1996; Anstett *et al.* 1997; Herre and West 1997; Herre 1999; Johnson *et al.* 1997; Foster and Delay 1998; Irwin and Brody 1998; Addicott and Bao 1999; Currie *et al.* 1999; Jaeger *et al.* 2000; Mueller 2002; Dufay and Anstett 2003; Dunn *et al*. 2008b).

The fig/ fig wasp interaction represents one of only a handful of obligate pollination mutualisms (yucca/ yucca-moth, senita/ senita-moth, globeflower/globeflower-flies, *Glochidion* tree/ *Epicephala* moth, New Caledonian *Phyllanthus/ Epicephala* moths). In each case, partners are reliant on one another in order to reproduce. Among monoecious fig trees, fig wasp larvae develop at the cost of potential seeds so that in the short-term, the presence of a seed represents only a missed opportunity for offspring development among pollinating wasps. This conflict over the fate of female flowers means that pollinating wasps should attempt to lay eggs in all *Ficus* ovules, whilst fig trees must limit wasp reproduction. If pollinating wasps did successfully lay eggs in all ovules, seeds would not develop and the mutualism would disintegrate. The interaction has however persisted for over 60 million years (Rønsted *et al*. 2005) suggesting that systems must be in place to limit the overexploitation of seeds by wasps (Yu *et al*. 2004).

7.2 Summary of results

I have considered different aspects of the fig/ fig wasp mutualism to investigate possible explanations for stability in this system. Recent evidence in monoecious fig species (Nefdt and Compton 1996; Anstett 2001; Dunn *et al*. 2008b) has highlighted that pollinator larvae are often laid in inner ovules, closest to the fig wall whilst seed development is predominantly limited to outer ovules. The short ovipositor hypothesis (SOH) was a theory proposed by Ramirez (1970a) to explain how the continuously distributed style lengths in monoecious *Ficus* may make outer flowers, closest to the fig wall, inaccessible to wasp ovipositors. In Chapter 2, I investigated its applicability in

Ficus rubiginosa, a monoecious fig tree home to four cryptic species of pollinating fig wasps. Genetic data identified the presence of three of the four pollinating species in Townsville, Australia, with all three species capable of accessing the vast majority of *F. rubiginosa* ovules. Although the SOH was clearly not a valid explanation for stability in this system, applying it to externally ovipositing NPFWs, and in particular to parasitoid genera, helped to explain why pollinating wasps display an oviposition preference for short-styled flowers. A significant proportion (33%) of the longest flowers (inner ovules) were out of reach to *Sycoscapter* parasitoids, potentially providing pollinator larvae developing in the innermost ovules with enemy-free space from parasite attack, and providing good evidence to support the optimal foraging hypothesis (Yu *et al.* 2004).

In chapter 3, I considered the situation in *Ficus racemosa* where adult wasps in some syconia (about 4% of the population) fail to emerge. Wasps and pollen from these syconia do not disperse. Direct manipulation of the number of male pollinators within syconia identified that the failure of wasps to emerge is a consequence of low male count. With less than ten males (and with NPFWs relying on male pollinators to produce exit holes), the likelihood of wasp emergence from syconia is significantly reduced. Syconia where wasps did not emerge had lower proportions of male and female pollinators, but not significantly more parasitoids of pollinators compared with syconia where wasps emerged normally. This suggests that low foundress number is likely to be responsible for the low male count and the consequent failure of wasps to exit these syconia. Moth and beetle larvae that burrow into *F. racemosa* syconia later on in their development may provide wasps trapped within syconia with some means of escape. Whether or not these larvae can be considered indirect mutualists of the system is largely dependent on whether they predate the trapped wasps and how common they are among unexited figs to provide wasps with a regular means of dispersal.

Chapter 4 focused on the African fig species, *F. burkei*, and its internally ovipositing sycoecine NPFW, *Philocaenus barbarus*. *Philocaenus barbarus* offspring were only found in syconia also containing the legitimate pollinator, *E. stuckenbergi*. In their absence, however, *E. stuckenbergi* occupied longer flowers than in syconia shared with *P. barbarus.* This species therefore has a negative effect on pollinator oviposition but no observable effect on seed set.

The impact of the green tree ant (*Oecophylla smaragdina*) on parasitism rates among *F. racemosa* syconia was considered in chapter 5. Syconia where ants had been excluded produced higher proportions of emerging NPFWs and lower proportions of pollinators. By predating externally ovipositing NPFWs (Schatz *et al.* 2006, 2008) in particular, parasitoids of the pollinators, *O. smaragdina* workers can be considered indirect mutualists of fig trees and their pollinators in this system.

Finally, chapter 6 considered mutualism stability from a comparative coevolutionary perspective, and illustrated that the conflict (or its absence) over the production of seeds in monoecious (or dioecious) fig species influences the selective pressures acting on the morphology of fig trees and their associated wasps. Monoecious and dioecious fig species with larger syconia had more flowers, bigger galls, bigger pollinators, thicker fig walls and parasitoids with longer ovipositors compared to species with smaller syconia. Pollinators of larger monoecious (but not dioecious) syconia had more elongate heads and longer ovipositors than pollinators of smaller syconia, whilst larger dioecious, but not monoecious, syconia had walls that were disproportionately thick and parasitoids with disproportionately long ovipositors. These results indicate that the fig breeding system can influence the trajectory of coevolution between fig trees and their associated wasps.

7.3 Conclusions

From the results of the preceding chapters, it is becoming increasingly evident that, like a Russian Babushka doll, the fig/ pollinator mutualism often involves more species than just the pollinating wasps that live within individual syconia. Moreover, whilst it is important to recognise that organisms external to the mutualism can (and do) exploit the system, it is also true that the mutualistic partners themselves can also be opportunistic exploiters. Fig trees and their pollinators are therefore not automatically excluded from the mechanisms needed to maintain the stability of the system. For example, a recent study of six Panamanian fig species identified that fig trees can impose ‘host sanctions’ on their pollinators by reducing the reproductive success of wasps that cheat the system by failing to pollinate (Jandér and Herre 2010). Whilst passively pollinated fig trees appeared to impose no sanctions on their pollinator wasps (because pollination occurs largely via accidental transfer and the probability of wasps entering syconia without pollen is low), actively pollinated trees were more likely to abort fruit that housed these ‘free-loaders’ (Jandér and Herre 2010).

With regards to a general mechanism for mutualism stability in the fig- wasp system, its existence seems increasingly unlikely (see Dunn *et al.* 2008b; Wang *et al.* 2009). A more likely outcome is that different fig/ fig wasp partnerships have one, or a number of mechanisms in place that contribute to stability in that particular system. Wang and others (2009) have recently identified that in *F. racemosa* in China, syconia are often underexploited by wasps in the summer (producing few seeds and galls), and overexploited in the winter (producing few seeds and many galls) because pollinator wasp lifespans are shorter and longer in these seasons, respectively. Host control appears to occur, at least in part, via density-dependent closure of the ostiole which allows more foundresses entry into syconia thereby increasing wasp (and seed) production in the summer months when wasps are limiting. In the winter months wasps are plentiful; however, given that interference competition increases with foundress number, by extending the period the ostiole remains open, fig trees are able to intensify competition among ovipositing females and increase seed production at a time when the conflict between fig tree and wasp is highest (Wang *et al*. 2009).

Results from this thesis have identified that selective forces imposed by fig trees (e.g. thickened fig walls and narrow ostioles) can constrain the size and therefore fecundity of their associated pollinators and these selective forces can be influenced by the fig reproductive system. Moreover, species external to the mutualism (e.g. non-pollinating fig wasps and predatory ants) can be important third parties in the interaction. In *F. rubiginosa*, the short ovipositor hypothesis (with respect to externally ovipositing NPFWs) suggests that inner ovules furthest from the fig wall are those least accessible to NPFW ovipositors. Consequently, these ovules are likely to be favoured oviposition sites for pollinator foundresses whose offspring are likely to suffer reduced rates of parasitism. Outer ovules, closest to the fig wall are less favoured by foundresses, and, if pollinated, are more likely to become seeds. Meanwhile, in *F. racemosa*, patrolling by the green tree ant, *Oecophylla smaragdina*, reduced the proportion of all NPFWs emerging from syconia and therefore reduced parasitism rates among pollinators. These results present two very different mechanisms to explain how different fig/ wasp systems are not overexploited by pollinating and non-pollinating fig wasps, respectively. Further work to investigate the more general applicability of the parasitoid short ovipositor hypothesis in other fig/ fig wasp pairs, and the incidences and impacts of internally ovipositing non-pollinating fig wasps are both imperative to elucidate how important these are as general, if only partial, explanations for stability in the fig/ fig wasp mutualism.

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