

OVERVIEW

Speciation in fig wasps

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Abstract. 1. There are over 700 species of fig trees in the tropics and several thousand species of fig wasps are associated with their syconia (inflorescences). These wasps comprise a monophyletic family of fig pollinators and several diverse lineages of non-pollinating wasps. The pollinator larvae gall fig flowers, while larvae of non-pollinating species either initiate their own galls or parasitise the galls of other wasps.

2. A single fig species has one to four pollinator species and also hosts up to 30 non-pollinating wasp species. Most wasps show a high degree of host-plant specificity and are known from only a single fig species. However, in some cases wasps may be shared across closely related fig species.

3. There is impressive morphological co-evolution between figs and fig wasps and this, combined with a high degree of partner specificity, led to the expectation that figs and pollinators have cospeciated extensively. Comparison of deep phylogenies supports long-term codivergence of figs and pollinators, but also suggests that some host shifts have occurred.

4. Phylogenies of more closely related species do not match perfectly and may even be incongruent, suggesting significant roles for processes other than strict cospeciation. Combined with recent evidence on host specificity patterns, this suggests that pollinator wasps may often speciate by host shifts between closely related figs, or by duplication (the wasp speciates but the fig doesn't). The frequencies and biological details of these different modes of speciation invite further study.

5. Far less is known about speciation in non-pollinating fig wasps. Some lineages have probably co-evolved with figs and pollinators for most of the evolutionary history of the symbiosis, while others appear to be more recent colonisers. Many species appear to be highly host-plant specific, but those that lay eggs through the fig wall without entering the syconium (the majority of species) may be subject to fewer constraints on host shifting than pollinators. There is evidence for substantial host shifting in at least one genus, but also evidence for ecological speciation on the same host plant by niche shifts in other cases.

6. Finally, recent work has begun to address the issue of 'community phylogeny' and provided evidence for long-term co-divergence of multiple pollinating and non-pollinating wasp lineages with their host figs.

Key words. Biogeography, co-evolution, ecology, fig, fig wasp, host shift, phylogeny.

Introduction

Insects and flowering plants are arguably the most dominant and conspicuous taxa in terrestrial ecosystems today. They

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Conflicts of interest: the authors have not declared any conflicts of interest.

interact strongly through both herbivory and pollination and show contemporaneous diversification in the fossil record (Labandeira, 2002). There is evidence that adoption of herbivory is associated with increases in the species richness of insect clades (Mitter *et al.*, 1988) and a plethora of evidence that host shifts between plant groups play a major role in insect herbivore radiation (e.g. Ehrlich & Raven, 1964; Farrell 1998; Lopez-Vaamonde *et al.*, 2003; Stone *et al.*, 2009). At the next trophic level, parasitoids of

insect herbivores also comprise a very substantial component of terrestrial biodiversity (essentially, every herbivore is attacked by parasitoids), although one whose speciation patterns are arguably less well understood than those of the herbivores (Godfray, 1994; Quicke, 1997; Lopez-Vaamonde *et al.*, 2005). Consequently, speciation in insect herbivores and the parasitoids that attack them contributes greatly to the patterns of terrestrial biodiversity that we see today and there is increasing evidence that diversification is linked across trophic levels (Abrahamson & Weis, 1997; Kankare *et al.*, 2005; Stireman *et al.*, 2006).

Interactions between insects and plants are largely antagonistic, because the insects eat the plants. Plants fight back in evolutionary terms with a range of defences, including secondary plant chemistry, and insect – plant coevolution emerged as a well-defined field following the publication of Ehrlich and Raven's (1964) classic study relating butterfly host-plant use to chemistry and taxonomy. However, insects and plants also interact, generally to mutual benefit, through the medium of insect pollination (Thompson, 2005; Waser & Ollerton, 2006). This varies from quite diffuse relationships between plant and insect species, increasingly studied in the form of pollination webs, to highly partner-specific systems, amongst which figs (*Ficus* species) and their pollinating wasps (family Agaonidae) provide the classic example (Anstett *et al.*, 1997; Cook & Rasplus, 2003).

Figs and their pollinators engage in an obligate mutualism, with each partner dependent on the other for successful reproduction. Agaonidae are the only pollen vectors for *Ficus*, while the wasp larvae develop and feed only in the syconia of *Ficus* species. The syconia (fig 'fruits') are inflorescences that, depending on species, contain from tens to thousands of individual unisexual flowers (Cook & Rasplus, 2003; Kjellberg *et al.*, 2005). Pollinator females enter receptive syconia through a narrow bract-lined tunnel called the ostiole, which closes soon afterwards, sealing the foundress wasps in a 'tomb blossom' (Weiblen, 2002). Inside, the wasps pollinate the female fig flowers, but also lay eggs into some of them. The foundress wasps then die and the seeds and wasp offspring mature over a period of a few weeks. The wingless male wasps hatch first from their galls and search for galls containing females. In at least some species, the males bite holes into the female galls and insert their genitalia in order to mate. Female wasps then emerge into the fig cavity and at this time the male fig flowers are mature and liberating pollen. In some species, the wasps actively collect pollen into thoracic pollen pockets and later deposit it deliberately onto flower styles (Galil & Meiri, 1981; Kjellberg *et al.*, 2001; Joussetin *et al.*, 2003a). In others, pollination is passive via transport on the wasp's body, as in most other insects (Waser & Ollerton, 2006). The male wasps then chew exit tunnels through the fig wall, allowing the winged, mated, pollen-bearing female wasps to disperse and search for new receptive figs, generally on other trees.

The fig–pollinator interaction is famous for its degree of reciprocal partner specificity (Wiebes, 1979; Cook & Rasplus, 2003). Until recently, it was widely thought that there is generally a one-to-one match of *Ficus* and pollinator species. However, this paradigm has been overturned in the last decade,

as a growing number of studies on all tropical continents reveal exceptions. In many cases, a single fig species hosts more than one (Michaloud *et al.*, 1985, 1996; Lopez-Vaamonde *et al.*, 2002; Molbo *et al.*, 2003; Machado *et al.*, 2005; Peng *et al.*, 2008; Su *et al.*, 2008), and up to four, pollinator species (Haine *et al.*, 2006). In a smaller number of cases, wasp species are shared between different fig species (Molbo *et al.*, 2003; Jackson, 2004; Machado *et al.*, 2005; Su *et al.*, 2008).

The interaction between figs and their pollinators is the core of the system, but each *Ficus* species also hosts a community of up to 30 non-pollinating fig wasp (NPFW) species (Bouček *et al.*, 1981; Bronstein, 1991; Compton *et al.*, 1994; West *et al.*, 1996; Kerdelhue *et al.*, 2000; Cook & Rasplus, 2003). These include species that induce galls inside the figs and others that take over the galls of pollinators (or other wasps) by either killing them directly as parasitoids (Tzeng *et al.*, 2008) or outcompeting them in their own galls as lethal inquiline (Joseph, 1955; West *et al.*, 1996; Cook & Rasplus, 2003). In most cases, we do not know whether a given species is a true parasitoid or an inquiline, or exactly which of the other wasp species can act as hosts (Cook & Rasplus, 2003); however, recent studies have begun to address these important issues (Yang *et al.*, 2005; Pereira *et al.*, 2007; Tzeng *et al.*, 2008; Wang & Zheng, 2008; Zhai *et al.*, 2008). There are certainly several thousand NPFW species (see Figweb: <http://www.figweb.org/>), most of which remain undescribed, but it is difficult to estimate their full diversity without better information on the degree of host-plant specificity of different wasp taxa (Cook & West, 2005). It is also important to emphasise that while the pollinating wasps form a single monophyletic clade (Wiebes, 1982; Machado *et al.*, 2001; Lopez-Vaamonde *et al.*, 2009), the NPFW represents several different lineages of wasps (mostly Chalcidoidea) that have colonised the syconium habitat independently (Rasplus *et al.*, 1998; Cook & Rasplus, 2003).

The case for coevolution and cospeciation

Figs and fig pollinating wasps provide excellent evidence for phenotypic coevolution (Kjellberg *et al.*, 2001; Joussetin *et al.*, 2003b; Weiblen, 2004). In general terms, many details of the life cycle of fig wasps and the development of fig syconia depend on co-adaptation and synchronisation for reproductive success (Galil, 1977; Cook & Lopez-Vaamonde, 2001). However, since the wasp larvae eat fig seeds, there is conflict over the fate of individual female flowers, which can give rise to either a seed or a wasp, but not both. Selection on wasps favours individuals that exploit seeds to enhance their own reproductive success, but selection on figs favours production of both seeds and female pollinator wasps (pollen vectors). These underlying tensions may be partly responsible for the diverse evolutionary trajectories of the basic symbiosis (Herre, 1989, 1999; Cook & Rasplus, 2003).

One striking and important dichotomy is between wasp species that pollinate figs passively, like most other insect pollinators, and those that do so actively, paralleled only by a few other insect lineages such as *Yucca* (Pellmyr *et al.*, 1997; Pellmyr & Leebens-Mack, 1999) and Senita moths (Holland & Fleming, 1999). Female wasps from species that pollinate

actively emerge from their galls and collect pollen into thoracic pollen pockets using special combs of hairs on their legs. They then disperse and enter receptive figs where they use their forelegs to remove pollen from the pockets and place it onto the stigmas of female flowers (Galil & Meiri, 1981; Kjellberg *et al.*, 2001). This remarkable behaviour is mirrored by large changes in pollen investment by the plants. In fig species with active pollinators, only about 10% of flowers are male, while in those with passive pollinators the figure is about 30% (Kjellberg *et al.*, 2001). Moreover, phylogenetic evidence suggests that there have been several changes between active and passive pollination syndromes and in each case both partners have changed (Kjellberg *et al.*, 2001; Cook *et al.*, 2004).

Another striking example of coadaptation is between fig breeding system and wasp ovipositor length. Long ovipositors are presumably costly to pollinators (Bronstein, 1992; Ganeshiah *et al.*, 1995), but allow access to flowers with longer styles, because wasps push their ovipositors down the flower style to lay an egg right next to the fig ovary at the bottom of the style (Jousselin *et al.*, 2001). In dioecious figs, the flowers of 'male' trees all have short styles and give rise to wasps, while those of 'female' trees have much longer styles and give rise to seeds. In contrast, monoecious figs have only one type of tree and one type of syconium, within which the flowers show continuous variation in style length, with some nurturing wasps and others seeds. Evolutionary transitions between monoecy and dioecy in figs are matched by transitions between long and short ovipositors in the wasps (Jousselin *et al.*, 2003b; Weiblen, 2004). This is because even a short ovipositor is sufficient to reach all flowers in male syconia of dioecious figs, while a longer ovipositor can allow access to a greater proportion of flowers in syconia of monoecious figs.

Figs and their pollinators show an impressive degree of reciprocal partner specificity and provide several clear examples of phenotypic coadaptation. This has encouraged the view that they have cospeciated (Wiebes, 1979), despite the general fact that coevolution need not lead to cospeciation (Thompson, 2005). It has been argued that high partner specificity might constrain fig speciation to occur only in complete allopatry (Michaloud *et al.*, 1996 and references therein). However, some exceptions to 1:1 reciprocal partner specificity have been long known (Michaloud *et al.*, 1996; Rasplus, 1996). Weak support for cospeciation comes from the good general correspondence between the classification of the plants and insects. There may be some circularity here, because the two classifications were not developed totally in isolation (Wiebes, 1979; Compton *et al.*, 1996). However, Kjellberg *et al.* (2005) argue that this has been overemphasised, because most wasps and figs can be placed easily into their respective genera/sections, which are increasingly well supported as natural units. In any case, rigorous tests of cospeciation require formal comparison of independent phylogenies of figs and wasps.

Classification and biogeography of figs and pollinators

Ficus is one of the largest genera of land plants with 735 species (Berg & Corner, 2005) divided into 19 intra-generic

sections (Rønsted *et al.*, 2008b) (Table 1). Meanwhile, fig-pollinating wasps form the hymenopteran family Agaonidae, which comprises 20 genera (Wiebes, 1979, 1982; Bouček, 1988, 1993; Weiblen, 2002; Lopez-Vaamonde *et al.*, 2009). In most cases, figs in the same section are pollinated by wasps in the same genus (Table 1). A notable exception occurs in African figs forming the section *Galoglychia*, which are pollinated by wasps from seven genera (Berg & Wiebes, 1992). However, these genera (1) appear to form a monophyletic group (Erasmus *et al.*, 2007; Lopez-Vaamonde *et al.*, 2009) and (2) do not pollinate figs from other sections (Erasmus *et al.*, 2007), so this in itself does not substantially change the general picture of a clade of wasps associated exclusively with a clade of figs. On the other hand, figs in some subsections of *Galoglychia* are pollinated by more than one of these closely related genera and some individual trees may host wasps from two genera (Greeff *et al.*, 2003), so clearly strict cospeciation is not the only process involved in radiation of these African wasps.

Figs are found on all tropical continents and play important roles in many ecosystems, including both rainforests and more arid habitats. *Ficus* is the only genus to appear in the top three tree genera in long-term study plots of lowland rainforest on all tropical continents (Harrison, 2005). The ecological importance of figs stems from their unusual year-round production of ripe fruits, which helps sustain populations of diverse frugivorous animals including birds, bats, rodents and primates (Shanahan *et al.*, 2001). About half of all fig species are monoecious and the other half dioecious (Berg & Corner, 2005). Each tropical continent has endemic radiation(s) of monoecious figs (Table 1), and there are smaller endemic radiations within the large islands of, for example, Madagascar and New Caledonia (Kjellberg *et al.*, 2005). While several sections of monoecious figs are restricted to one continent or region (e.g. *Galoglychia* to Africa), others (*Urostigma*, *Oreosyceae*, *Sycomoraceae*) have much wider geographic representation across the Old World. In the New World there are only two fig sections – *Americana* and *Pharmacosyceae* – both monoecious and endemic to the region. Dioecious figs are found only in the Old World, with the vast majority of species occurring in Asia and Papua (Kjellberg *et al.*, 2005). A few of these species spill over into northern Australia and there are a few endemic African species. However, the dioecious subgenus *Sycidium* ranges from Madagascar to the deep Pacific, and also occurs in both tropical China and Australia, so still achieves wide geographic representation.

Comparing fig and pollinator phylogenies

Molecular phylogenies have been compared between various groups of figs and wasps. These studies tend to focus on one of two taxonomic/biogeographic levels: (1) high/global – emphasising the global diversity and using a few species from several wasp genera/fig sections; and (2) low/regional – emphasising regional radiations of species within one or two wasp genera/fig sections.

At the global level, molecular phylogeny supports the identity of many (but not all) morphologically-defined fig

Table 1. Fig sections/subsections and pollinator genera (Berg and Corner, 2005).

<i>Ficus</i> subgenus	<i>Ficus</i> section or subsection	Pollinator genus	Distribution
<i>Pharmacosycea</i>	Sect. <i>Pharmacosycea</i>	<i>Tetrapus</i>	Neotropics
	Sect. <i>Oreosycea</i>	<i>Dolichoris</i>	Africa, Asia, Australasia
<i>Sycidium</i>	Sect. <i>Sycidium</i>	<i>Kradibia</i> *	Africa, Asia, Australasia
	Sect. <i>Paleomorphe</i>	<i>Lipporhopalum</i>	Asia, Australasia
<i>Sycomorus</i>	Sect. <i>Sycomorus</i> †	<i>Ceratosolen</i>	Africa and Asia
	Sect. <i>Sycocarpus</i>	<i>Ceratosolen</i>	Asia, Australasia
	Sect. <i>Adenosperma</i>	<i>Ceratosolen</i>	Asia, Australasia
	Sect. <i>Hemicardia</i>	<i>Ceratosolen</i>	Asia Australasia
	Sect. <i>Papuasyce</i> ‡	<i>Ceratosolen</i>	Australasia
	Sect. <i>Bosscheria</i>	<i>Ceratosolen</i>	Australasia
	Sect. <i>Dammaropsis</i>	<i>Ceratosolen</i>	Asia, Australasia
<i>Urostigma</i>	Subsect. <i>Urostigma</i>	<i>Platyscapa</i>	Africa, Asia, Australasia
	Subsect. <i>Conosycea</i>	<i>Eupristina</i> , <i>Deilagaon</i> , and <i>Waterstoniella</i>	Asia, Australasia
	Sect. <i>Americana</i>	<i>Pegoscapus</i>	Neotropics
	Sect. <i>Galoglychia</i>	<i>Allotriozoon</i> , <i>Agaon</i> , <i>Courtella</i> , <i>Elisabethiella</i> , <i>Nigeriella</i> , <i>Alfonsiella</i> , and <i>Paragaon</i>	Africa
	Subsect. <i>Stilmophyllum</i>	<i>Pleistodontes</i>	Asia
<i>Ficus</i>	Subsect. <i>Malvanthera</i>	<i>Pleistodontes</i>	Australasia
	Sect. <i>Ficus</i>	<i>Blastophaga</i>	Asia, Africa, Europe
	Sect. <i>Eriosycea</i>	<i>Blastophaga</i>	Asia, Australasia
<i>Synoecia</i>	Sect. <i>Kissosycea</i>	<i>Wiebesia</i>	Asia, Australasia
	Sect. <i>Rhizocladus</i>	<i>Wiebesia</i>	Asia, Australasia

*Two species from subgenus *Sycidium* are pollinated by *Ceratosolen* species.

†Subgenus *Sycomorus* is largely dioecious, but with one section secondarily monoecious.

‡This small section of three species contains two monoecious and one dioecious taxa and is nested within a largely dioecious subgenus.

sections (Rønsted *et al.*, 2005, 2008a) and wasp genera (Machado *et al.*, 2001; Lopez-Vaamonde *et al.*, 2009) and therefore the strong general matching suggested by traditional classifications. However, many aspects of the deep branching order of clades are resolved poorly for either the plants or the insects, or both, making it difficult to estimate the true level of congruence. There are also a few notable deep ‘misfits’ that are too strong to be attributable to errors in phylogeny estimation (Kjellberg *et al.*, 2005). Nevertheless, it seems likely that figs and pollinators have diversified together (though not necessarily through strict cospeciation) to a significant extent. In this paper we use the term ‘cospeciation’ to refer to a strict linkage of speciation in fig and wasp in which they speciate together at the same time, leading to mirror image phylogenies. We also use the term ‘codivergence’ to describe a looser linkage between speciation in the two groups, in which their long-term radiation is linked, such that their true phylogenies are broadly similar (but not identical), but which allows a significant role for events other than strict cospeciation.

Rønsted *et al.* (2005) used ‘double dating’ techniques to compare the ages of matching fig and wasp clades and found

a strong correspondence, supporting a history of coupled divergence for over 60 MY. However, the role of strict sense cospeciation in this diversification awaits convincing tests. Jackson (2004) analysed available data and concluded that there was not significant support for cospeciation, but was only able to use relatively few species in his tests. The taxon sampling in the latest studies (Rønsted *et al.*, 2008a; Lopez-Vaamonde *et al.*, 2009) is far denser and more representative than in earlier ones, but resolution of key deep nodes will probably require data from more genes. Only when we have large and well resolved phylogenies for both groups will conclusive tests become possible.

At the lower taxonomic level, studies vary in the degree of congruence reported. There is evidence for significant cospeciation between a set of pollinators and figs mostly from Papua (Weiblen & Bush, 2002; Silvieux *et al.*, 2008). However, the figs actually include members of six sections within subgenus *Sycomorus*, so this has a regional focus but intermediate taxonomic scope. In contrast, phylogenies of African figs and pollinators match less (Erasmus *et al.*, 2007; Joussetin *et al.*, 2008) and presence of multiple wasp genera on some fig species suggests an ancient duplication of wasps

that has persisted. Finally, studies in Latin America (Molbo *et al.*, 2003; Machado *et al.*, 2005; Su *et al.*, 2008) suggest a complex situation in New World figs (sections *Americana* and *Pharmacoscycea*), with lack of congruence and extensive cases of wasps shared between figs (Jackson, 2004; Machado *et al.*, 2005; Marussich & Machado, 2007; Jackson *et al.*, 2008; Su *et al.*, 2008).

These cases might actually be less contradictory than they appear if some more recent radiations of figs represent hybridising swarms that have yet to evolve species barriers and sort lineages of wasps (Machado *et al.*, 2005), while in other clades of figs deeper splits separate fig species that no longer hybridise or share wasps. In addition, we also suggest that complex short-term sorting (Fig. 1) and switching of wasps within fig sections is consistent with a longer-term pattern in which wasp genera map well onto fig sections, as long as host switches occur only rarely between fig sections.

Phylogeny estimation is subject to error from various sources and quality is often limited by both taxon and gene sampling. The problem is worse when one then aims to compare two phylogenies, each with errors, as in cospeciation studies. The most recent phylogenies of both pollinators (Lopez-Vaamonde *et al.*, 2009) and figs (Rønsted *et al.*, 2008a; Lopez-Vaamonde *et al.*, 2009) contain many more species and better coverage of higher taxa than most earlier studies. They also include more than one gene locus, but the increase to two/three, while a very useful step, leaves room for further improvement. Phylogenies should continue to improve as sequencing gets cheaper and easier and taxon sampling improves. Existing wasp markers are quite good at resolving low taxonomic levels, but less useful for the crucial deeper nodes. In contrast, fig markers often provide poor resolution at low taxonomic levels, which may sometimes be due to hybridisation between closely related species (Machado *et al.*, 2005).

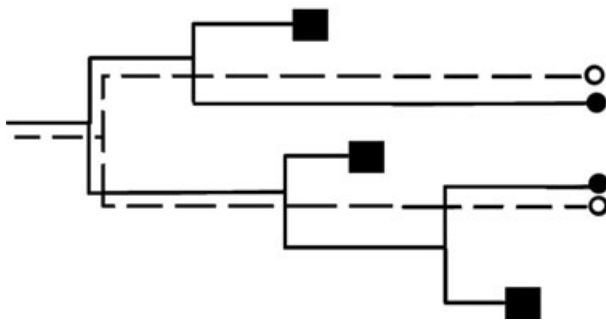


Fig. 1. An ancestral fig species (broken lines) has a single pollinator species (solid lines). These then cospeciate to produce two matched species pairs. However, sometimes a wasp speciates without the fig species doing so, leading to two wasp species associated with a single fig species. Later, there is sorting / extinction (squares) of some wasp species such that we return to the single fig/single wasp scenario. Despite several wasp speciation events that do not involve co-speciation, if we sample extant fig and wasp species (circles) and estimate their phylogenies, we will reveal strong congruence. Such a 'split and sort' process of pollinator speciation would be hard to detect from deep phylogeny, but can be suggested if extant co-pollinators of a single fig are often sister species.

Even if the underlying phylogenies are excellent, there is ongoing debate about how best to compare them (see Page, 2002). There is currently no single best approach and it is important to be aware that results may often depend on the method used (Jackson, 2004; Marussich & Machado, 2007). In addition, as phylogenies get larger, some methods may become impossible, or impractical due to computational demands. Simple tests can be used initially to detect whether there is any correlation between two phylogenies (see Lopez-Vaamonde *et al.*, 2001), but in the case of figs and pollinators we are interested in quantifying the role of cospeciation. Maximum cospeciation analysis, implemented in TREEMAP (Page, 1995), has been used extensively, for example to show significant speciation between Papuan figs and pollinators (Weiblen & Bush, 2002). A more sophisticated 'Jungles' approach was developed in TREEMAP 2 (Charleston & Page, 2002), and this allows exploration of different event (cospeciation, host shift, duplication) weighting options, but is very computationally intensive. A quite different approach (Legendre *et al.*, 2002), implemented in PARAFIT, still seeks the solution that maximises the amount of cospeciation in the system, but does so by comparing branch lengths between taxa rather than focusing on topology. Recent experience suggests that this kind of approach may currently be the only practical way of dealing with large phylogenies containing over a hundred species (N. Rønsted, pers. comm.). We do not aim to give a detailed review of these methods and issues, which are discussed in several recent papers (e.g. Page, 2002; Taylor & Purvis, 2002; Jackson, 2004; Marussich & Machado, 2007; Jackson *et al.*, 2008; Jousselin *et al.*, 2008), but it is essential to be aware that there are considerable inherent problems in this type of analysis.

How well do pollinators fit our expectations?

Recent studies of diverse *Ficus* sections in Australia (Lopez-Vaamonde *et al.*, 2002; Haine *et al.*, 2006), Africa (Michaloud *et al.*, 1985, 1996; Kerdelhue *et al.*, 1999), Latin America (Molbo *et al.*, 2003; Machado *et al.*, 2005; Su *et al.*, 2008), and Asia (Peng *et al.*, 2008) show that several monoecious *Ficus* species have more than one pollinator species and some have as many as four (Haine *et al.*, 2006). To date, pollinators have been sampled from 142 of the 396 monoecious fig species and there are 45 published cases of multiple pollinators on a single fig (J.-Y. Rasplus, pers. comm.). This suggests that about one-third of monoecious figs have multiple pollinators, but there are several potential sources of error, including misidentifications, under-sampling, and bias towards availability of data from common, widespread species that perhaps have more pollinators.

The excess of wasp species implies that many wasp species do not arise through strict cospeciation. In principle, the pattern could arise from our greater ability to recognise wasp than fig species, but there are good reasons to refute this. First, there are over 700 *Ficus* species described formally, but only about 150 agaonid species (Lopez-Vaamonde *et al.*, 2009), so any bias should be in the other direction. Second, co-pollinators often occur simultaneously and repeatedly in the same individual trees (Michaloud *et al.*, 1996; Lopez-Vaamonde *et al.*, 2002;

Greeff *et al.*, 2003; Haine *et al.*, 2006) and even syconia, so these species really do share a host. For whatever reasons, it appears that the wasps speciate more often than the figs.

Dioecious figs may present a slightly different picture, as studies in Papua suggest most figs have one local pollinator. However, it may be that co-pollinators of dioecious figs are largely allopatric (e.g. see Lin *et al.*, 2008) so have yet to be recorded as much as the often sympatric co-pollinators of monoecious figs. There is evidence to suggest that pollinators of dioecious figs disperse shorter distances, closer to ground level (Harrison, 2003; Harrison & Rasplus, 2006), which is consistent with them being more likely to develop geographic isolation and allopatric species. However, one recent study supported long distance dispersal and mixing of wasp populations between mainland and island populations of dioecious figs (Zavodna *et al.*, 2005), so further study is needed.

The converse pattern – one wasp across two figs – is also observed, but appears variable between monoecious fig lineages. Studies in Latin America support considerable sharing of pollinator species across apparently separate, but closely related, fig species (Molbo *et al.*, 2003; Machado *et al.*, 2005; Su *et al.*, 2008). In contrast, similar studies of monoecious Australian figs (Lopez-Vaamonde *et al.*, 2001, 2002; Rønsted *et al.*, 2008b) and Papuan dioecious figs (Silvieus *et al.*, 2008) have reported almost no cases of pollinators shared across fig species. This may sometimes be due to differences in the ages of taxa. For example, several figs in the Australasian subsection *Malvanthera* appear to be old species long diverged from their closest relatives (Rønsted *et al.*, 2008b), while other groups of figs in other sections may share more recent common ancestors or even still hybridise (Machado *et al.*, 2005).

Where a single pollinator species is repeatedly found in more than one fig species, it usually involves two figs from the same *Ficus* section. However, long (taxonomic) distance pollinator host shifts do occur and could provide valuable insight into the basis of host specificity. Without competition from the coadapted ‘normal’ pollinator introduced *Ficus* species, or those at the edge of their ranges, are sometimes colonised by local pollinators from other *Ficus* sections (Ramirez & Montero, 1988; Compton, 1990; Machado *et al.*, 2005). In some cases this results in viable hybrid figs. For example, the Asian species, *Ficus microcarpa* (subsection *Conosycea*) and *F. religiosa* (subsection *Urostigma*) have been introduced to the Americas and were both found to be pollinated by local *Pegoscapus* species from section *Americana* figs (Ramirez & Montero, 1988). In Costa Rica, *F. microcarpa* was pollinated by *Pegoscapus tristanti*, the usual pollinator of *F. padifolia*, whereas in Florida *F. religiosa* was pollinated by the usual pollinator of *F. aurea*. In both cases, viable hybrids were produced. Such cases might permit occasional gene flow between figs from different sections and possibly even occasional origin of major phenotypic novelty prompting the evolution of, for example, a new section of figs and associated pollinator lineage.

From the wasp perspective, phenotypic matching constrains host shifts. First, small wasps may be able to enter large figs, but not vice versa. For example, we have recently found

that, where *F. macrophylla* is planted outside its natural range, its syconia are sometimes entered by *P. imperialis*, the normal pollinator of *F. rubiginosa* (J. M. Cook and D. W. Dunn, unpublished). These figs both belong to the same subsection (*Malvanthera*) but *P. imperialis* is smaller than *P. froggatti*, the normal pollinator of *F. macrophylla*. Where the two figs occur in natural sympatry, we have also detected *P. imperialis* in about 1% of *F. macrophylla* fruits, but have never found *P. froggatti* in *F. rubiginosa* syconia (J. M. Cook & E. Webster, unpublished). Second, while wasps may enter figs, they may not be able to reproduce. In the example above, seeds are set in the *F. macrophylla* fruits but no wasp offspring are produced. This may well be because the ovipositor of *P. imperialis* is too short to reach down the longer flower styles to lay eggs.

Reconsidering pollinator speciation

Figs and pollinator wasps have cospeciated to some extent, but we do not yet know what percentage of wasp speciation is due to the underlying events of cospeciation, host shifts, and duplication (i.e. the wasp speciates, but the fig does not) (Page, 2002). Most consideration of fig and pollinator speciation has concentrated on coevolution, and on how population subdivision could lead to phenotypic codivergence and cospeciation (e.g. Ramirez, 1970; Kiester *et al.*, 1984). If a fig/pollinator system is split into allopatric populations then divergence by adaptation or genetic drift could generate reproductive incompatibilities in one or both taxa upon secondary contact. Candidate traits include physical size matching, timing of fig phenology (Kiester *et al.*, 1984), habitat use (e.g. forest and savannah patches – Michaloud *et al.*, 1985, 1996), or the spectra of volatile chemicals produced, which differ between even closely related fig species (Ware *et al.*, 1993; Grison-Pige *et al.*, 2002a,b). Once two subpopulations of wasps are restricted to different subpopulations of figs (whether by a geographical barrier or by, for example, chemical or habitat differences), they then begin to act, in turn, as reproductive isolating agents for the figs by partitioning pollen flow between the fig subpopulations.

However, the apparent excess of wasp over fig species means that many extant wasp species have not arisen through cospeciation. It also seems likely that wasp speciation can proceed more rapidly in absolute time than fig speciation, because fig generation time is about 100 times longer than wasp generation time. We therefore explore further how wasps might speciate through duplication (Page, 2002); i.e. without fig speciation, or any host shift. This could manifest itself as follows under geographic isolation:

- 1 Fig and wasp populations split into geographically and genetically isolated sub-populations, for example as climate change fragments tropical forests.
- 2 Divergence occurs between populations due to selection and/or drift, but proceeds more rapidly in wasps than in figs due to generation time.
- 3 Secondary contact occurs because forest expands again, or the fig species evolves to exploit a wider range of habitats along an ecological gradient.

- 4 On contact, figs but not wasps can interbreed so the two wasps begin to co-occur and may later become largely sympatric.
- 5 Over longer time periods, one pollinator species may go extinct due to competition.

If the above scenario applies, then co-pollinators of a given fig species should be sister species. In contrast, if they arise by host shifts, they will generally not be sister species. Although a small proportion of host shifts could give rise to sister co-pollinators, if a high proportion of co-pollinator cases involve sister species, a major role for duplication in wasp speciation is suggested. This is an eminently (if laboriously) testable prediction and testing it would add considerably to our understanding of wasp speciation in this system.

An intriguing question is what happens to co-pollinators in the long-term? Standard ecological theory suggests that two species should not be able to coexist in the same niche (Zhang *et al.*, 2004). In some cases, co-pollinators are segregated by habitat or have essentially allopatric distributions (Michaloud *et al.*, 1985, 1996; Kerdelhue *et al.*, 1999); however, many cases involve sympatric wasp species (Cook & Rasplus, 2003; Machado *et al.*, 2005; Haine *et al.*, 2006; Peng *et al.*, 2008; Su *et al.*, 2008). These could coexist if their niches differ in subtle ways. So far there is no evidence for subtle differences in flower use within syconia (Molbo *et al.*, 2003). However, anecdotal evidence suggests that yellow or pale pollinators are attracted to light and disperse at night, whereas brown/black pollinators do not come to light and disperse by day. Perhaps such a dispersal difference could contribute to coexistence in some cases.

Even more intriguingly, it has been hypothesised that they can coexist through frequency-dependent sex ratio shifts (Zhang *et al.*, 2004). According to this model, population growth rate is frequency dependent because when a species is rare, females tend to be single foundresses and therefore produce highly female biased broods (increasing population growth rates) due to local mate competition (Herre, 1985). In contrast, when a species is common, females tend to form multi-foundress groups and produce a higher proportion of males (decreasing population growth rates) (Zhang *et al.*, 2004). This is an intellectually satisfying idea, but its applicability may be limited by details of the biology. First, the number of foundresses entering a syconium appears to be under considerable control from the fig and not just a passive feature of wasp density (e.g. Herre, 1989). Second, this model seems inappropriate for coexistence of pollinators in fig species such as *F. obliqua* (Lopez-Vaamonde *et al.*, 2002) that are rarely entered by more than one foundress.

This leaves the distinct possibility that one species will outcompete the other, but over a long time period. Such long transient dynamics would allow us to often observe two extant co-pollinators, even though one may ultimately leave no daughter species. If co-pollinators have often arisen by duplication with one species subsequently going extinct, this might have little impact on the phylogenies of extant fig and wasp species, which could appear to derive largely from strict cospeciation (Fig. 1). In other words, such a 'split

Table 2. Major taxa of non-pollinating fig wasps.

NPFW taxon	Larval biology	Enter figs	Distribution
Epichrysomallinae	Large gallmakers	No	Old World
Eurytomidae	Parasitoids/inquilines	No	Old World
Sycophaginae	Mostly gallmakers*	No [†]	Pantropical
Sycoryctinae	Parasitoids/inquilines	No	Mostly Old World
Otitellinae	Gallmakers	No [†]	Mostly Old World
Sycoecinae	Gallmakers	Yes	Old World

*But at least one shown to be a parasitoid (Yang *et al.*, 2005).

[†]A few species do enter figs.

and sort' process could be common, but we will need to test for it by looking at extant pairs of co-pollinators, not deep history. The main process that can disrupt long-term matching of fig and wasp phylogenies is host switching, especially between distantly related figs. Interestingly, there does appear to have been ancient duplication in the pollinators of African *Galoglychia* figs and this has not been followed (yet) by sorting, as we observe fig species pollinated by extant wasps from more than one genus (Table 1). Here, duplication leaves a signal, but if sorting has occurred, it may be more or less invisible, even if common (see Fig. 1).

Another little studied factor that could be important in fig wasp speciation is the disruptive action of microbial endosymbionts, including *Wolbachia* bacteria. It is likely that *Wolbachia* bacteria infect the majority of arthropod species (Hilgenboecker *et al.*, 2007) and studies in Panama (Shoemaker *et al.*, 2002) and Australia (Haine & Cook, 2005) have shown that they occur in about three quarters of the fig-pollinating wasp species surveyed, as well as about half of the NPFW species studied. *Wolbachia* often cause reproductive incompatibilities between infected and uninfected individuals and can lead to partial or complete reproductive isolation between sympatric or allopatric subpopulations of the same species. In theory, *Wolbachia* can drive host insect speciation on their own, but only under relatively restrictive circumstances. However, under a wide range of circumstances, they could act to facilitate or reinforce speciation, allowing it to proceed more rapidly than otherwise. There is clearly room for a lot more work on this topic, although the natural history of fig wasps does not lend itself easily to controlled crosses and mating experiments.

Speciation in NPFW

The fig-pollinating wasps form a single monophyletic clade and probably number about a thousand species. In contrast, NPFW belong to several independent lineages that have colonised figs at different times in their evolutionary history (Rasplus *et al.*, 1998) and number several thousand species (Cook & West, 2005). Some of these lineages are only distantly related to each other and our understanding of their relationships with other wasps not associated with figs is limited (Rasplus *et al.*, 1998). The current classification recognises five large NPFW

subfamilies, all from the super family Chalcidoidea, that are associated exclusively with fig fruits. In addition, there are various small to large radiations on figs of other wasp families (e.g. Eurytomidae) that are also well represented on hosts other than figs and fig wasps. Most of the NPFW subfamilies are thought to consist largely of gall-forming species (Table 2), but the biology of relatively few species has been investigated in detail. The subfamily Sycoryctinae contains species that have been shown to be parasitoids or inquilines of other wasps (Joseph, 1959; Yang *et al.*, 2005; Tzeng *et al.*, 2008; Zhai *et al.*, 2008), including the pollinators, and it is likely that most species in this subfamily lay eggs into the galls of other wasp species and kill or outcompete them. In contrast, most Sycophaginae are gall-makers, but one *Platyneura* (= *Apocryptophagus*) species has been shown to be a parasitoid of pollinators (Yang *et al.*, 2005), while an *Idarnes* species is a facultative feeder on seeds (Pereira *et al.*, 2007). Nevertheless, there is a great need for further work on the larval biology of NPFW species (Cook & Rasplus, 2003).

For ecological studies, NPFW can be placed into four groups according to their trophic roles and time of attack: (1) large gallers that lay eggs early in syconium development and that may prevent the abortion of unpollinated syconia, (2) large parasitoids of these large gallers, (3) smaller gallers, including the pollinators, that oviposit slightly later in syconial development, and (4) parasitoids of the small gallers. In at least some cases, the large and small wasps may form essentially separate modules of the fig wasp community, even though they develop inside the same syconia (Compton *et al.*, 1993; West *et al.*, 1996).

Broad patterns of host use by NPFW

NPFW taxa differ considerably in their distribution across continents and fig sections (see Figweb: <http://www.figweb.org/>). The most striking difference is between New and Old World communities. The former are either complex and dominated by Sycophaginae (section *Americana* figs), or simple and dominated by a few Sycoryctinae (on section *Pharmacoscycea*). In contrast, communities on Old World monoecious figs often contain a mix of higher taxa, involving several of Sycophaginae, Sycoryctinae, Oritesellinae, Sycoecinae, Epichrysomallinae, and Eurytomidae to produce more taxonomically diverse assemblages.

There are also differences between monoecious sections on the same continent; for example, in Australasia, Oritesellinae are prevalent in communities on subsection *Conosycea* figs but absent from those on subsection *Malvanthera* figs. Overall, there are major differences in both presence and dominance of NPFW taxa across fig sections and while some of the major groups may have coevolved with figs and pollinators for tens of millions of years over wide areas of the globe, others are more recent and local radiations. For example, to date *Megastigmus* species (Family Torymidae) are known only from three fig species in subsection *Malvanthera*, but the wider genus is large and associated with a range of host plants, including oaks and conifers. We should therefore expect considerable variation in the radiation patterns of different lineages. Nevertheless, the framework of thinking in terms of cospeciation, host shifts and

duplication can still be useful. However, it is more likely (than with the pollinators) that similar fig and NPFW phylogenies represent delayed 'phylogenetic tracking' by the NPFW rather than true cospeciation (e.g. Silvieus *et al.*, 2008). In addition, with parasitoid/inquiline taxa we should also consider how divergence has proceeded relative to the insect hosts (e.g. pollinators) as well as the host plants (Lopez-Vaamonde *et al.*, 2001; Marussich & Machado, 2007; Silvieus *et al.*, 2008).

Cospeciation of NPFW and hosts

Several studies have compared the phylogenies of regional NPFW radiations with phylogenies of their *Ficus* or insect hosts (Machado *et al.*, 1996; Lopez-Vaamonde *et al.*, 2001; Weiblen & Bush, 2002; Jackson, 2004; Joussetin *et al.*, 2004; Jiang *et al.*, 2006; Joussetin *et al.*, 2006; Marussich & Machado, 2007; Joussetin *et al.*, 2008; Silvieus *et al.*, 2008). Most of these involve wasps from one or two fig sections, but one study of Asian *Philotrypesis* wasps, most of which are parasitoids/inquilines of pollinators, found little correspondence between *Philotrypesis* phylogeny and *Ficus* sections and the authors argue that 'rampant host shifting' has occurred (Jiang *et al.*, 2006). Further studies of both parasitoid and galler taxa across fig sections are needed to test if, for example, host switching is more common for parasitoids than gallers.

There is evidence for considerable cospeciation between NPFW and figs of the endemic African *Ficus* section *Galoglychia* (Joussetin *et al.*, 2004, 2006, 2008). Phylogenetic analyses demonstrated that both the parasitoid/inquiline genus *Philotrypesis* and two distinct species groups of the galling genus *Oritesella* have cospeciated with their *Galoglychia* host figs, with support from both topological congruence and molecular dating. In fact, in this case, the NPFW lineages show greater congruence with the figs than do the pollinators, due partly to the ancient pollinator duplication mentioned in the section above. However, the overall picture from this body of work is that whole communities have tended to codiverge (Joussetin *et al.*, 2008). This means that there is a discrepancy between the *Philotrypesis* patterns found in Asia (Jiang *et al.*, 2006) and Africa (Joussetin *et al.*, 2004, 2006, 2008). This deserves further study, but one possibility is that *Philotrypesis* may be a long-term component of *Galoglychia* fig wasp communities, but a more recent invader of some Australasian ones, as suggested by low levels of genetic divergence between distinct *Philotrypesis* species in Asia (Jiang *et al.*, 2006) and Australia (S. T. Segar, unpublished).

Another interesting example involves the galler genus *Platyneura* (= *Apocryptophagus*) and figs in the subgenus *Sycomorus*. Weiblen and Bush (2002) concluded that there was no significant congruence in their phylogenies, but a later study (Silvieus *et al.*, 2008) involving more species found evidence for cospeciation. This emphasises the wider point that some studies may have failed to detect significant cospeciation due partly to the low statistical power available when using small phylogenies. Silvieus *et al.* (2008) subsequently used molecular dating to support the idea that the congruence stemmed from delayed phylogenetic tracking by the gallers, rather than strict sense cospeciation. Indeed, studies of many

insect radiations on plants support delayed colonisation by the insects with a subsequent radiation that may, or may not, reflect plant phylogeny (Ehrlich & Raven, 1964; Lopez-Vaamonde *et al.*, 2003, 2005; Stone *et al.*, 2009). In some cases, molecular divergences suggest waves of radiation, involving first plants, then herbivores, then parasitoids (Lopez-Vaamonde *et al.*, 2005). This may be a common theme in tri-trophic systems and fig wasp communities offer a fertile testing ground for such ideas.

Studies on the pollinators and NPFW of some section *Americana* figs reveal complex patterns of specificity and radiation and only limited evidence for congruence between any of the lineage phylogenies (Marussich & Machado, 2007). The authors conclude that host-shifting and duplication events have dominated the interaction between pollinators and NPFW. However, there was also evidence for long-term codivergence between some ecologically interacting guilds, including pollinators and their competitors, and large galls and their parasitoids. This detailed study again shows how conclusions may be influenced by choice of analytical methods when comparing phylogenies, a point emphasised by Jackson (2004), who also showed that choice of congruence measure and the level of taxonomic sampling have strong effects on cospeciation tests.

NPFW host specificity and host shifts

We lack extensive data on NPFW host specificity and even those we have are often limited by the alpha taxonomy or sampling effort involved (Cook & Rasplus, 2003; Cook & West, 2005).

Nevertheless, it seems likely that most NPFW are specific to just one or a few fig species. Silvieus *et al.* (2008) describe patterns of host association in *Platyneura* gall makers and *Apocrypta* parasitoids from dioecious figs in Papua. They found that *Platyneura* species were on average only slightly less host-plant specific than the associated pollinators, while the few *Apocrypta* parasitoid species were rarer and some used three different host insects/plants. Since gall makers are less common than pollinators in figs, *Apocrypta* parasitoids of gall makers face a low abundance of any one host species and this may select for more generalist host use. It also suggests that we might find different levels of host specificity between parasitoids of pollinators, which are generally very abundant, and those of other gall makers that are more patchy and less abundant (Silvieus *et al.*, 2008). This is consistent with some results from our ongoing work (J. M. Cook and S. T. Segar, unpublished) on monoecious figs in the section *Malvanthera*. Here, the most prevalent and numerous NPFW species belong to the genera *Sycoscapter* and *Philotrypesis*, which are parasitoids/inquilines of the pollinators (Joseph, 1959; Tzeng *et al.*, 2008; Zhai *et al.*, 2008). To date, we have not found evidence that any of the several species in these two genera occur on more than one fig species.

Is host shifting more common in NPFW than in pollinators? The ostiole (entrance tunnel) is a major hurdle for wasps that enter syconia and this is reflected in striking convergent morphological evolution between pollinators and sycoecine

NPFW that enter the same figs (van Noort & Compton, 1996). Internally ovipositing NPFW should therefore be highly host-plant specific. However, most NPFW bypass the ostiole by injecting eggs directly through the fig wall, which is likely to reduce constraints on host-shifting (Lopez-Vaamonde *et al.*, 2001; Marussich & Machado, 2007). In addition, trophic role may be more labile within NPFW genera than once thought (Yang *et al.*, 2005; Pereira *et al.*, 2007) and this might facilitate host shifts too. However, even NPFW need to be well adapted to their host plants. Consequently, the impressive phenotypic diversity of syconia across fig species (Herre, 1989; Berg & Wiebes, 1992; Cook & Rasplus, 2003) still presents formidable constraints. If we consider just syconium size, then across fig species these structures vary from about the size of a pea to a tennis ball, and contain from about 10 to 10 000 flowers. The ovipositor of a small NPFW has short absolute length, even if it is long relative to body size, and since larger syconia tend to have thicker walls, many potential host shifts may be ruled out by the inability of wasps to reach the appropriate fig ovules (see Dunn *et al.*, 2008). A successful host shift also requires that the invader is not outcompeted by better adapted 'native' wasps, thereby making the number of available niches and competing species in new hosts important determinants of host-shift success (van Noort & Compton, 1996).

The degree of fig/wasp reproductive synchrony required by developing larvae and the specificity of chemical recognition may also add important constraints to host switching. Volatile cues are important in maintaining species specificity amongst pollinators (Ware *et al.*, 1993; Grison-Pige *et al.*, 2002a,b) and it has been suggested that NPFW that oviposit at the same stage of fig development and use these cues will demonstrate a correspondingly high level of specificity (Jousselin *et al.*, 2006). An interesting study by Proffit *et al.* (2007) also suggested that NPFW attacking figs at other developmental stages also use volatile cues to gauge when a fig is suitable for them. As syconia develop, volatile emissions change and NPFW species were attracted only to the volatile cocktail emitted at the appropriate window of opportunity (Proffit *et al.*, 2007). A natural progression is to consider how fig species-specific these 'secondary' volatile emissions are. If this volatile variation is low within *Ficus* sections, then NPFW, host specificity may be reduced and shifts between similar sized figs with similar developmental times may be more common.

Ecological speciation in a community context

New NPFW species could arise by changes in ecological role in the same community (niche shift) or by moving to a new community (host shift). Both occur in a community context and the number of available niches and their degree of saturation by existing species (Compton & Hawkins, 1992) will be important factors influencing speciation potential. There is evidence from African communities that parasitoid diversity tracks galler diversity and that galler diversity may be constrained by the historical incidence of colonisation events (Compton & Hawkins, 1992). In addition, there is an intriguing pattern that dioecious fig wasp communities are much less diverse

than those on monoecious figs (Kerdelhué & Rasplus, 1996). This may reflect the simpler internal structure of dioecious figs (a single flower layer) limiting the opportunities for niche divergence (Kerdelhué & Rasplus, 1996).

An interesting example is provided by the community on *F. racemosa*. This is both an unusually well-studied community (e.g. Ghara & Borges, in press; Yang *et al.*, 2005; Wang & Zheng, 2008) and an unusual one. It is unusual because this fig is a member of a small radiation of secondarily monoecious figs (section *Sycomorus*) within a larger clade of otherwise dioecious figs (subgenus *Sycomorus*). Its NPFW community of six species is small in comparison to communities from most well-studied monoecious figs in other sections, but large relative to those on most dioecious species (Kerdelhué & Rasplus, 1996). In China, there are three *Platyneura* species and two *Apocrypta* species, with species in both genera separated by ovipositor length, which reflects the timing of oviposition. Two *Platyneura* species are gall-makers and each has a specialist *Apocrypta* parasitoid. Surprisingly, the third *Platyneura* species appears to be a parasitoid of the pollinator (Yang *et al.*, 2005), a previously undescribed niche for a member of the subfamily Sycophaginae. This community shows the effects of both evolutionary history and ecological speciation. First, it hosts wasps from only two NPFW genera like its close dioecious relatives, but in contrast to the more taxonomically diverse communities of most monoecious figs from other sections. Second, there is niche separation between congeneric pairs of galls and pairs of parasitoids, as well as a major niche shift (galler to parasitoid) by one species (*P. agragensis*) that probably arose through a host shift from a dioecious fig.

More evidence for ecological speciation comes from *Platyneura* galls associated with dioecious *Sycomorus* figs in Papua (Weiblen & Bush, 2002). Here, sister species exploiting the same fig show marked divergence in relative ovipositor length. Such ecological speciation events (Johannesson, 2001) may be an important factor in explaining NPFW biodiversity and divergences from one to one cospeciation. If Kerdelhué and Rasplus's (1996) appealing explanation for the difference in wasp diversity between monoecious and dioecious figs is correct, we might also expect large syconia to have greater potential for niche divergence than small ones and correspondingly richer communities. However, several factors probably combine to determine community diversity and composition (Compton & Hawkins, 1992), including phylogenetic and biogeographic constraints on colonisation, as well as ecological factors such as host-plant density and geographic range.

Acknowledgements

We would like to thank the NERC, BBSRC, Operation Wallacea and the University of Reading for supporting our research on fig wasps. We are also grateful to Jean-Yves Resplus and an anonymous referee for valuable comments on the manuscript and to many colleagues working with figs and fig wasps for discussion of ideas relating to speciation and coevolution over several years.

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First published online Accepted 3 October 2009