

Biology, Ecology, and Evolution of Fig-pollinating Wasps (Chalcidoidea, Agaonidae)

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ABSTRACT

The *Ficus*-agaonid wasp association is a classical example of mutualism and coevolution. Agaonids breed in the *Ficus* inflorescence – the fig – and ensure pollination, often through an active behavior. The association is species-specific and seems to have evolved mainly through cospeciation.

The higher order classification of both Agaonidae and *Ficus* reflects convergent adaptation and not monophyly. A remarkable match exists between the classification of *Ficus* and agaonids, suggesting parallel phylogenies resulting from cospeciation; most exceptions cited in the literature arise from imperfect taxonomy. The distribution of extant species coupled with molecular phylogenies allows us to propose a phylogeographic reconstruction of the history of the association which most probably diversified during the breaking up of Gondwana.

Despite an identical basic life cycle, there is much variation among species in details of their biology. Receptive figs attract the wasps by producing a relatively simple, but specific chemical message. Wasps pollinating monoecious species seem to generally disperse over much larger distances than wasps pollinating dioecious species. This correlation seems to be indirect and to result from a correlation between the *Ficus* breeding system and life form. Having entered a receptive fig, the wasps oviposit and pollinate. In monoecious figs, ovules are stacked in several layers, and wasps oviposit mainly in the ovules located close to the fig cavity, probably because offspring will emerge more easily from their galls. When depositing an egg, the female wasp also injects a drop of fluid that will induce the enlargement of adjacent tissue. The emerging larvae then either induce the parthenogenetic development of the endosperm on which they will feed or, if the ovule has been fertilized, feed on the developing triploid endosperm. It seems that the double fertilization of the ovule facilitates successful galling of the ovule. Active pollination has probably evolved as a means to fertilize the ovules into which the wasps had oviposited and not as a means to increase seed set. Counter-adaptations of the host *Ficus* ensure that some of the actively deposited pollen grains are used for seed production instead of for wasp larvae development. Probably

as a result of the evolutionary conflict between the host *Ficus* and insect on which flowers should be fertilized, active pollination has been lost numerous times in the evolutionary history of the wasps.

Mature wasps trigger the ripening of the fig in which they are enclosed. The males emerge from their galls, mate with the females enclosed in their galls, and finally often cut an exit hole from the fig allowing females to exit. At this stage, the fig may have a large internal cavity or almost none. This, associated with variation in male behavior, leads to diversified patterns of female emergence and can lead to male fighting. In female figs, the ripening process is quite different from that in figs hosting wasps. We propose that the ripening process of female figs prohibits the successful reproduction of pollinating wasps as well as that of most other wasps associated with the system. For each of the successive steps of the wasps' life cycle, wasp behavior and variation in wasp behavior and morphology can be interpreted in terms of coadaptation.

INTRODUCTION

Fig wasps include the monophyletic fig-pollinating Agaonidae (Hymenoptera: Chalcidoidea) and a number of other chalcid wasp lineages and even some braconid wasps that have evolved independently to exploit the Agaonidae-*Ficus* association (Rasplus et al. 1998). They include ovule gallers and parasitoids or cleptoparasitoids. Because of the complexity of the taxonomic and biological assemblage, the present chapter will mainly deal with the fig-pollinating wasps, that is, Agaonidae (sensu stricto). The numerous studies on the mutualistic association between Agaonidae and *Ficus* (Moraceae) have provided a wealth of information on this classic example of coevolution (Anstett et al. 1997, Weiblen 2002, Cook and Rasplus 2003).

In the following sections, we present details of the agaonid wasp-*Ficus* interaction, and also present some elements of variation within this large assemblage of species. Then we describe some elements of their taxonomy, phylogeny, and biogeography. Finally we shall outline the life cycle of Agaonidae, following the successive steps of wasp behavior, presenting available knowledge and the selective forces that can explain the observed traits. This will allow us to present a series of new ideas about the functioning of the interaction. Surprisingly little is known precisely in terms of wasp behavior and variation. Often, knowledge stems from observations, often casual, of a few species. We have chosen to describe what is known to provide evidence for a series of open lines of research. As figs constitute the habitat influencing the evolution of Agaonidae, we will treat aspects on *Ficus* as well as those of the wasps.

AGAONID WASP BIOLOGY

Life Cycle

Each of the 700 plus species of *Ficus* (Berg 1989) is exclusively pollinated by one or, rather often, a few specific agaonid wasp species (Rasplus 1996, Cook and Rasplus 2003, Molbo et al. 2003), which breed only in their specific host species. The inflorescence of *Ficus*, the fig (or syconium - from the Greek *sykos* meaning fig), is an urn-shaped receptacle, closed by apical bracts

forming the ostiole, and lined internally by uniovulate female flowers and male flowers. When the fig is receptive it produces a specific odor which attracts the mated pollen-laden female agaonid wasps (Grison-Pigé et al. 2002a). The wasp enters the fig through the ostiole, often losing wings and antennae beyond the third segment. Once inside, the wasp walks on the stigmatic surface and probes the flowers, introducing its ovipositor through the style (Fig. 1). The egg is deposited beside the ovule (Jousselin et al. 2001a). Hence, if the style is too long, no egg is deposited. While ovipositing, the wasp also pollinates. Then the females die and each wasp larva develops at the expense of a single ovule transformed into a seed-sized gall. Some weeks later, the offspring become adults. Males emerge from their galls, and search for galls containing females. They gnaw a mating hole in the gall and, using their telescopic genitalia, mate with the female that lies entrapped within the gall. At the end of the mating period, the males cut an exit hole out of the fig through which the females loaded with fresh pollen leave the fig. The freshly emerged females fly rapidly away in search of a new, receptive fig.

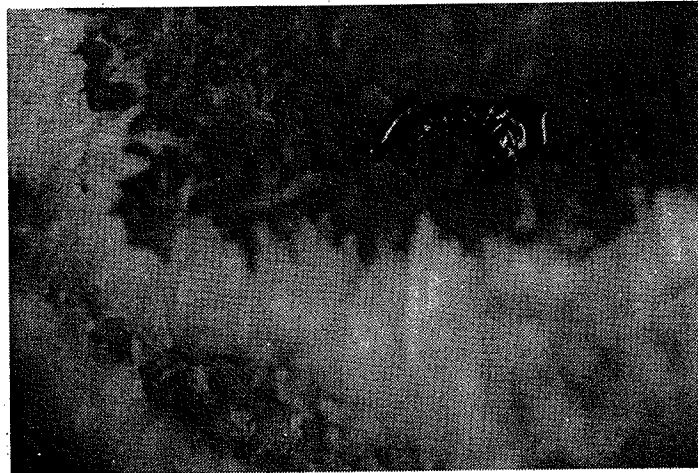


Fig. 1 Ovipositing female *Courtella armata* Wiebes. The wasp has lost the wings and the terminal part of the antennae while passing through the ostiole. The stigmas are intermingled, making it difficult for the wasp to deposit pollen precisely on the stigmas of the flower into which it oviposits. The stigmas have conspicuous papillae allowing pollen tubes to grow from one stigma into another. (Photograph by J.-Y.R.)

The Fig-wasp Community*

A community of up to 30, mostly specific, chalcid wasp species develops within figs of each *Ficus* species. In almost all instances they oviposit into fig ovules and each galled ovule produces one insect. These species are mainly gall inducers, but a number of species develop in galls initiated by another species (cleptoparasitism, Joseph 1958). Most nonpollinating fig wasps oviposit into ovules from outside the fig using their long ovipositors. Larger gall inducers and their parasites oviposit early in fig development and

*Based on Cook and Rasplus (2003).

initiate large galls. They are generally present in low numbers per fig and in few figs per population. Smaller gall inducers (including the agaonid pollinating wasps) are much more speciose and numerous. They oviposit just before, during, or just after fig receptivity and are parasitized by similar sized wasps. Some groups of nonagaonid gall-inducing fig wasps enter figs to oviposit at the same time as the pollinator. The association with figs has evolved several times independently, and wasps belonging to the same functional group may have widely different evolutionary origins. The biology of nonagaonid fig wasps is poorly known despite their impact on the fig-agaonid mutualistic interaction; often figs produce more nonagaonids than agaonids.

Monoecy and Dioecy

About half the *Ficus* species are monoecious (Berg 1989). Each fig produces both wasps and seeds. At receptivity, flowers are stacked in several layers within the fig, but all the stigmas reach the same height due to variable style length (Ganeshiah and Kathuria 1999). Wasps preferentially oviposit in shorter styled flowers, located close to the fig cavity (Jousselin et al. 2001a, 2003a). Longer styled flowers, located close to the fig wall, mainly produce seeds (Nefdt and Compton 1996, Anstett 2001). After wasp emergence, the fig ripens and becomes attractive to a very large array of fruit consumers, some of which disperse the seeds (Shanahan et al. 2001).

The remainder of *Ficus* species are functionally dioecious, male trees producing wasps (pollen vectors) and pollen and female trees producing only seeds. Female trees bear figs in which all the styles of female flowers are too long to allow wasp oviposition (e.g., Nefdt and Compton 1996). The wasps will successively probe the styles but, as the tip of the ovipositor does not reach the fig ovule, no egg is laid. Figs of female trees produce only seeds and neither wasps nor pollen. Male trees bear figs in which all-female flowers bear very short styles. These flowers either produce a wasp or remain empty, so that, although the male figs include both male and female flowers, they are functionally male, producing only pollen and attracting pollen vectors (Kjellberg et al. 1987).

Active and Passive Pollination

In about one-third of *Ficus* species (Kjellberg et al. 2001), pollination is passive. At the time of wasp emergence, abundant pollen is shed, thus dusting the wasps completely with pollen. Just after leaving the fig, the females carefully clean themselves, but some pollen is trapped between the abdominal pleura, as the gaster retracts on exposure to the external air. When the wasps enter a receptive fig, the gaster progressively swells in the moisture saturated internal atmosphere. This enables the progressive dispersal of pollen within the fig cavity (Galil and Neeman 1977).

In other *Ficus* species, pollination is active. Anthers are few and pollen is not shed. Female wasps, before leaving their natal fig, go to the anthers, pick some pollen with their forelegs and introduce it into specialized sternal

structures called pollen pockets. Once the wasps have entered a receptive fig, each time they deposit an egg, and before withdrawing the ovipositor from the style, they pick some pollen with their forelegs and deposit it on the stigmas (Galil and Eisikowitch 1974). In actively pollinated *Ficus* species, little pollen is produced so that no passive pollen transport occurs (Galil and Meiri 1981, Kjellberg et al. 2001). In the actively pollinated female figs, although the wasps do not deposit eggs, each time they introduce their ovipositor into a style they deposit some pollen (Galil 1973, Balakrishnan Nair and Abdurahiman 1987).

Active pollination is rare in nature. Other than fig wasps, it has been shown only in *Yucca* (Pellmyr 1997) and in *Senita* cactus (Cactaceae) (Holland and Fleming 1999).

Morphological Traits of Agaonid Wasps

Wings of female agaonids are exceptionally large (Bouček 1988; J.-Y.R., unpublished observation), a trait which may be associated with gliding in the wind to reach host trees. The head is modified to enter the fig: it is flat and wedge-shaped, appearing as an extension of the thorax. The mandibles bear appendages that help the wasp move through the fig ostiole. The antennae are deeply modified; the distal segments beyond the third have numerous sensillae, linearia, or chaeticha, for locating the host *Ficus* and are intended to break off when penetrating the fig ostiole. The first three segments are strong, the third one usually with a toothlike expansion, which is used for entering the fig ostiole. The antennae are located forward on the frons, but the part of the frons behind the antennae generally includes a longitudinal depression into which the first three antennal segments can fold back. The fore and hind femurs are strong. The front tibia and hind tibia bear teeth. The legs are adapted for pushing the wasp body through the ostiole. The body is smooth (Bouček 1988).

Male agaonids are wingless, amber colored, and their heads projecting forward, and their eyes often reduced; the antennae are generally located in the antennal groove at the front of the head, project forward, and are quite simple. The pronotum is well developed, the mesonotum, metanotum, and propodeum are more or less fused; the entire mesosoma is relatively long. The front legs project forward and have large, flattened, strong femora, stout tibiae, and a short but strong tarsus. These legs are modified for digging among the flowers to reach the galls containing the females. The hind legs also have large, strong femora, but the tarsi are not as short as the fore ones and project laterally, thus enabling the wasps to remain steady. The intermediate legs are slimmer and may be reduced. The gaster is folded, or can fold forward under the thorax, so that its tip can project beyond the head. Hence, males can insert their genitalia into galls containing females without releasing their grip on the gall. Most male agaonid wasps are so modified to move among flowers that they cannot walk on a flat surface (F.K., unpublished observations).

Because so many wasp traits are constrained by fig traits, great care should be taken to separate traits shared due to common ancestry from convergent adaptations. Indeed, among nonagaonid gall-inducing fig wasps, those that enter the figs to oviposit have similar adaptations to female agaonid wasps, such as smooth body, head projecting forward, teeth on the tibiae (Bouček 1988), and some species even have mandibular appendages (van Noort 1994). They all seem, however, to lack the peculiar antennae that are able to be broken off.

Variation in Agaonid Wasp Behavior

While the functioning of the *Ficus*-pollinator interaction is quite homogeneous among species, variation occurs at all stages of the cycle. Such variation provides the basis for a comparative analysis.

Upon landing on a receptive fig, wasps may either cooperate, teaming up to lift the outer ostiolar scale (e.g., *Blastophaga psenes* L. [F.K., unpublished observation]), or compete aggressively with each other (e.g., *Pegoscapus mexicanus* Ashmead [M.H., unpublished observation], *Eupristina verticillata* Waterston [M.H. and E.J., unpublished observations]). To enter the fig, most pollinating wasps use the third antennal segment which generally is denticiform. They press the antennae forward and use the 'teeth' to lift the ostiolar scales. While entering the fig, they often lose the distal part of the antennae and their wings. In some species, however, there is no apical projection of the third segment and the distal part does not break off (e.g., *Tetrapus* [Ramirez B. 1970], *Alfonsiella fimbriata* Waterston [Michaloud and Deveze 1982], and *Elisabethiella baijnathi* Wiebes [Baijnath and Ramcharun 1988]). On entering the fig cavity, the female wasps may either ignore or attack each other (e.g., *Pegoscapus* [Ramirez B. 1970], *Pegoscapus mexicanus* [M.H., unpublished observation], *Platyscapha awekei* Wiebes [J.M. Greeff, S. van Noort and F.K., unpublished observations]). Then the wasps may present a set of behaviors associated with pollen pockets specifically aimed at achieving pollination (active pollination) or this set of behaviors may be totally lacking (passive pollination). While removing pollen from the pockets, the wasp may brush the fore legs directly on the stigmas, or it may brush the fore legs together above the stigma dusting them in the process, or it may deposit the pollen on the hypopygium which will be dragged over the stigmatic surface afterwards (Frank 1984). However, more precise observations are necessary to confirm this variation. Once the wasp has oviposited, it may either die in the cavity or it may re-emerge from the fig (Gibernau et al. 1996, Moore et al. 2003) and may even enter a second fig (e.g., *Blastophaga psenes* [Gibernau et al. 1996], *Wiebesia nipponica* Grandi [Okamoto and Tashiro 1981], *Liporrhopalum tentacularis* Grandi [Moore et al. 2003]). Then each larva will develop at the expense of an ovule. Larvae and seeds develop, the pedicels of the flowers elongating so that most often, but not in all species, the developing ovaries fill the internal cavity of the fig. In some species (notably subgenus *Sycomorus*), the cavity becomes drenched with a liquid (Weiblen 2002), and in such species the cavity may remain open throughout development.

When the wasps are adults, the fig usually softens and enlarges so that the flowers become loose allowing wasp movements. The internal cavity usually remains small, almost nonexistent. However, in some species there is a large internal cavity (e.g., subgenus *Sycomorus* [Weiblen 2002], subsection *Galoglychia* and part of subsection *Caulocarpae* of section *Galoglychia* [Greeff et al. 2003], *Ficus eximia* Shott of section *Americana* [Pereira et al. 2000], one species of section *Oreosyceae* [S. Ungricht, unpublished observation]). Males emerge from their galls and search for females to mate. In most cases males ignore each other, but in some species they even engage in active fights (Greeff et al. 2003). Males cut a hole in the galls containing females and mate by introducing their telescopic abdomen. Mated females may stay in their galls (e.g., *Platyscapha quadraticeps* Mayr [Galil et al. 1973]), waiting for males to cut an exit hole from the fig or for the ostiole to loosen, or they may emerge rapidly (Michaloud and Devez 1982, Greeff et al. 2003), crowding the cavity, while waiting for males to cut an exit hole. In some species, females, after mating, enlarge the mating hole and emerge freely without any help from the males. In other species, the males, after mating, enlarge the mating hole (e.g., *Courtella*, *Alfonsiella* [Michaloud and Devez 1982]). In some instances, the males grab the females by the antennae, drawing them out of their galls, and often remove the remainder of the exuvia around the antennae of the females in the process (e.g., *Alfonsiella fimbriata* [Michaloud and Devez 1982], *A. binghami* Wiebes, *Allotriozoon heterandromorphum* Grandi [Greeff et al. 2003]). Once most females have been mated, a few males may chew small exit holes through the fig wall, while in some species most males team up and chew one or two large exit holes. In species in which many males chew the exit hole, when the males emerge from the fig, they may disperse in a ring around the hole, thus offering themselves as prey for ants and protecting the emerging females effectively (Zachariades 1994). In some species, most emergences from a fig occur within a few minutes in a synchronized flush, while in other species, despite a grouped peak emergence, these emergences may extend over several days (e.g., *Blastophaga psenes* [F.K., unpublished observation], *Platyscapha soraria* Wiebes [Greeff and Ferguson 1999], *Pleistodontes frogatti* Mayr [Bean and Cook 2001]). Exit holes may be chewed through the ostiole or laterally. In some species males do not usually chew an exit hole and the ostiole loosens naturally (e.g., *Blastophaga psenes* [Grandi 1929], *Wiebesia nipponica* [Okamoto and Tashiro 1981]). In a few species, some males leave their natal fig and enter another one, accessing more females (Greeff 2002, Greeff et al. 2003). Before leaving the fig, females may walk to the anthers and load pollen into their sternal pollen pockets. Anthers may be periostiolar or disperse, but the males can also cut off the filaments of the ostiolar male flowers so that the anthers disperse (Galil and Eisikowitch 1974). The anthers may be dehiscent or nondehiscent. In the latter case, females use their mandibles and the dentiform third antennal segment to slit the anther open (e.g., *Platyscapha soraria* [Baijnath and Naicker 1989], *Allotriozoon heterandromorphum* [Newton and Lomo 1979], *Pegoscaphus mexicanus*, *P. franki* Wiebes [Frank 1984]). As the

numbers of ovules within a fig may vary from one (in female figs of some forms or individuals of *Ficus deltoidea* Jack [Corner 1969]) to 21,600 (in *F. sakalavarum* Baker [Kjellberg et al. 2001]), crowding conditions vary widely. Relative numbers of male and female flowers are also highly variable (in *F. sakalavarum*, 250 anthers for 21,600 ovules; in *F. punctata* Thunb., 16,000 anthers for 18,000 ovules [Kjellberg et al. 2001]). Obviously, the conditions encountered by the wasps are much diversified.

FICUS AND WASP TAXONOMY AND BIOGEOGRAPHY

Taxonomy

The taxonomy of Agaonidae was revised by Wiebes (Berg and Wiebes 1992, Wiebes 1994, 1995) and *Ficus* taxonomy was revised for Asia by Corner (1960, 1965, 1967) and for Africa by Berg (Berg and Wiebes 1992).

The higher order classification of *Ficus* corresponds broadly to a combination of breeding system and growth form. Most monoecious freestanding trees are classified into subgenus *Pharmacosycea*. Monoecious hemiepiphytic (or epilythic) trees are classified into subgenus *Urostigma*. Dioecious *Ficus* were long united into a single subgenus, but they are now classified into two subgenera, subgenus *Sycomorus* and subgenus *Ficus*. Subgenus *Sycomorus* includes a few species that have reverted to monoecy (Weiblen 2000) but which present obvious reproductive and vegetative morphological similarities with their dioecious ancestors (Corner 1958, Berg 1989, Weiblen 2000).

Agaonidae are divided into two subfamilies, the Agaoninae and the Blastophaginae (Wiebes 1994). All wasps associated with figs with spiral ostioles (interlocking ostiolar bracts) are classified as Blastophagini. All wasps associated with figs with linear ostioles (all ostiolar bracts turned inward) are classified as Agaonini. Although assigning a female wasp to a tribe is straightforward, the morphological traits involved are submitted to strong directional selection linked with ostiole shape of host *Ficus* (Ramirez B. 1991, van Noort and Compton 1996) and no similar simple subdivision can be established using males only (Bouček 1988). Indeed, Wiebes proposed Blastophaginae as a monophyletic unit but Agaoninae as polyphyletic, with genus *Tetrapus* as the outgroup to all other genera (Wiebes 1994).

No good description of the traits separating the different genera of Agaonidae exists. Nevertheless, most genera are easy to recognize and probably constitute valid monophyletic entities, or maybe in some instances well delimited subentities of monophyletic entities (e.g., *Liporrhopalum* Waterston could branch within *Kradibia* Saunders, or *Nigeriella* Wiebes and *Alfonsiella* within *Elisabethiella* Grandi). The taxonomy of most species looks convincing; however, that of some species (e.g., species of *Blastophaga*) needs re-evaluation. Clearly, *Tetrapus* presents the most ancestral traits, having, for instance, a typical chalcid antenna. The genera associated with section *Galoglychia* seem to form a monophyletic unit, with the female pronotum reduced to lateral plates. The genera *Blastophaga* subgenus *Blastophaga* and

Wiebesia (sensu Wiebes 1994) seem closely related, as genera *Kradibia* and *Liporrhopalum*, and these four genera constitute together *Blastophaga* (sensu lato). Present morphological knowledge does not, however, allow greater precision in grouping genera.

Does Parallel Cladogenesis Exist between *Ficus* and Agaonidae?

It has been suggested that parallel cladogenesis between *Ficus* and Agaonid wasps could be the rule (Thompson 1989, Herre et al. 1996). This is supported by each *Ficus* section or subsection being pollinated by its own genus or genera of Agaonidae (Table 1). At this level of investigation, there are only some slight discrepancies which have often been emphasized (e.g., Wiebes 1994), without critical re-evaluation of the taxonomic assignment of species. Many of the mismatches are, however, clearly due to imperfections of wasp or *Ficus* classification (F.K. and J.-Y. R., unpublished observations). It has also been suggested that because descriptions of agaonid wasp species followed *Ficus* classification, there was an inherent bias favoring an

Table 1 The association between *Ficus* and Agaonidae morphological classification

Subgenus	Section	Subsection	Agaonidae genus
Pharmacosyceae	Pharmacosyceae		Tetrapus
	Oreosyceae		Dolichoris
Urostigma	Malvanthera		Pleistodontes
		Galoglychia	Allotrioazon
		Galoglychia	Agaon
		Cyathistipulae	Courtella
		Caulocarpae	Elisabethiella,
		Platyphyllae,	Nigeriella,
		Chlamidodonae,	Alfonsiella,
		Crassicostae	Paragaon
		Americana	Pegoscapus
		Urostigma	Platyscapa
	Conosyceae	Eupristina, Deilagaon, Waterstoniella	
Ficus	Ficus	Ficus	Blastophaga
		Eriosyceae	Blastophaga
	Rhizocladus		Wiebesia
	Kalosyce		Wiebesia
	Sycidium	Sycidium	Kradibia
		Palaeomorphe	Liporrhopalum
Sycomorus	Sycomorus		Ceratosolen
	Neomorphe		Ceratosolen
	Adenosperma		Ceratosolen
	Sycocarpus	Sycocarpus	Ceratosolen
		New Guinea group^a	Ceratosolen

bold: linear ostioles (all the bracts turned inward), Agaoninae

italics: dioecious species

^a*F. microdictya* and *F. pritchardii* are monoecious.

artificial parallelism between the two classifications (Wiebes 1994, Weiblen 2002). This criticism does not stand close examination: wasp identification to genus is generally straightforward and on over 200 species we have found only few cases of erroneous assignment in the revisions by J.T. Wiebes (J.-Y.R. and F.K., unpublished observations) and host *Ficus* assignment to section is also generally straightforward (F.K., personal observation), with few species appearing problematic.

However, some situations show that cospeciation is not always the rule. Within the section *Galoglychia*, the subsections *Platyphyllae*, *Chlamydodora*, and *Crassicostae* are each pollinated by a combination of the closely related genera *Elisabethiella*, *Alfonsiella*, *Nigeriella*, and *Paragaon* (Berg and Wiebes 1992); and the discrepancies between wasp and *Ficus* classification can probably not be completely explained by the difficulties in classifying the host *Ficus* species. Further, in East Africa, individual trees of both *F. natalensis* Hochst. and *F. thonningii* Bl. are each pollinated by both a species of *Alfonsiella* and a species of *Elisabethiella* (Greeff et al. 2003). This shows that host shifts do sometimes occur. Another example will be section *Sycomorus*. It is mainly present in continental Africa and Madagascar. *Ficus sycomorus* L. is the sole species of the section present both on Madagascar and on the African mainland. Throughout its range of distribution, it hosts two species of agaonid wasps, an active pollinator, *Ceratosolen arabicus* Mayr, belonging to a Malagasy species group, and the sole known nonpollinating agaonid wasp, *C. galili* Wiebes, belonging to the African species group (Kerdelhué et al. 1999). Finally, in Panama, Molbo et al. (2003) have shown that in situations where two species pollinate the same host they are not necessarily sister species.

Preliminary molecular phylogenies of both *Ficus* and agaonid wasps suggest (1) monophyly of *Ficus* (Herre et al. 1996) and of fig-pollinating wasps (Rasplus et al. 1998, Machado et al. 2001), (2) monophyly of *Ficus* sections (Weiblen 2000, Jouselin et al. 2003b) and agaonid genera (Machado et al. 2001, Weiblen 2001), (3) nonparallel branching order of agaonid genera and *Ficus* sections (Machado et al. 2001), and (4) some support for fine scale cospeciation of wasps and *Ficus* (Weiblen 2001).

However, the molecular evidence supporting point (3) is weak. Molecular results (Weiblen 2000, Jouselin et al. 2003b) show that *Ficus* can be divided into three strongly supported lineages (Fig. 2), a basal one containing section *Pharmacosycea*, and two groups of equal rank; Group 1 (subgenus *Urostigma* except section *Urostigma*); and Group 2 (all other species, i.e., section *Oreosycea* [subgenus *Pharmacosycea*], section *Urostigma* [subgenus *Urostigma*], subgenus *Sycomorus* and subgenus *Ficus*).

The global arrangement of *Ficus* according to molecular data, although surprising in terms of some morphological traits, is consistent with several others. It allows grouping within a single lineage, Group 2, all species that present dissected leaves (some species of section *Oreosycea* and subgenus *Ficus*); all the species that have specialized ostiolar male flowers (species of subgenus *Sycomorus* and sections *Urostigma*, *Ficus*, *Rhizocladus*, *Sycidium*, and

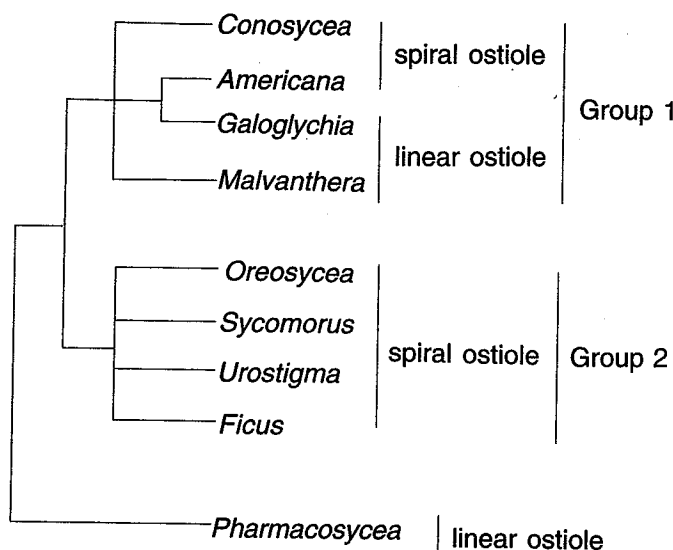


Fig. 2 A molecular phylogeny of *Ficus* using ITS and ETS sequences. Three monophyletic groups are defined: Group 1, Group 2, and the outgroup, section *Pharmacosycea*. Spiral ostioles seem to have evolved three times independently. (Modified, after Jousselein et al. 2003b)

Oreosycea); and all species with bisexual flowers (section *Oreosycea* and *Sycidium* for any male flower, but also *Ficus* and *Rhizocladus* for flowers located in the transition zone between male and female flowers). In agaonid wasps the analysis of structures is not yet sufficiently advanced to enable the characterization of genera and groups of genera. Nevertheless, Wiebes proposed in 1994: "*Platyscapa* [pollinating section *Urostigma*] and *Dolichoris* [pollinating section *Oreosycea*] are related to *Blastophaga* [pollinating section *Ficus*]." Further, males of the *Kradibia* pollinating *Ficus bambusaefolia* Seem. of section *Sycidium* can easily be confused with *Dolichoris* males (J.-Y.R., unpublished observation). Hence there seem to be morphological indications that the pollinators of *Ficus* Group 2 form a distinctive unit.

Further, the structure of the third antennal segment fits the phylogenetic results. This segment is simple, unspecialized, similar to that of typical chalcid wasps, or only slightly specialized in the basal group, *Tetrapus*. It is specialized, has a tooth, is undivided or divided into two in pollinators of Group 1, and it is divided into three in pollinators of Group 2 (J.-Y.R. and F.K., unpublished data). This categorization works at the generic level as the most frequent and strongest differentiation of the third antennal segment, but, within genera, often a few species present less specialized segments with reduced division and may even lack a tooth, as in *Pleistodontes mandibularis* Wiebes (Wiebes 1977), in *Alfonsiella* (Berg and Wiebes 1992), in some species of *Elisabethiella* (Bajjnath and Ramcharun 1988), in some species of *Waterstoniella* (J.-Y.R. and F.K., unpublished observation), and in *Dolichoris vasculosae* Hill (J.-Y.R., unpublished observation). This reduction is probably always associated with loose ostiolar bracts allowing easy entry into the fig. Third antennal segment structure is therefore highly adaptive. Nevertheless, while similar ostioles in sections *Americana* (Group 1), *Conosycea* (Group 1), *Oreosycea* (Group 2), and *Urostigma* (Group 2) (F.K., unpublished

observation) have led to a generally similar aspect of the wasps, they have not led to similar segmentation of third antennal segments. Thus the third antennal segment seems to convey a strong phylogenetic message. We predict that molecular investigation of the wasps will reveal a parallelism between the deeper nodes of wasp and *Ficus* phylogenies.

Hence, available evidence supports the idea that (1) parallel cladogenesis is the general rule in the '*Ficus*-pollinating wasp' association, but that (2) some host shifts occur between related species. The '*Ficus*-agaonid wasp' association is, therefore, a unique model for testing molecular phylogenies and morphological classification, as it is possible to compare wasp and *Ficus* phylogenies and to make morphological inferences.

Biogeography and Biogeographic History

Fossil evidence provides limited information on the biogeographic history of *Ficus*. The only nonambiguous agaonid wasp fossil is a 20–25 million yr old *Pegoscapus*, pollinator of section *Americana*, found in Dominican amber (Poinar 1993, Wiebes 1994). This fossil can clearly be identified to species group within *Pegoscapus* (J.-Y.R., unpublished observation). The often cited Oligocene *Tetrapus* fossil from Colorado (Brues 1910) is probably neither a *Tetrapus* nor even an agaonid wasp (J.-Y.R., F.K., unpublished observation). *Ficus* species are described from the late Cretaceous, but assignment of the fossils to *Ficus* is dubious (Tiffney 1986). The first fossil seed record is about 50 million yr old (Chandler 1962) and the first fossil pollen record is about 45 million yr old (Muller 1981). Hence, these records offer limited information on the biogeographic history as the '*Ficus*-agaonid wasp' association is probably close to 100 million yr old (Machado et al. 2001).

The basal group within *Ficus*, section *Pharmacosycea*, is restricted to America. Within Group 1, each of the sections occupies a different continent, with some minor overlap in Australasia. Within Group 2, each of the four subunits is spread all over the Old World, but is rather under-represented in continental Africa (Berg and Wiebes 1992), suggesting secondary colonization of Africa. So we may propose the following biogeographic scenario (Fig. 3). (1) *Ficus* originated on Gondwana. (2) The ancestors of Group 1 colonized the whole of Gondwana before differentiation of modern groups. (3) The ancestor of Group 2 diversified into modern groups within the continent constituted by the Indian–Antarctic–Australian plates and its species groups spread all over that continent. (4) The members of Group 1 were separated by continental drift, giving rise first to three units (Africa and South America [common ancestors of sections *Galoglychia* and *Americana*], India [section *Conosycea*], Australia [section *Malvanthera*]) and finally to four units with the separation of Africa from South America. Should this biogeographic scenario have happened, then section *Conosycea* would have colonized Asia about 50 million yr ago with the collision between India and Asia. (5) Continental Asia would have been colonized by the sections or subgenera of Group 2 first through India and much more recently maybe also through Wallacea. (6) Continental Africa would have been colonized

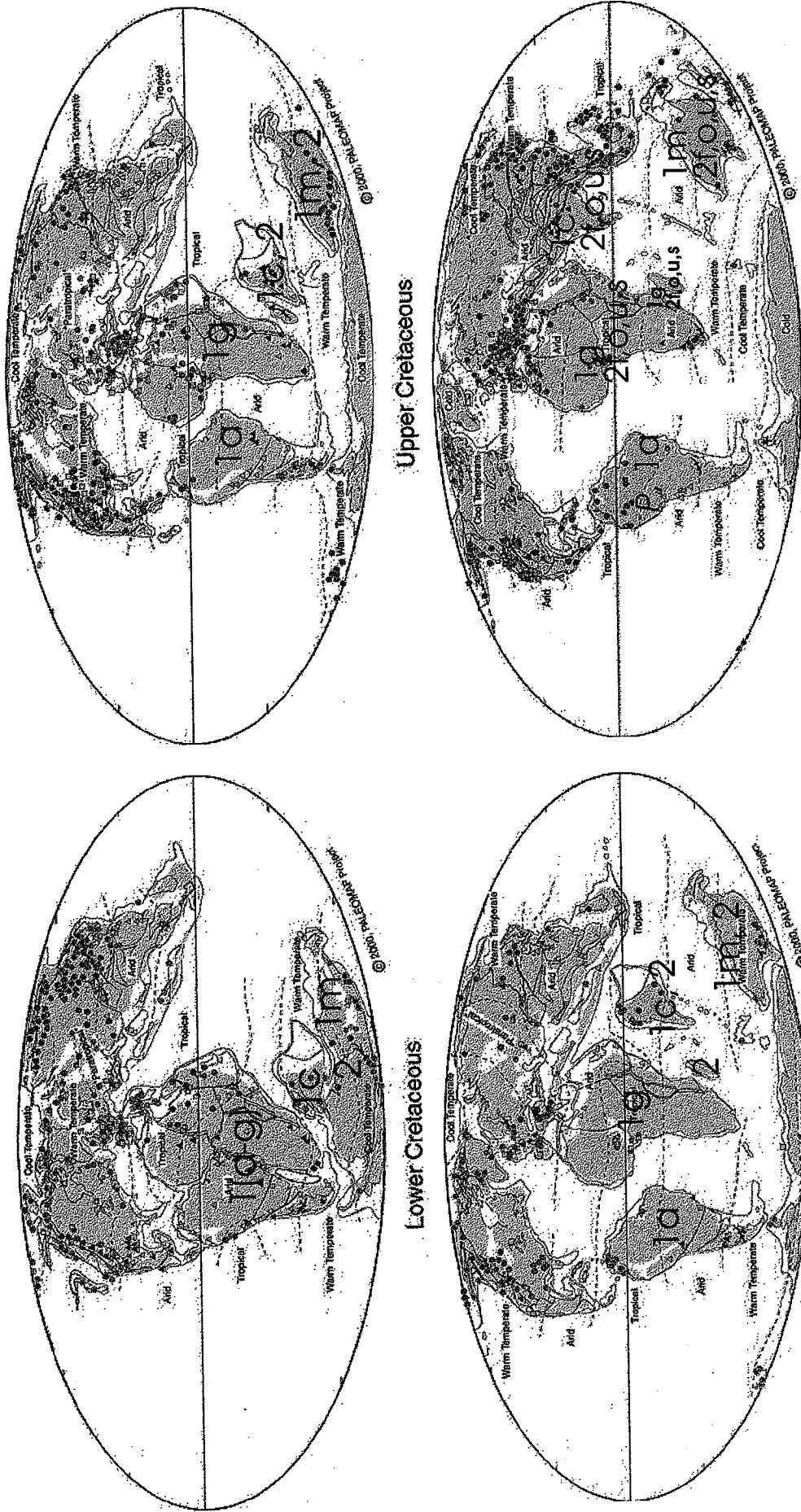


Fig. 3 A tentative reconstruction of *Ficus* paleogeography. P: the basal section *Pharmacosyceae*, 1: phylogenetic Group 1, 2: phylogenetic Group 2, a, b, c, m, o, u, (a-g): respectively, sections *Americana*, *Galglychia*, *Conosyceae*, *Malvanthera*, *Oreosyceae*, *Urostigma*, putative common ancestor of sections *Americana* and *Galglychia* f. s. respectively, subgenera *Ficus* and *Sycomorvus*, 2 indicates the presence of phylogenetic Group 2 with all its four lineages. (Background maps reproduced from Scotese 2002)

by Group 2 from Madagascar (section *Sycomorus*, section *Oreosycea*) and maybe also from Asia through Arabia (candidates: section *Sycidium*, section *Urostigma*). Hence, available evidence suggests that the diversification of *Ficus* and their associated agaonid wasps is ancient, mirroring the splitting up of Gondwana. This agrees with the result that Agaonidae is basal among Chalcidoidea (Rasplus et al. 1998).

LIFE CYCLE

A wealth of information is available on the successive steps of the Agaonidae biological cycle. Often researchers have worked on a limited number of species or on a limited number of taxonomic groups, and variation within the system has, therefore, been largely overlooked. We have presented here what is known about the wasps' life cycles, consolidating the present knowledge and extracting emerging patterns. We have chosen a linear description of the successive steps of the cycle. Each step is a tale in itself about adaptation and coevolution.

Free Life

Finding Receptive Figs

Reaching a receptive fig is a major challenge for the adult agaonid wasps. The life span of a free-flying female is short, from a few hours to 2 d (Kjellberg et al. 1988). This challenge is especially daunting for some *Ficus* species that may occur at low densities (less than 1 tree per 10 ha), synchronized within individual trees, but highly asynchronous between trees. Furthermore, a diversity of *Ficus* species often occurs in the same habitat, and the agaonid wasps rarely enter the figs of nonhost species (Weiblen 2002).

Data on how the wasps reach a tree bearing receptive figs are available for a single species, *Elisabethiella baijnathi*. On leaving their natal tree, the wasps fly up, and then they glide downwind, relatively high in the air column. When they pass a receptive tree, they fly down and fly upwind to the tree (Ware and Compton 1994a, b). This ability to fly is compatible with the larger wings of agaonid wasps, as compared with other chalcid wasps (Boucek 1988), suggesting adaptation to gliding. This scenario is compatible with the production of volatile compounds by receptive figs. Indeed, receptive figs produce blends of volatiles that are specific to the species, and composed of one to three major chemicals, such as benzyl alcohol, linalol oxide, and linalol in *Ficus carica* L. (Grison-Pigé et al. 2001a, 2002b). These volatiles attract specifically the associated wasp species (Gibernau et al. 1998, Grison-Pigé et al. 2002a).

Despite the importance of odors in ensuring fig visitation, and despite insufficient visitation of figs by wasps often limiting seed and wasp production (e.g., Bronstein 1988a), the energy investment by the fig tree in volatiles is limited, representing less than 0.1 percent of the tree's investment into figs (Grison-Pigé et al. 2001b). This suggests that attracting too many wasps may be counteradaptive. The risk of lack of pollination may however be

limited by individual figs of some species remaining receptive for up to 3 wk if unvisited, a remarkably long period for an inflorescence (Khadari et al. 1995).

These features of how wasps reach their hosts could explain why, in monoecious hemiepiphytic *Ficus* of Panama, the wasps reaching a tree may travel a distance of over 10 km (Nason et al. 1998). This long-distance attraction of wasps can, however, hardly be as efficient for the *Ficus* species fruiting at ground level in rain forests, as wind effect may be negligible. Indeed, for such species very local wasp transfers have been conjectured and transient local wasp extinctions have been witnessed (Harrison 2000a, b). Globally, more local-pollinating wasp dispersion for *Ficus* species of the forest understory seems to lead to the evolution of dioecy and to high endemism, while longer-distance pollinating wasp dispersion for *Ficus* species fruiting in (or close to) the canopy seems to lead to monoecy and limited endemism.

Within Figs

Oviposition and Gall Initiation*

Upon entering a fig, a female wasp oviposits by inserting its ovipositor through the stylar canal. When the ovipositor reaches the curved part of the stylar canal, elongated cells bordering the raphal strand guide the ovipositor which punctures the tissue at the junction of the raphe and the fused integuments. The lack of differentiated inner integument in that zone probably facilitates the penetration of the ovipositor. The wasp egg is deposited between the raphe and the nucellus. Near the wasp egg some drops of a secreted fluid staining green with PAS-methylene blue are deposited. Oviposition is followed by rapid growth of the ovule which reaches its final size in about 10 d. Fertilization is rapid, but the embryo sac hardly enlarges until the wasp egg hatches. The nucellar cells enlarge manifold and the integuments thicken. Expansion of the nucellar tissue occurs mainly on the side of the ovule where the egg is located, probably due to the drop of a secretion, and leads to the development of a round gall. Then the egg hatches, the larva migrates to the embryo sac and enters it when the ovule has reached final size. The globular stage of the fig embryo rapidly degenerates; the endosperm continues to grow and is progressively consumed by the wasp larva, while the nucellus is resorbed into the plant tissue. The gall wall is strikingly different from a seed wall in structure, and tissue layers involved, but a structural variation occurs among species. In female figs of *Ficus asperifolia* Miq., the elongate cells bordering the raphal strand are absent and the inner integument is not reduced, suggesting that these two anatomical peculiarities found in all female flowers of monoecious species and in female flowers of male figs are adaptations facilitating oviposition. No description using modern techniques of gall development in the absence of fig ovule fertilization is available. Nevertheless, gall development probably follows a similar course to that in the development of a parthenogenetic endosperm.

*Based on Verkerke (1986, 1987, 1988).

Choosing a Flower for Oviposition

In monoecious *Ficus*, at receptivity, the flowers are stacked in several layers, those located close to the fig wall presenting long styles and those located close to the fig cavity presenting shorter styles, so that all the stigmas reach more or less the same height and form a platform (Ganeshaiah and Kathuria 1999). The wasps' ovipositors in many species allow them to reach most flowers (Nefdt and Compton 1996). Nevertheless, eggs are preferentially deposited in the short-styled flowers located close to the fig cavity (Galil and Eisikowitch 1971, Verkerke 1986, Joussein et al. 2001a, 2003a), and consequently at fig maturity, galls containing wasps are mainly located close to the fig cavity while seeds are mainly located close to the fig wall (Galil and Eisikowitch 1971, Nefdt and Compton 1996, Herre 1999, Anstett 2001). Male agaonid wasps start by mating with females in galls located close to the fig cavity, and only afterwards mate with females located in ovules situated deeper down among the flowers. As a result, when males have cut the exit hole from the fig, females from galls located close to the cavity emerge very rapidly, while those located close to the fig wall are still unmated and could remain unmated (Anstett 2001). Delayed emergence from figs could be energy expensive for the wasps because of predation by ants. An alternative explanation for wasps ovipositing in flowers located close to the fig cavity was that pollinator eggs deposited close to the fig wall are more exposed to parasitism by wasps ovipositing through the fig wall, but in fact even such wasps preferentially use short-styled flowers (West and Herre 1994).

It could, however, be expected that when several foundresses enter a receptive fig, once the flowers located close to the cavity have been used up, the wasps would begin using all the flowers; however, they do not. Although the number of eggs laid usually increases with number of foundresses, and even when the foundresses bear more than enough eggs to fill up all the fig flowers, many flowers escape oviposition (Herre 1989, Joussein et al. 2001a, Moore et al. 2003) and foundresses die with many unlaidd eggs in their ovarioles (Kathuria et al. 1999). It could be that wasps will initially attempt to oviposit mainly in flowers located close to the cavity, and that in the process they damage longer-styled flowers, making them unsuitable for later oviposition. Competition could also potentially lead to several agaonid eggs being laid in the same ovule. We have only observed this to be frequent in *Tetrapus* pollinating section *Pharmacosycea*, despite histological checks of ovules after oviposition in sections *Americana*, *Galoglychia*, *Conosycea*, *Sycomorus*, *Urostigma*, and *Ficus* (E.J., unpublished data). Nevertheless, two wasps have never been observed to develop within the same gall. These observations suggest that in the basal section *Pharmacosycea* the mechanism of oviposition is subtle and different from that in the remainder of *Ficus*. They also suggest that in most *Ficus* sections the deposition of two eggs would lead to systematic failure of development, whereas in section *Pharmacosycea* deposition of two eggs within a flower could lead to the successful development of a *Tetrapus*.

In dioecious *Ficus*, a different situation prevails. In male figs, the flowers are usually presented in a single layer, the styles are short, and each stigma

is individualized. In *Ficus carica* there is a linear increase in number of eggs laid in a fig in response to foundress number (Ibrahim 1985) and in extreme situations with numerous foundresses, nearly every ovule produces a wasp (F.K., unpublished data). Hence it seems that male figs of dioecious species have adaptations facilitating wasp oviposition. Indeed the most reliable trait separating pollinators of monoecious and dioecious species is ovipositor length, which is always short in wasps associated with dioecious species and long in species associated with monoecious species (Wiebes 1994, Weiblen 2001).

In female figs, styles are longer than the ovipositor. The wasps systematically probe the stigmatic surface and introduce their ovipositor into the styles, but they do not deposit eggs in the flowers, probably because the ovipositor does not reach the fig ovule. Nevertheless, once foundresses have died within a female fig, some eggs can be found scattered among the stigmas (E.J., unpublished data).

Pollination

As described above, pollination may be passive or active. In passively pollinated species, pollen is abundant and wasps are completely dusted with the pollen. Among the actively pollinated species, pollen is scarce, and the wasps load pollen into specialized structures on the mesosterna called pollen pockets (Galil and Meiri 1981, Kjellberg et al. 2001).

Passive pollination is considered ancestral. Species of *Tetrapus* have remained passive pollinators throughout their evolutionary history. Active pollination has evolved once in a common ancestor of all other agaonid wasps (Machado et al. 2001, Jouselin et al. 2003b). Active pollination behavior would then have been lost within *Eupristina*, *Platyscapa*, *Pleistodontes*, *Ceratosolen*, *Dolichoris*, and *Wiebesia* (Jouselin et al. 2003b; F.K. and J.-Y.R., unpublished observations), all these genera being today polymorphic among species for mode of pollination. Active pollination would have been lost in an ancestor of *Deilagaon* and *Waterstoniella*, and in an ancestor of *Blastophaga* subgenus *Blastophaga* (Kjellberg et al. 2001). There are some indications pointing towards passive pollination also occurring in some species of *Pegoscapus*, *Liporrhopalum*, and *Blastophaga* subgenus *Valisia*. There have hence been many shifts in mode of pollination. Further, within *Platyscapa*, one passively pollinating species is present in the Réunion island, whereas the two other known passive pollinators occur in Peninsular Asia and the Australasian region, suggesting independent evolution. Similarly, within *Pleistodontes*, a close relative of the passively pollinating *P. rigisamos* Wiebes is the actively pollinating *P. cuneatus* Wiebes (Lopez-Vaamonde et al. 2001, Kjellberg et al. 2001), suggesting independent origin of passive pollination from other passively pollinating *Pleistodontes*. This shows that active pollination has often been lost over evolutionary time and hence that the selective forces stabilizing active pollination can be relaxed.

Why has active pollination evolved and why can it be lost? In a number of actively pollinated *Ficus* species, foundresses deprived of pollen have been introduced into figs. The figs always develop normally and completely, enabling the production of wasp offspring (Galil and Eiskowitch 1971, Nefdt

1989, Joussein et al. 2003a, Joussein and Kjellberg 2001). A reduction in brood size has been observed several times, suggesting larval mortality (Joussein et al. 2003a). Nevertheless, in the sole instance where offspring size has been measured, no reduction in the size of the offspring has been evident (Joussein et al. 2003a). Our current interpretation is that all agaonid wasps are gall inducers, but the fertilization of the fig ovule facilitates the successful transformation of the flower into a gall. Indeed, the early development of the wasp larva depends on the rapid expansion of the nucellar cells adjacent to where the egg was laid, before the larva migrates towards the embryo sac and begins to feed on the developing endosperm (Verkerke 1986, 1987). The rapid expansion of the nucellus would result from a galling process initiated by the drops of liquid deposited at the same time as the egg. The large size of the vesicle that stores this liquid in the adult females reinforces the importance of this liquid (Grandi 1929). Hence, all agaonid wasp larvae are gall inducers feeding first on the raphal cells and the nucellus, before consuming cells of the endosperm. Fertilization of the ovule would be a factor facilitating the initiation of endosperm development and thus facilitating the onset of the development of the resources on which the larva will feed.

In section *Pharmacosycea*, which has retained ancestral passive pollination by *Tetrapus*, long-styled flowers have elongate stigmas that project into the fig cavity, and short-styled flowers have short stigmas. The elongate stigmas brush the surface of the ovipositing wasps. As a result, mainly the flowers that do not receive a pollinator egg are pollinated and fertilized, and the flowers that receive a pollinator egg are not pollinated (Joussein et al. 2003a). Hence it may be suggested that in the ancestral situation, most wasp larvae developed as true gall inducers in unfertilized ovules.

Wasps would have developed the active mode of pollination as a means to ensure pollination and hence fertilization of the flowers into which they oviposited, that is, flowers that did not get pollinated by passive pollination. In the first stage, active pollination would have had no effect on seed set as the very strong differentiation between the stigmas of long-styled and short-styled flowers allowed precise pollen deposition on the stigmas of short-styled flowers. Such a situation may still be present in *Ficus macrophylla* Desf. ex Pers. pollinated by *Pleistodontes froggatti* Mayr. *Ficus macrophylla* has the numerous dehiscent male flowers of passively pollinated *Ficus* and indeed *P. froggatti* is abundantly dusted with pollen. Nevertheless, *P. froggatti* has well-developed pollen pockets that it fills with pollen before emerging from its natal fig. Hence *F. macrophylla* has the traits of a passively pollinated *Ficus* while its associated agaonid wasp is an active pollinator.

The step after the wasps had started pollinating actively would have been the acquisition of stigma traits in the host *Ficus* that made the pollen deposited for gall induction available for seed formation. In all actively pollinated monoecious figs we have observed at receptivity, the stigmas form a tightly packed surface and the stigmas are interlaced, limiting the precision of pollen deposition. Further, in most species, the stigmas remain

clustered together through their elongate papillae, so that pollen deposited on a given stigma may grow into the stigma of another flower (Jousselin et al. 2003b). (An exception is in the Asian and Australasian species of section *Oreosyce* in which the stigmas, though packed together, do not stick together [F.K. and S. Ungricht, unpublished data].) As a result, the pollination behavior of the wasps results in seed set. This would have allowed the reduction in anther number associated with active pollination in all instances other than *Ficus macrophylla*. The reduction in number of anthers would also have resulted in increased difficulty for the wasps in filling the pollen pockets. In all the actively pollinating wasps, combs on the forecoxa appear to gather pollen into pollen pellets to be pushed into the pockets; the combs are absent in all passively pollinating species. The sole exception to date is *Pleistodontes froggatti*, which lacks coxal combs, but its host tree produces abundant pollen (Kjellberg et al. 2001).

Hence, while active pollination has often been viewed as a wasp behavior selected to increase seed set it may be more realistic to view active pollination as a behavior evolved to subtract pollen from seed production. Seed production would be ensured by counter-adaptations of the host *Ficus*. Because of the underlying evolutionary conflict between the tree and the wasps, active pollination can hardly be expected to be optimal for both seed set and wasp larvae development. Indeed, in some species, the resulting pollination intensity is limiting for seed set as well as for wasp larvae development, as about half the ovules may go unfertilized and the association between presence of a pollen tube and presence of an egg in an ovule is random (Jousselin et al. 2003a). This may lead to loss of active pollination. Indeed, active pollination must involve a cost for the wasp, including time spent loading and unloading pollen and energetic cost associated with carrying a pollen load. If the tree becomes too efficient in generating pollen tubes to grow into seed flowers instead of into galled flowers, it may be expected that benefits for the wasps of pollinating actively become smaller than the costs involved, so that wasps may lose the behavior. There is within-species variation among trees in quantities of pollen produced, so that abundant pollen production could rapidly evolve in response to wasps giving up active pollination. Secondarily passively pollinated *Ficus* also rapidly evolve stigmas that project into the fig cavity, thus brushing pollen off the wasp's body.

Constraints associated with active and passive pollination are somewhat different in dioecious *Ficus* species (Jousselin and Kjellberg 2001). Active pollination is ancestral in dioecious species. Actively pollinated male figs present well-separated tubular stigmas. As a result, wasps manage to deposit pollen precisely on the stigmas of the flowers into which they oviposit and very little pollen ends up on the stigmas of nonoviposited flowers. The separation of the stigmas prevents growth of a pollen tube from one flower into another. This explains why active pollination is maintained as the wasps effectively benefit from the fertilization of the ovules into which they oviposit but it also explains why male figs produce hardly any seed. In female

figs, the stigmas are also tubular but are tightly packed and stick together (Fig. 4). In these figs, each time a wasp introduces its ovipositor into a style it does not oviposit, since the ovipositor can not reach the ovules; despite this, however, the wasp deposits pollen before removing the ovipositor (Galil 1973, Balakrishnan Nair and Abdurahiman 1987). Pollen is secondarily dispersed through lateral growth within the synstigma (Jousselin and Kjellberg 2001). The stigmas in female figs are tubular and lack an elongate branch intertwining with other stigmas as in monoecious actively pollinated figs. This may be explained through male–female developmental correlation in stigma structure: it could be that selection in male figs for tubular stigmas also results in tubular stigmas in female figs.

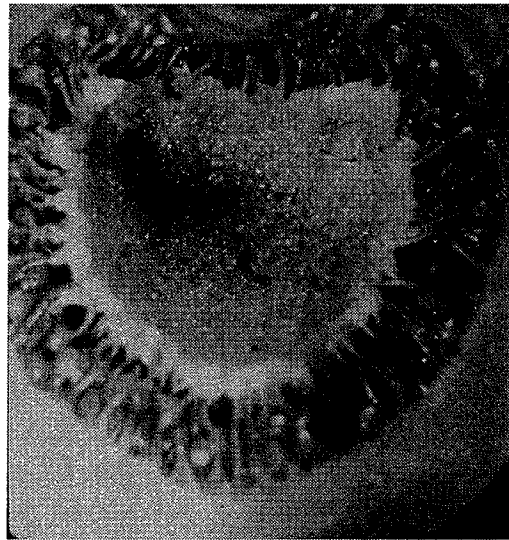


Fig. 4 The stigmatic surface in a female fig of the actively pollinated *F. condensa*. The stigmas are fused together through their papillae, limiting the precision of pollen deposition and allowing pollen tube growth from one stigma into another. (After Jousselin and Kjellberg 2001, used with permission from Blackwell Publishing)

In passively pollinated dioecious species, the wasps disperse pollen randomly within male figs. The stigmas are, however, hardly sticky so that most pollen ends up between the flowers and few pollen grains adhere to the stigmas. Further, pollen tube growth is more or less impeded. As a result, in passively pollinated species male figs produce no seed as the ovules are not fertilized and the wasps always develop as true galls in unfertilized flowers (Jousselin and Kjellberg 2001). In male figs, the stigmas are generally slightly bifid, whereas in female passively pollinated figs the stigmas are strongly bifid, elongate, and project into the cavity brushing the body of the wasps and they are sticky so that pollen attaches to them (E.J., F.K., unpublished observations). Pollen tubes germinate and grow efficiently in female figs, but there is no pollen tube growth from one style into another.

The precise association, in male figs of actively pollinated species, of pollination of a flower and oviposition within it confirms the hypothesis that wasps benefit from fertilization of the ovule into which they oviposit. The strict association throughout *Ficus* between mode of pollination, *Ficus*

breeding system, and stigma structure shows that the relationship is adaptive (Jousselin et al. 2003b). The sole exception to the association rules are the secondarily monoecious species of subgenus *Sycomorus* that have retained the tubular stigmas of dioecious *Ficus*.

Given how passive pollination is effected, it can be inferred that, in passively pollinated *Ficus* species, any wasp that emerges from figs at the same time as agaonid wasps and that would enter figs to oviposit at the same time as agaonid wasps would efficiently transfer pollen. Further, given internal conditions within figs, timing of entry into and emergence from figs is always synchronized with that of the agaonid wasp. A number of nonagaonid chalcid wasps have evolved fig-entry behavior. Of those, *Robertsia*, *Diaziella*, *Eujacobsonia*, and *Lipothymus* are associated with passively pollinated *Ficus* species and are hence pollinators (Jousselin et al. 2001b). Interestingly, fig-entering nonagaonid wasps seem to be evolutionarily much more recent than agaonid wasps and no situation has yet been found in which they have replaced the agaonid wasps as pollinators. These wasps can probably not evolve into active pollinators as they probably deposit their eggs within the ovule and hence consume the embryo sac before the triploid endosperm has had time to develop (Galil and Eisikowitch 1970, Jousselin 2001). The precise location at which the agaonid egg is deposited seems to be an important factor in the evolution of the *Ficus*-agaonid wasp mutualism. While two species of pollinating agaonid wasps have yet to be shown to coexist in the same habitat, co-occurrence of pollinating *Waterstoniella* (Agaonidae), *Diaziella* (Sycoecinae), and *Lipothymus* (Otitesellinae) on the same host within the same habitat seems to be usual, suggesting that each genus exploits somewhat different resources within figs (Jousselin et al. 2001b).

Brood Sex Ratio

An ovipositing female wasp, further to choosing in which ovules to lay eggs and whether it will pollinate, should also decide on the sex of its offspring. Agaonid wasps breed under typical Local Mate Competition conditions (Hamilton 1967): all matings are between individuals that hatch and develop within the same fig before the dissemination of mated female offspring. Hence, if only one mated female wasp enters a fig it should lay mainly daughters and just enough sons to fertilize their sisters and cut the exit hole from the fig. If many females enter a fig, they should produce about as many sons as daughters as the situation will be close to panmixis. Modeling the system allows to predict that females are selected to lay increasing proportions of males with increasing numbers of foundresses. This is what has been observed in agaonid wasps: females seem to adjust the sex ratios within their broods in response to the numbers of other foundresses they encounter within a fig (Herre 1985, Herre et al. 1997). Sex ratio adjustment is most precise under conditions the wasp species frequently encounters in nature (Herre 1987, West and Herre 1998). A full understanding of the sex ratio strategies of the different foundresses will however require thorough experimental studies and the use of genetic markers to assess the order in

which eggs of the different sexes are laid and the individual contribution of each foundress and hence establish the fine individual strategies. Indeed, when several foundresses colonize a fig, some lay few eggs, and this should strongly affect their sex-ratio strategy towards laying mainly male eggs (Kathuria et al. 1999).

Sex ratio adjustment has a major consequence for wasp strategies. If only one female wasp colonizes a fig it will lay a strongly female-biased clutch of eggs and, as a result, for each egg laid it will produce about 0.90 to 0.95 mated females. On the contrary if the wasp encounters another foundress, it will produce a more male-biased sex ratio, so that for each egg laid, it will produce fewer mated females, maybe approximately 0.80 mated females. This represents a 10–20 percent reduction in contribution to the next generation (for an extensive overview of sex-ratio response to number of foundresses see Herre et al. 1997). Hence, in itself, sex ratio is a strong selective force in favor of females trying to avoid figs that have already been entered by other females. As this combines with potential competition with other females for oviposition sites, there is a very strong selective pressure to avoid already entered figs, and this may explain the often under-dispersed distribution of numbers of foundresses per fig (when the few aberrant figs are removed, Janzen 1979).

Wasp sex ratio is also important for the fig tree. As only female wasps disperse pollen, strongly female-biased sex ratios will make the tree's investment into pollen vectors much more efficient. Tight ostioles can be seen as the result of selection on the tree to oblige wasps to lay all their eggs within the same fig, thus giving rise to local mate competition conditions.

A second consequence of sex ratio adjustment occurs when two agaonid wasp species occupy the same host *Ficus* in sympatry. In *Ficus* species in which there are often several foundresses per fig, it may be expected that the least common agaonid species is more often present as one foundress per fig. If so, the least common species should produce more female-biased sex ratios and hence benefit from a demographic advantage. This factor should facilitate agaonid species coexistence on the same host. Surprisingly, when geographic distribution of co-occurrence of two pollinating agaonid wasps on the same host species has been investigated, this co-occurrence seems to be limited to contact zones between habitats, each species occurring in a different habitat (Michaloud et al. 1985, Kerdelhué et al. 1997). Hence, the limited available data suggest that sex-ratio adjustment only rarely provides a sufficiently strong frequency-dependent selective factor to allow species coexistence. The suggested co-occurrence of cryptic species of agaonid wasps (Molbo et al. 2003) has yet to be confirmed.

Fig Development

After wasp oviposition and/or pollination, the flowers within the fig swell rapidly, generally filling up the fig cavity. Developing wasps seem to be highly sensitive to excessive temperatures and fig transpiration limits their internal temperature, thus allowing wasp survival (Patiño et al. 1994).

Agaonid wasps may also be subject to parasitism by other fig wasps and competition for nutrients with nonpollinating fig wasps. Ants on the tree can benefit the agaonid wasps by disturbing parasites ovipositing from outside (Compton and Robertson 1988, Cushman et al. 1998, Harrison 1996), although the ants may also feed on the agaonid wasps trying to enter the receptive figs (Bronstein 1988b), on emerging wasps (Harrison 1996, Zachariades 1994, Dejean et al. 1997), and even on unripe figs (Dejean et al. 1997). The relationship with ants is relatively complex, as some ant species may ensure protection against other ant species through exclusion (Dejean et al. 1997). The presence of more ants on male than on female trees, as observed on a few species of section *Sycocarpus*, suggests that the higher availability of fig wasps on male trees is sufficient to attract predatory ants (Harrison 1996; M.H. and B. Schatz, unpublished observations).

In dioecious *Ficus*, seed size and wasp size may be very different, seeds being either much smaller (e.g., section *Kalosyce*) or much larger (e.g., *F. deltoidea*) than the wasps. Further, development duration of wasp figs and seed figs may be different and in general seed figs develop more slowly (Patel and McKey 1998, Harrison et al. 2000). (An exception is *Ficus carica* [Kjellberg et al. 1987].) In monoecious *Ficus*, agaonid wasps and seeds seem always to retain similar sizes and figs generally ripen shortly after wasp emergence. Further, monoecious fig development time is similar to that of male figs (Harrison et al. 2000). Hence, the simultaneous development of pollen vectors and seeds within a fig seems to put a strong constraint on the system.

Mating Period and Emergence from the Fig

The end of fig development is complex, especially in the monoecious species. First, the fig must modify its internal structure to allow wasp activities such as copulation, pollen loading, and emergence from the fig, and then the fig must become attractive to fig consumers. In general, wasp behavior is also strongly synchronized within fig and among figs ripening on the same day, so the hours of beginning of male activity and of female wasp emergence from figs are predictable.

Change in Fig Structure

Clearly, before the ripening period, the fig does not provide a suitable environment to enable wasp activities. This is illustrated by the fact that all wasps, agaonid wasps, and parasites of the system emerge simultaneously. In a majority of *Ficus* species, after oviposition, the fig cavity disappears by expansion of the flower pedicels and ovule growth. The fig becomes tightly packed, preventing any wasp movement inside. Just before male wasp emergence, the fig swells a little, so that the ovules become less tightly packed, allowing wasp movement among the flowers. In some species, final fig swelling is much more pronounced so that a large internal cavity is formed. Alternatively, in a number of *Ficus* species, a cavity remains throughout fig development (Weiblen 2002), but in most, if not all, of those species the fig becomes drenched with liquid that will only be resorbed when male wasps become active.

Wasps are important in determining the timing of change in fig structure preceding wasp activities: when wasp larvae are absent, the figs remain hard much longer (Galil and Eisikowitch 1971; F.K., unpublished observations). There must be some form of messenger synchronizing wasp maturation: when the males start chewing mating holes, some females are not yet receptive, but become rapidly so during the following hour (F.K., unpublished observation). Cross introductions of wasps into legitimate and illegitimate hosts provide further evidence for fig and wasp mediation (Dull 1992). *Pegoscapus franki* on its host, *F. citrifolia* P. Miller, develops more rapidly than *P. mexicanus* on its host, *F. aurea* Nuttall. When *P. mexicanus* was introduced into *F. citrifolia*, it managed to extend the development time of the figs as measured from time of visitation to time when the exit hole was cut. But when *P. franki* was introduced into *F. aurea*, it did not manage to shorten the development time of the figs. In both cases the situation led to decrease in brood size and to inability of many wasps to exit their galls. We suggest that fig swelling, allowing mating behavior, is determined by two factors: (1) the fig must have reached the proper physiologically responsive stage and (2) the wasp larvae produce the message triggering swelling. This hypothesis is sustained by the fact that *F. sycomorus* figs show an autoinhibition of ethylene formation in the figs during wasp development that is lifted at the time the wasps begin to become active, allowing the induction of ripening (Zeroni and Galil 1976).

Matings and Male Behavior

Generally there is a very limited cavity at wasp maturity. The agaonid males exit their galls and proceed to mate with the females. They locate a gall containing a receptive female and gnaw a mating hole into it, introduce their genitalia, mate with the female, and then move on to find another gall containing a receptive female, digging among the flowers with their stout fore legs and hind legs. After mating, the females stay in their galls, waiting for the males to cut an exit hole through the fig wall. As the females stay in their galls, they may be mated several times. Males begin by mating with females in galls located close to the fig cavity and only afterwards search for galls located deeper among the flowers (Anstett 2001). Males return several times to the most accessible galls, located close to the cavity, gnawing anew at galls presenting mating holes and remating (or attempting to remate) the females (J.M. Greeff, S. van Noort, and F.K., unpublished observations). When many, but not all, females are mated, some males begin to cut exit holes from the fig, individually or in pairs (F.K., M.H., unpublished data). Once an exit hole has been cut, females rapidly emerge from their galls, load pollen if they are active pollinators, and leave the fig. In this situation, the male behavior of cutting an exit hole may be interpreted as the means, for a male, to prevent the females he has mated from being mated by other males. It seems that females stay in their galls as long as they are submitted to an atmosphere rich in CO₂ and that gas exchange through the exit hole could be the mechanism triggering their emergence (Galil et al. 1973)

Another pattern of male behavior is observed in *Ceratosolen* and some species of other genera. The figs present a large internal cavity filled with liquid before wasp activity (Weiblen 2002). Males often emerge into the cavity before all the fluid has been resorbed and are therefore adapted to moving around and breathing in a liquid environment. This may explain the very peculiar organization of male anatomy. The propodeum of male *Ceratosolen* overhangs the gaster as a free flap and has large to very large spiracular peritremata, a unique disposition in Agaonidae, so that the propodeum functions as a respiratory organ (Compton and McLaren 1989). In *Ceratosolen* subgenus *Rothropus* Wiebes, the hind legs are modified, elongate with numerous bristles (Wiebes 1963), and are probably used to swim. Matings go on as in the previous pattern with the occurrence of multiple matings (Murray 1990). However, at one stage, all females emerge into the cavity (Galil and Eisikowitch 1974), so that no more females (or only a few) are available to be mated. In such species, females are not sensitive to CO₂ (Galil and Eisikowitch 1974). The males then team up and cut cooperatively a large exit hole through the fig wall, allowing rapid emergence of all the females from the fig (Harrison 2000b). It may be suggested that male cooperation results from the fact that no more females are available to be mated, so that in these species males do not have a choice between cutting an exit hole and mating with more females. There is, however, a problem with this interpretation, at least in some species. Males may show an additional behavior: they cut off the filaments of the anthers so that the anthers drop into the fig cavity (Galil and Eisikowitch 1974), and this behavior starts while females have not yet emerged into the cavity. In some species in which the cavity disappears during development and only reappears at fig maturity, male behavior is similar, but they lack adaptations to swimming.

The third pattern of male behavior has been observed in some pollinators of section *Galoglychia*. In these species a large internal cavity is also present at fig maturity. The male, after mating a female, enlarges the mating hole, grabs the female by the antennae, and pulls her out into the cavity (Michaloud and Devez 1982, Greeff et al. 2003). Therefore each female is mated only once. As in the previous case, once all the females have reached the cavity, the males collectively cut an exit hole from the fig. The main difference from the previous situation is that the availability of mateable females progressively decreases, increasing competition among males for females. In a number of such species, the males present adaptation to fighting and indeed begin to fight when mateable females become rare (Michaloud and Devez 1982, Greeff et al. 2003). Adaptations to fighting include falcate mandibles, broad pronotum, rather fused mesonotum, metanotum, and propodeum, thinner, often longer legs, but also rapid movement. Adaptations to fighting do not seem compatible with digging deep among the flowers, so that male fighting does not seem to have evolved in figs in which flowers are stacked in many layers at maturity. Male fighting also occurs in some species of the first type (females staying in their galls), but could be restricted to figs producing few wasps and presenting a very simple internal structure at

maturity. Strong relatedness among interacting males does not seem to interfere with the evolution of fighting behavior.

Male Dispersal

In most species, males are adapted to burrowing among flowers, and the associated morphology does not allow them to walk on a flat surface. Therefore, those that emerge from the fig after cutting the exit hole fall over helplessly on the fig surface. Often the emerging males are also exhausted, and dying. This has led to the belief that exposure to the external air rich in oxygen kills the males. But we have regularly opened figs during male mating activity and the male wasps readily survive exposure to air and continue to mate females for several hours (F.K., M.H., J. Greeff, unpublished observations). However unlike the peaceful males that fall over helplessly, fighting males can walk on a flat surface and males of some species may walk from one fig to another. In some species they may just enter another fig after wasp emergence, through the exit hole, and have access to some yet unmated females (Greeff 2002). In other species, however, a male wasp may cut an entrance hole into a fig in which matings will soon begin (Greeff et al. 2003).

Dispersing males seem to have large to very large eyes for agaonid male wasps, which can readily be explained by their exposure to free life. Nevertheless, nondispersing males of many other agaonid species also have large eyes while some have reduced eyes. This is surprising as vision would seem to be useless within the dark cavity of the fig. In fact, light within a fig cavity has only been measured once, in *F. sycomorus*, and, while faint (about 0.07 percent of incident light), is not negligible (Galil and Eisikowitch 1974). It may be suggested that in species for which sufficient light reaches the fig cavity, male wasps make use of vision.

Emergence of the Females

Once the males have cut an exit hole, females emerge from the fig. In actively pollinating species, the wasps rapidly fly off in search of a new receptive fig. In passively pollinating species, however, the emerging wasps are covered with pollen and first must spend some time cleaning themselves. This probably makes them more exposed to ant predation than active pollinators. Rapid emergence from the fig in species in which all females pool into the cavity and in which males collectively cut an exit hole could also constitute a coadapted set of traits favored in ant-rich environments. Further, the males emerging from such figs and forming a ring around the exit hole through which the females emerge could also constitute a further protection against ant predation on the females by becoming the prey first encountered by the ants (Zachariades 1994).

Fig Ripening

In monoecious *Ficus*, there must be a mechanism that prohibits fig final ripening before wasp emergence. Data from one species suggest that fig final ripening is climacteric, mediated by a production of ethylene. Before fig maturity, the internal cavity would be high in CO₂ inhibiting ethylene

production and hence fig ripening. Cutting the exit hole would allow this CO₂ concentration to decrease, leading to ethylene production and hence fig climacteric ripening (Galil et al. 1973). Whether this mechanism is true or not, it is clear that the cutting of an emergence hole is in several, maybe all, monoecious *Ficus* species a major factor for the onset of final ripening. Figs that have gone through the first swelling allow wasp movements within the fig. Some fail to go through final ripening from a crop in which most figs have already ripened: these figs generally lack exit holes, either because they contain males only, females only, or because they only or almost only contain parasites that normally use the pollinator's exit hole. Alternatively to the CO₂ hypothesis, it may be suggested that ethylene production is stimulated by the bruising of fig tissue that results from an exit hole being cut.

In dioecious *Ficus*, the questions associated with fig maturation are completely different. Male figs do not become attractive to seed dispersers after wasp emergence (or become much less attractive than female figs): there is no climacteric ripening. Hence, they merely need to relax their internal structure just before the onset of male wasp activity. In a number of dioecious species (belonging to all sections of subgenus *Ficus*), the ostiolar bracts become less tight at the moment of wasp emergence, and wasps may emerge without the males having to cut an exit hole. The lack of any report of a similar trait in monoecious *Ficus* species supports the idea that in these species the cutting of an exit hole has a functional link with fig ripening.

In female figs on the other hand, fig ripening cannot result from wasp behavior as no wasps are hatched in female figs. Further there is no necessity to allow wasp movement. Hence the internal cavity generally remains quite tightly packed until the climacteric ripening has started. Female fig development time may also strongly differ from wasp (male fig) development time. As a consequence of this, even if a wasp oviposited in a female fig, there would probably be no possibility for matings to proceed normally and for offspring to emerge from the fig. This may explain why no pollinator and almost no hymenopteran ovule parasite of the system have evolved the capacity to oviposit in female figs. The only exception to the rule are wasps (*Neosycophila Grandi* [Grandi 1923]) that cut an exit hole directly from their natal gall out of the fig and that mate outside figs. Hence these wasps are not submitted to the same selective pressures as wasps that must pass through the fig cavity.

Conclusions on the Life Cycle

The extensive variation observed at the successive steps of the wasps' biological cycle seems to be largely explained by differences in the ecology of the interaction, which represent divergent selective pressures. Each time we formulate a hypothesis on how these selective pressures could vary, we find examples allowing the hypothesis to be tested. At each step, the wasps' behavior plays a central role in the interaction. Surprisingly, as can be seen from the above treatment, only limited quantitative data and few clear descriptions are available on this behavior. Simple descriptions of subtle aspects of behavior of a number of species will probably constitute the major future contribution to our understanding of the Agaonidae-*Ficus* interaction.

CONCLUSION

The mutualistic interaction between Agaonidae and *Ficus* has become an impressive textbook example of coevolution. Although it has been intensively studied for many years, it is only now that the logic of its functioning and the scope of the diversity of its functioning are becoming apparent. Broad repeated patterns are being recognized. It is also only now that global molecular phylogenies are beginning to offer clarity to the history of the association. The number of publications on the association is so great (Weiblen 2002) that they are barely accessible to the nonspecialist. Joining variation in the functioning of the association with knowledge of phylogenies will provide a wealth of information on adaptation.

Agaonid wasps have traditionally been described as seed parasites. They are probably better seen as ovule gallers especially as in the basal genus, *Tetrapus*, most larvae develop in unpollinated ovules. This is in accordance with results on other, supposedly seed-eating chalcid wasps: *Megastigmus* Dalman has been shown to develop indiscriminately in pollinated and unpollinated ovules (Rappaport et al. 1993) and one may wonder whether many chalcid wasps are true seed eaters. A unique feature of agaonid wasps is that they seem to perform better in fertilized ovules (a trait that has still to be confirmed in passively pollinating species). The fact that agaonid wasps are ovule gallers differentiates them from other systems in which active pollination has evolved, as these involve seed eaters. Further, as agaonid wasps transfer pollen from their natal figs, *Ficus* are selected to facilitate oviposition, and this is illustrated by the anatomic features of the fig ovule facilitating early larval development. This is strikingly different from instances of mutualistic pollination by seed eaters in which the insects do not disperse pollen from their natal plant: in these systems, the plant is selected to kill developing pollinator larvae. Another major difference is that in other systems, insects seem to have evolved active pollination to increase seed set (Huth and Pellmyr 2000), whereas agaonid wasps seem to have evolved active pollination to counteract fig adaptations that ensured that mainly flowers that did not receive pollinator eggs were pollinated.

Hence, in many aspects the agaonid wasp-*Ficus* mutualistic association is unique. A surprising feature is how rapidly, in evolutionary terms, such a unique system became established and diversified. In molecular terms, Agaonidae are a basal lineage within Chalcidoidea and it is very difficult to position *Ficus* within Moraceae. There seems to be a connection between the distribution of extant *Ficus* taxonomic groups and agaonid wasp genera and events in terms of continental drift occurring before 90 million yr ago.

How much of the natural history of a given association can be inferred from observing a male and a female agaonid wasp preserved in alcohol has yet to be revealed. We may surmise that simple morphological observations will enable us to tell whether the host trees are dense and/or aggregated as opposed to disperse and low density; if the ostiole is linear or spiral; if spiral, whether it has a long or short linear portion; how deep is the ostiole

and is it loose or tight; whether the fig is monoecious or dioecious; whether it presents many anthers; if actively pollinated whether the wasps pollinate most flowers or not; whether there are many or few layers of flowers; whether foundresses fight; whether the fig fills with liquid that resorbs late in development; and whether males fight. This tentative list only gives traits for which we strongly suspect there are causal relationships between wasp morphology and host-fig traits. Reciprocally, we could propose a number of predictions on wasp morphological and behavioral traits just by looking at a developing fig. Many of the predictive traits probably still elude us. The *Ficus*-agaonid wasp association is progressively becoming an impressive story of coadaptation. Improved morphological taxonomy and phylogenies are progressively adding a perspective of multiple evolutionary origins of trait associations.

Some broad patterns, however, still largely elude us. For instance, has evolution basically gone from pachicaul trees with large figs and linear ostioles towards leptocaul trees with smaller figs and spiral ostioles, accompanied by convergent evolution of the wasps? Does mode of pollination cycle, at least in some secondarily passively pollinating species, re-evolving active pollination? To what extent could aspects of the *Ficus*-agaonid wasp interaction result in (and from) differential species survival? Is the extensive diversification of *Ficus* linked with the association with Agaonidae? How much of the biology of the *Ficus*-Agaonidae interaction depends on the complex web of interactions with other organisms?

Acknowledgments

We are indebted to the community of fig biologists. Exchange of ideas and unpublished results has always been very free and productive. This has resulted in our liberal use of unpublished results from colleagues.

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