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Pollination mode in fig wasps: the predictive power of correlated traits

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The over 700 species of *Ficus* are thought to have co-speciated with their obligate pollinators (family Agaonidae). Some of these wasp species pollinate figs actively, while others are passive pollinators. Based on direct observations of mode of pollination in 88 species, we show that mode of pollination can confidently be predicted from fig traits only (anther-to-ovule ratio) or from wasp traits only (presence of coxal combs). The presence of pollen pockets is not a predictor of mode of pollination. Data, direct and indirect, on 142 species, demonstrate numerous cases of the loss of active pollination and suggest one or few origins of active pollination. Hence, active pollination, an impressive example of the sophisticated traits that may result from mutualistic coevolution, depends on selective forces that can be overcome in some species, allowing reversions. Despite frequent loss, active pollination remains the predominant mode of pollination in *Ficus*.

Keywords: Agaonidae; *Ficus*; fig; pollination; coevolution; mutualism

1. INTRODUCTION

How mutualisms arise, persist and diversify is a challenging question. The general importance of selective conflicts between mutualists in shaping their evolution is widely recognized (Bronstein 1994; Anstett *et al.* 1997; Herre *et al.* 1999). How these selective conflicts translate into longer-term evolution can be visualized by determining the evolutionary trajectories of coevolved traits. There is, however, hardly any documentation on the distribution of variation in coevolved traits in a large diversified coevolved mutualistic association. Co-speciating systems are particularly well suited for such studies because trait evolution in different lineages is largely independent, and hence repeated or divergent trajectories of the co-evolutionary process can be revealed.

The *Ficus*–Agaonidae pollination mutualism is an appropriate model for investigating such trajectories. It is obligate and species specific, and there are over 700 *Ficus* species (Berg 1989), which are thought to have co-speciated with the associated agaonid wasps. There is, however, no general overview of the distribution of variation in the association, except for the distribution of monoecy and dioecy in *Ficus* (Weiblen 2000), a fig trait that has important evolutionary consequences for the wasps (Anstett *et al.* 1997). Here, we investigate the taxonomic distribution of active pollination in *Ficus*.

The fig is an urn-shaped inflorescence, the inside of which is lined by variable numbers of uniovulate female flowers and male flowers. When female flowers are receptive, pollen-loaded females of the associated agaonid wasp species are attracted to and enter the fig. Once inside, they oviposit in some ovules and pollinate. Either the wasps have pollen loaded into thoracic structures called pollen pockets (figure 1*a*–*c*,*e*) and each time they deposit an egg they also discharge some pollen with their forelegs (active pollination; Frank 1984), or they show no such behaviour. In the latter case, pollen trapped in the abdominal pleura is progressively dispersed throughout the fig as the wasp's gaster swells in the water-saturated fig cavity atmosphere (passive pollination; Galil & Neeman 1977). The founding females then die and each larva develops at the expense of a single, galled ovule. Offspring wasps reach adulthood some weeks later. Males emerge from their galls and mate with the females. Females then emerge from their galls. Either they go to the anthers and pick up pollen with their forelegs, deposit it on the ventral part of the thorax and shovel it into the mesothoracic pollen pockets using their fore coxae (active pollination), or they totally lack this behaviour (passive pollination). Females then leave their natal fig in search of a receptive fig. Wasps emerging from actively pollinated figs do not bear visible pollen grains on their body, except in the pockets. In contrast, passively pollinating wasps are covered with pollen on emergence. They immediately clean themselves carefully, so that pollen is no longer seen. At least in the well-studied species *Blastophaga psenes* (Galil & Neeman 1977), pollen is trapped in the abdominal pleura when the gaster shrinks on exposure to the external air, which is drier than the internal atmosphere

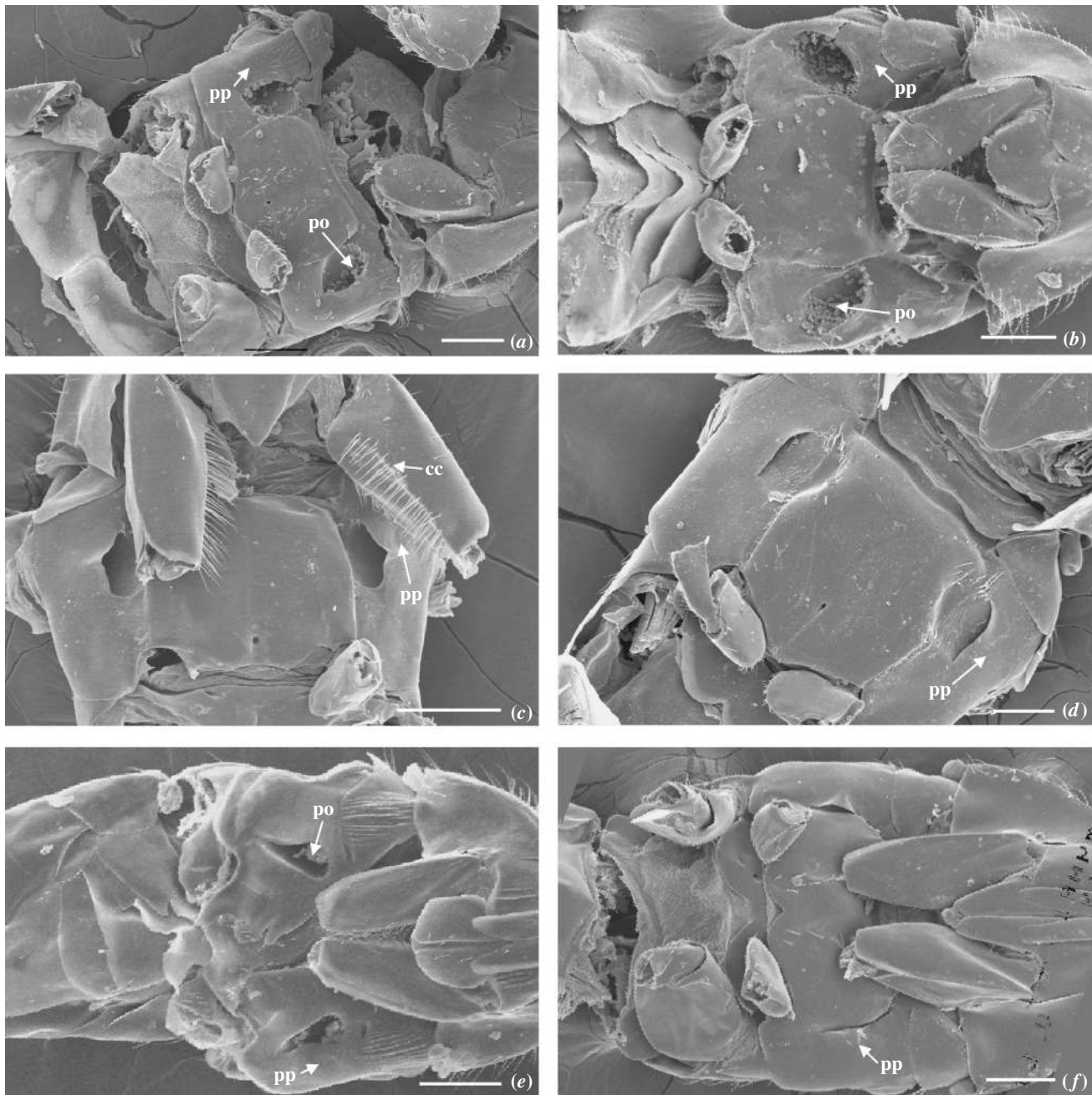[*]Author for correspondence (kjellberg@cefe.cnrs-mop.fr).

Figure 1. Ventral views of female agaonid mesosoma from mainly actively pollinating genera: (*a*) *Pegoscapus longiceps* (host *Ficus dugandii*), (*b*) *Blastophaga* (*V.*) *confusa* (host *Ficus grossularioides*), (*c*) *Pleistodontes imperialis* (host *Ficus rubiginosa*), (*d*) *Pleistodontes addicotti* (host *Ficus crassipes*), (*e*) *Ceratosolen constrictus* (host *Ficus condensa*) and (*f*) *Ceratosolen galili* (host *Ficus sycomorus*). The head is pointing right or upward. Species pollinating actively are shown in *a–c* and *e*. Species pollinating passively are shown in *d* and *f*. Abbreviations: pp, pollen pocket; po, pollen seen through pollen-pocket opening; cc, coxal comb. Scale bar, 100 μm.

of the fig. It is widely accepted, though not proven, that actively pollinated *Ficus* species produce little pollen because wasps born within a fig will only load a limited amount of pollen into their pockets. If this is true then passively pollinated *Ficus* should produce much more pollen so that emerging insects become covered with it (Galil & Meiri 1981). This increase in pollen production would be accompanied by an increase in the number of anthers. Furthermore, anthers should shed their pollen in passively pollinated fig species but not in actively polli- nated ones, in which wasps go directly to the anthers to

pack pollen into their pockets (Ramírez B. 1970; Galil & Meiri 1981).

We present the first systematic survey of the distri- bution of active and passive pollination and of associated fig and wasp traits based on observations of 142 species chosen to cover all the major taxo- nomic subdivisions of both *Ficus* and Agaonidae. This survey enables us to propose a set of wasp and fig traits sufficient to predict mode of pollination. We show that active pollination, a striking example of the complex morphological and behavioural traits that

may be produced by mutualistic coevolution, has been repeatedly lost.

2. MATERIAL AND METHODS

(a) *Direct evidence of mode of pollination*

For as many species as possible, we established mode of pollination using our own observations and published data. Any one of the following three criteria was considered, by itself, as direct evidence of active pollination: observation of pollen-loading behaviour; observation of pockets filled with pollen; or observation of pollen-deposition behaviour (the correlation between active pollen collection and active pollen deposition has been documented for about 20 species). For passive pollination, the only criterion was the presence of pollen scattered over the body with no particular concentration in the pollen-pocket zone. Wasps emerging from figs producing large amounts of pollen are covered with pollen but clean themselves afterwards, therefore, our observations were made on wasps that had had time to clean themselves. Hence, to demonstrate passive pollination, the wasps were allowed to emerge from their natal figs into mesh bags and were collected only after they had died some hours later. They were preserved dry and the location of pollen was observed under a microscope on wasps crushed between slides.

(b) *Traits correlated with mode of pollination*

For all the wasp species to which we had access and for which the mode of pollination was determined by direct evidence, we closely examined the fore coxae, which are involved in loading pollen into the pockets, and the ventral and lateral parts (mesosternae and mesopleurae) of the female thorax (mesosoma). This allowed us to establish whether the fore coxa bore a comb (a line of setae), whether pollen pockets were present, either fully developed or reduced, and, when no pockets were observed, whether traces of pockets were present. We also compiled all previously published data on these traits. To these data we added observations on other agaonid wasp species for which we had access to sufficient material for dissections.

For *Ficus* species in which mode of pollination was known, we established whether pollen and anthers were abundant by visual inspection of several figs from one or several trees. We confirmed the visual impression by counting numbers of anthers and female flowers in one or several figs in order to test the hypothesis that actively pollinated fig species produce less pollen than passively pollinated species and hence have lower anther-to-ovule ratios. Counts were always performed on whole figs, because counts on the two halves of a fig regularly produced quite different ratios. As male flowers appear early in fig development, counts could be made at any stage from receptivity to maturity. Counts were, however, easier to perform late in fig development, but before wasp emergence, in most species. For species to which we did not have access we used published data on flower counts. To these data, we added observations on species for which mode of pollination was not determined by direct observation but for which we had access to data or to preserved figs that could be dissected.

In three species belonging to three different subgenera (*Ficus aurea* (subgenus *Urostigma*, section *Americana*), *Ficus hispida* (subgenus *Sycomorus*, section *Sycocarpus*) and *Ficus exasperata* (subgenus *Ficus*, section *Sycidium*)) counts on several hundred figs (379, 545 and 483 figs, respectively) were used to establish the within-species distribution of the anther-to-ovule ratio.

(c) *Taxonomic considerations*

Species identification within *Ficus* can be somewhat difficult. However, the internal aspect of a fig allows one quite readily to determine without ambiguity at least the section to which the species belongs. Hence, we expect no errors in the section assignment of the figs we counted. In a number of cases we had data on several forms (named subspecies or varieties) within a *Ficus* species. We treated these forms as distinct fig species when they occurred as well-differentiated entities within a locality and were pollinated by unique species of wasps. We have used species names according to Corner (1965, 1972), Berg *et al.* (1984), Berg & Wiebes (1992) and Wiebes (1994, 1995). The arrangement of *Ficus* sections into subgenera is according to Berg (1989) and Berg & Wiebes (1992), pending a thorough revision of higher-level classification of *Ficus*.

(d) Ficus *classification reflects the classification of Agaonidae*

Ficus and Agaonidae taxonomy have been independently and *ca.* 280 species associations are known (Wiebes 1994). For four agaonid genera, pollinating three related subsections of section *Galoglychia*, there are serious misfits between the classification of figs and the classification of wasps (*ca.* 20 associations). For the remaining 260 associations, there is a nearly perfect correspondence between wasp classification into 16 genera and fig classification (using the taxonomic criteria for figs defined in Berg & Wiebes (1992)). The main deviation is that, within the section *Conosycea*, the species pollinated by *Eupristina, Waterstoniella* and *Deilagaon* are somewhat intermixed (Wiebes 1994). Preliminary molecular-phylogeny data (E. Jousselin, J.-Y. Rasplus and F. Kjellberg, unpublished data) supports the idea that the classification of this section has to be revised following the classification of the associated wasps. Apart from this case, there are only three documented mismatches between fig and wasp classifications. This strongly supports the idea that these 16 genera of agaonid wasps constitute good monophyletic entities and that each of them pollinates a monophyletic group of figs. This idea is further supported by a molecular phylogeny of *Ficus* (Weiblen 2000) and the presently available wasp molecular phylogenies (Machado *et al.* 2001).

3. RESULTS

The global data on mode of pollination and on the fig and pollinator traits associated with it (143 species) are presented in electronic Appendix A (available on The Royal Society's Web site). The data set covers 16 out of 17 fig sections (missing *Sinosycidium*, one species) and all 20 pollinator genera. Mode of pollination was established directly for 88 fig species (published data were used for 32 of these species). This enabled us to define traits that allowed us to predict mode of pollination. Mode of pollination was then inferred, based on correlated traits of both fig and wasp species, for 32 other associations, and inferred from fig traits only for 23 additional species.

The female wasps known by direct observation to be active pollinators invariably possessed pollen pockets and coxal combs on their fore coxae (figure 1*c*), except for *Pleistodontes froggatti*, pollinating *Ficus macrophylla*, which lacked coxal combs (table 1). Furthermore, the fore coxae of actively pollinating species always presented sharp edges and an elongate depression that was bordered by the comb (figure 1*c*). Agaonid species known to be

Table 1. *Association between presence of coxal comb and mode of pollination*

(χ^2, $p < 0.05$).

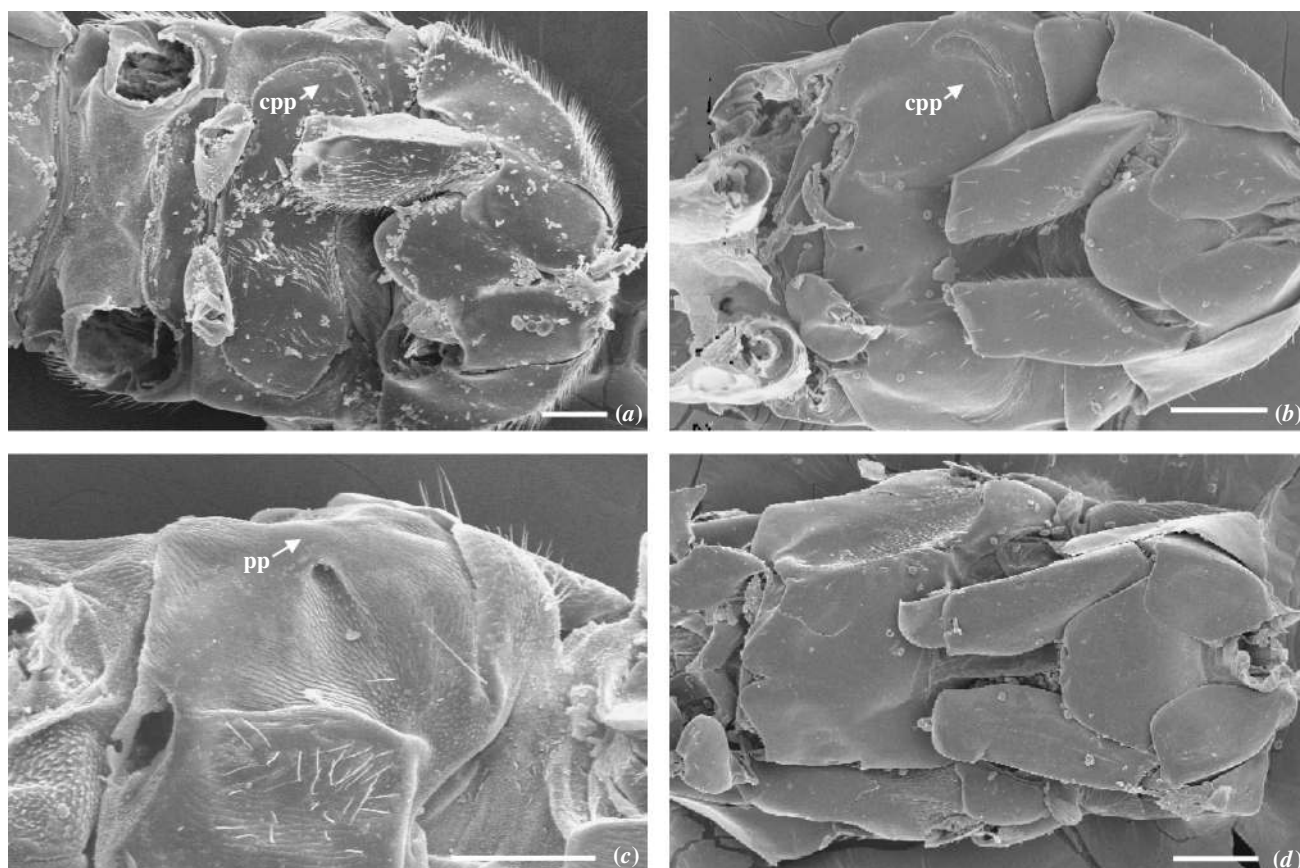|	active pollination (direct evidence)	passive pollination (direct evidence)	passive pollination (direct evidence or non-functional pollen pockets)
coxal comb	68	0	0
no coxal comb	1 (*Ficus macrophylla*)	12	21

Figure 2. Ventral view of female agaonid mesosoma from mainly or exclusively passively pollinating genera: (*a*) *Wiebesia contubernalis* (host *Ficus punctata*), (*b*) *Blastophaga* (*B.*) *psenes* (host *Ficus carica*), (*c*) *Waterstoniella sp.* (host *Ficus xylophylla*), (*d*) *Tetrapus americanus* (host *Ficus maxima*). The head is pointing right. All species shown pollinate passively. Abbreviations: pp, pollen pocket; cpp, closed pollen pocket. Scale bar, 100 μm.

passive pollinators never possessed a coxal comb (although they sometimes bore setae on the coxae, figure 2*a*) (table 1). Their fore coxae had more rounded edges and did not exhibit the elongate depression. While passive pollinators often possessed pollen pockets (figures 1*d,f* and 2*c*), pollen was never observed to be concentrated within them.

There was a highly significant difference in the anther-to-ovule ratio between actively and passively pollinated figs for which there was direct evidence for mode of pollination (figure 3 and table 2; Mann–Whitney *U*-test, $p < 10^{-3}$); in fact, there was no overlap even in the most extreme species values, except for a single species (*F. macrophylla*). When pollination was known to be active (78 species), the anther-to-ovule ratio was low (*F. macrophylla* excluded, mean ± s.d. = 0.077 ± 0.03; range

of 0.01–0.15; *F. macrophylla*, 0.53). In the 11 cases in which pollination was known to be passive, the ratio was almost an order of magnitude higher (mean ± s.d. = 0.61 ± 0.32; range of 0.29–0.92; figure 3 and table 2) and the results remained similar when species with obviously non-functional pollen pockets (strongly reduced or totally lacking) were included (26 species, mean ± s.d. = 0.61 ± 0.27; range of 0.22–1.12; figure 3 and table 2). This result remained valid over the whole 200-fold range of variation in the number of ovules per fig (from below 100 to over 20 000). Hence, the anther-to-ovule ratio appears to be a reliable indicator of mode of pollination in *Ficus*. The counts systematically confirmed the conclusions drawn from visual inspections of the figs except in a few instances for figs collected very early in development (anthers still very small).
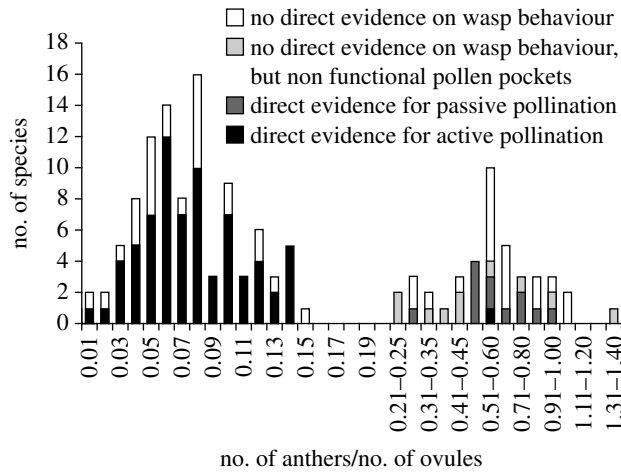
Figure 3. Interspecific variation of the anther-to-ovule ratio according to mode of pollination.

Table 2. *Association between the anther-to-ovule ratio and mode of pollination*

$(\chi^2, p < 0.05)$.

number of anthers/ number of ovules	active pollination (direct evidence)	passive pollination (direct evidence)	passive pollination (direct evidence or non-functional pollen pockets)
< 0.16	77	0	0
> 0.21	1	11	26
	(*Ficus macrophylla*)		

Two additional traits were correlated with passive pollination. At fig maturity in passively pollinated species, the anthers were projecting into the fig cavity, and pollen was abundantly shed into the fig cavity through anther dehiscence. As these traits were only expressed at fig maturity, they could not be observed on a systematic basis. Nevertheless, we observed both traits in some species pollinated passively by *Tetrapus, Waterstoniella, Deilagaon, Pleistodontes, Blastophaga* subgenus *Blastophaga* and *Wiebesia*.

Surprisingly few figs needed to be examined to draw rigorous conclusions about a species' mode of pollination. This is evident from the pattern of within-species variation in the anther-to-ovule ratio for the three species investigated in depth (figure 4). *F. hispida* (subgenus *Sycomorus*) exhibited less within-species variation than *F. aurea* (subgenus *Urostigma*) and *F. exasperata* (subgenus *Ficus*) (mean ± s.d. (range): *F. hispida* 0.04 ± 0.02 (0.008–0.20), *F. aurea* 0.08 ± 0.04 (0.005–0.28) and *F. exasperata* 0.08 ± 0.05 (0.02–0.37); Levene test of homogeneity of variance, *F. aurea* versus *F. exasperata* non-significant, *F. hispida* versus *F. aurea* or *F. exasperata*, $p < 10^{-6}$). In all three species, the few individual figs with particularly high ratios were distributed across trees and across crops. Hence, in these species, examining a single fig from a single tree would usually have been sufficient to establish mode of pollination. The results would sometimes have been ambiguous (values outside the range of mean species
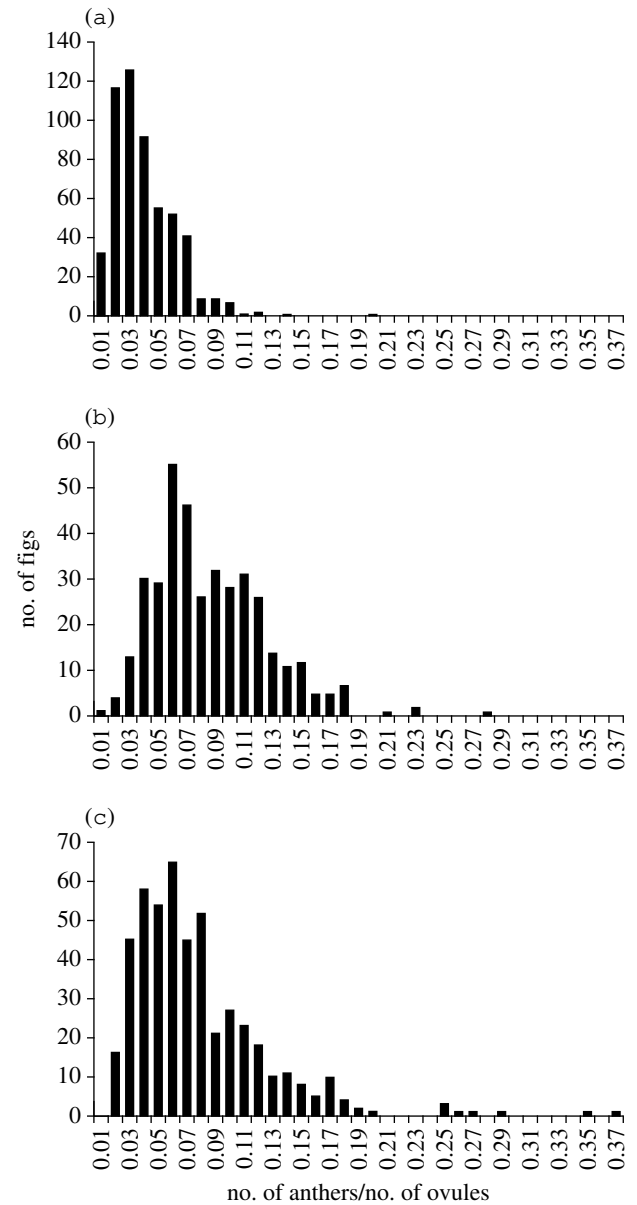
Figure 4. Intraspecific variation of the anther-to-ovule ratio in three actively pollinated species: (*a*) *Ficus hispida*, subgenus *Sycomorus*, (*b*) *Ficus aurea*, subgenus *Urostigma* and (*c*) *Ficus exasperata*, subgenus *Ficus*.

values for species known to be actively pollinated, i.e. above 0.14), necessitating the examination of a second fig. Counting two figs, even from a single crop, would almost certainly have removed any ambiguity (the probabilities of counts on two figs both giving ambiguous results for each of the three species were 2×10^{-5}, 10^{-3} and 8×10^{-3}, respectively).

Except for *F. lateriflora* (subgenus *Ficus*) and *F. thonningii* (subgenus *Urostigma*), in which visual inspection, confirmed by counts on a few figs, revealed individual trees consistently giving slightly outlying anther-to-ovule ratios (0.18 and 0.23, respectively) relative to the species median, determining anther-to-ovule ratios from a single tree gave qualitatively the same results as counts from several trees. In some species we could only count a single fig, but all counts giving ratios intermediate to the distributions for active and passive pollination, i.e. ratios

Table 3. *Association between the anther-to-ovule ratio and presence of a coxal comb*

(χ^2, $p < 0.05$).

number of anthers/ number of ovules	coxal combs present	no coxal comb
< 0.16	77	0
> 0.21	0	31

in the range 0.13–0.5, were based on at least two figs, often more. Furthermore, in almost all species we could add evidence from qualitative observations on several other figs from the same species.

In all the fig–wasp associations for which mode of pollination was not observed directly but for which wasps and figs were examined (32 associations), the modes of pollination inferred independently from wasp traits (presence or absence of coxal combs) and fig traits (anther-to-ovule ratio) were identical (table 3). Hence, in table 4 and electronic Appendix A, we have indicated

mode of pollination, whether observed directly or inferred from fig or wasp traits. In *F. montana* var. *purpurascens*, while passive pollination is suggested, further data are needed because observations were from a single cloned plant and no wasps were available.

In all wasp species with pollen pockets, these were located on the mesopleurae (figures 1 and 2). We did not observe any pollen pockets in specimens of the exclusively passively pollinating genus *Tetrapus* (figure 2*d*) nor in *Blastophaga psenes* (figure 2*b*) of the exclusively passively pollinating subgenus *Blastophaga* of genus *Blastophaga* and in a species of *Pleistodontes*. In all other passively pollinating species we observed the presence of more- or less-reduced pockets. In passively pollinating *Wiebesia contubernalis*, belonging to a genus including mainly passively pollinating species, the pockets were large but flat and had no opening, so that, although they could be seen clearly under a stereoscopic microscope, they could not be seen using scanning electron microscopy (SEM) (figure 2*a*). Only the anterodorsal chitinized margins of the pockets (which were also clearly apparent under optic microscopy) were visible on SEM images. The same

Table 4. *Variation of mode of pollination within and among agaonid and* Ficus *taxonomic entities*

(Polymorphic taxonomic entities are indicated in bold. Mode of pollination determined from direct evidence, or from correlated traits.)

wasp genus	fig section	mode of pollination	range of number of anthers/ number of ovules (number of species)	direct evidence for mode of pollination: number of species	coxal combs: number of species	
					cc present	cc absent
Tetrapus	*Pharmacosycea*	passive	0.22–0.29 (3)	1	0	2
Dolichoris	*Oreosycea*	active	0.04–0.15 (5)	0	3	0
Pegoscapus	**Americana**	active	0.04–0.14 (15)	15	8	0
		passive	0.53 (1)	0	0	1
group of related pollinator genera	*Galoglychia*	active	0.03–0.13 (17)	17	18	0
Platyscapa	**Urostigma and some species of section Conosycea**	active	0.04–13 (10)	7	8	0
		passive	0.27–0.57 (3)	0	0	3
Waterstoniella and *Deilagaon*	part of Conosycea	passive	0.32–0.92 (12)	6	0	9
Eupristina	**part of Conosycea**	active	0.05–0.13 (9)	7	6	0
		passive	0.42–0.57 (2)	0	0	1
?	*Stilpnophyllum*	passive	0.93 (1)	0	0	0
Pleistodontes	**Malvanthera**	active	0.11 (1)	1	1	0
		passive	0.65–0.90 (3)	0	0	2
	F. macrophylla	**active**	**0.53**	**1**	**0**	**1**
Ceratosolen	*Sycomorus*	active	0.01–0.10 (7)	8	7	
	C. galili (parasite)			1	0	1
Ceratosolen	*Neomorphe*	active	0.04 (1)	1	1	0
Ceratosolen	*Adenosperma*	active	0.05 (1)	1	1	0
Ceratosolen	*Sycocarpus*	active	0.01–0.08 (11)	6	7	0
Kradibia and **Liporrhopalum**	**Sycidium**	active	0.02–0.12 (13)	11	13	0
		passive	0.33 (1)	0	0	0
Wiebesia	**Rhizocladus, Kalosyce and some species of Ficus**	active	0.14 (1)	1	1	0
		passive	0.43–1.00 (9)	2	0	7
Blastophaga subgenus *Blastophaga*	*Ficus*, subsection *Ficus*	passive	0.5–1.20 (5)	2	0	4
Blastophaga subgenus **Valisia**	*Ficus*, subsection *Eriosycea*	active	0.07–0.14	5	5	0
		passive	0.85 (1)	0	0	0

structure was seen in SEM images of *B. psenes* (figure 2*b*). Hence, we interpret these structures as traces of pollen pockets in *B. psenes*, although an anatomical study is still needed. In the exclusively passively pollinating genus *Waterstoniella*, the pockets were strongly reduced in size but had a small opening, so that SEM was needed to confirm that the structures observed were, in fact, pollen pockets (figure 2*c*). Reduction in pollen-pocket size was variable in passively pollinating species of the mainly passively pollinating genus *Pleistodontes*. Some species clearly presented flattened but well-developed pockets (figure 1*d*), while in some species groups the pockets were very strongly reduced or even absent. Reduction in pollen-pocket size was more limited in passively pollinating species of the mainly actively pollinating genera *Pegoscapus* (Ramirez 1970), *Platyscapa*, *Eupristina* and *Ceratosolen* (figure 1*f*). We lack observations on the pollinator of section *Stilpnophyllum* and on passively pollinating species of the genus *Liporrhopalum* and of *Blastophaga* subgenus *Valisia*. In all actively pollinating species, pollen pockets were well developed and seemed to open much more widely than in passively pollinating species (figure 1*a*–*c*,*e*). Nevertheless, as the appearance of pollen pockets was highly variable among passively pollinating species (figures 1*d*,*f* and 2*a*–*c*), pollen-pocket observation alone was often not sufficient to infer mode of pollination, especially in species groups in which passive pollination was infrequent (figure 1*d*,*f*).

According to the complete set of results (based both on direct observation of mode of pollination and on indirect inferences), the pollinator genera *Tetrapus*, *Deilagaon* and *Waterstoniella* and *Blastophaga* subgenus *Blastophaga* were found to be exclusively passive pollinators. Genera *Dolichoris* and *Kradibia* and all the genera pollinating *Ficus* section *Galoglychia* (*Courtella*, *Agaon*, *Allotriozoon*, *Nigeriella*, *Alfonsiella*, *Elisabethiella* and *Paragaon*) were exclusively active pollinators. Genera *Pegoscapus*, *Platyscapa*, *Eupristina*, *Pleistodontes*, *Liporrhopalum* and *Wiebesia* and *Blastophaga* subgenus *Valisia* included both actively and passively pollinating species. Finally, all pollinators belonging to the genus *Ceratosolen* pollinated actively with the exception of one species (*C. galili*) that totally lacks pollination behaviour and is a parasite of its host fig (Galil & Eisikowitch 1969; Compton *et al.* 1991).

4. DISCUSSION

The data show nearly complete correspondence between one morphological trait of the fig (low or high anther-to-ovule ratio), one morphological trait of that fig's species-specific pollinator wasp (presence or absence of a coxal comb on the fore coxa) and mode of pollination (active or passive), suggesting that any one of these characters can be used to infer the others. It might have been expected that the presence of pollen pockets, in which the actively pollinating species transport pollen, would be the best indication of mode of pollination for a given fig–pollinator association. However, our results indicate that, in fact, many species that do not actively collect and deposit pollen nevertheless possess large pollen pockets.

As pollinator genera and their associated host figs appear to be valid monophyletic taxonomic entities, our results suggest that there have been many independent within-genus transitions in mode of pollination. Hence, the association of active pollination with coxal combs and low anther-to-ovule ratios and/or the converse association of passive pollination with lack of coxal combs and high anther-to-ovule ratios must have evolved repeatedly as a result of coadaptation. Further, at fig maturity in passively pollinated figs, anthers projected into the fig cavity and pollen was abundantly shed through anther dehiscence. Although these traits could not be observed on a systematic basis, they seem to be always present as they were observed in all passively pollinated fig species collected at the appropriate stage, i.e. fig species associated with six different wasp genera.

The predictive power of combs on the fore coxae is probably linked to the mechanism of pollen loading into the thoracic pollen pockets. Actively pollinating wasps begin by collecting pollen in the depression of the fore coxa, which is bordered by the coxal comb, before shovelling it from the coxal depression into the thoracic pollen pockets (Frank 1984). The coxal combs could play an important role in this process.

The predictive power of the anther-to-ovule ratio is probably linked to the efficiency of loading pollen onto the insects. In actively pollinated fig species, each wasp will only load a limited amount of pollen into its pockets and hardly any pollen is dispersed in the fig cavity during pollen loading. Producing more pollen would probably not increase the quantity of pollen dispersed by the wasps. Hence, actively pollinated fig species are selected to only produce enough pollen for the wasps to fill their pockets. In passively pollinated species, only pollen ending up by chance in places where the insect will not eliminate it by grooming will be transported to another fig. Hence much pollen is lost by not ending up on the insects or by being removed by the insect during cleaning. Therefore, passively pollinated fig trees are selected to produce much more pollen per fig than actively pollinated species.

The frequent occurrence of passively pollinating species with pollen pockets shows that the sophisticated behavioural and morphological traits, evolved to allow active pollination, depend on selective forces that can be overcome in some species, allowing reversions. This comes as a surprise because active pollination has been assumed to be highly beneficial for both wasp and tree. Indeed, our results show that active pollination benefits the host fig by allowing a strong reduction in anther production. It is also generally assumed that ovule fertilization benefits the wasp, either because it limits fig abortion (Janzen 1979) or because it ensures better larval nutrition (Verkerke 1987). To our knowledge there is, however, no published demonstration that would meet current scientific standards that shows that wasp larvae do effectively benefit from pollination. Similarly, there is clear evidence that a single actively pollinating wasp does not, or cannot, fully pollinate a fig: in numerous fig species, the entry of two wasps leads to the production of more seeds and/or wasps (see, for example, Herre 1989). Hence, active pollination could be limiting for the fig. Nevertheless, active pollination is probably generally beneficial for both wasp and fig, as active pollination usually remains stable within species and as most species are actively pollinated (in our subsample, only about one-quarter of the species were passively pollinated).

Interestingly, there is only one documented case in which an agaonid wasp (*C. galili*) lacks active-pollination behaviour and the associated *Ficus* (*F. sycomorus* and *F. mucuso*) have not developed a passive-pollination syndrome. In this case, the wasp is effectively a parasite (Galil & Eisikowitch 1969; Compton *et al.* 1991) and the trees are actively pollinated by another agaonid wasp, *C. arabicus*. This is also the only phylogenetically well-documented case of loss of active pollination behaviour. *C. arabicus* and *C. galili* are distantly related. The species group of *C. galili* includes seven species (Kerdelhué *et al.* 1999). *C. galili* is on a terminal branch of the molecular phylogeny. Behavioural observations directly show that all these species, except *C. galili*, are active pollinators (J.-Y. Rasplus, unpublished data). Hence, loss of active pollination and the acquisition of a parasitic mode of life are strictly linked in this case and probably represent a relatively recent evolutionary event. Furthermore, *C. galili* has large pollen pockets (figure 1*f*), suggesting that reduction of pollen-pocket size is a gradual process after the loss of active-pollination behaviour.

In a number of wasp genera, most species pollinate actively and only a few pollinate passively. These passively pollinating species could either be the few species maintaining the ancestral mode of pollination in an originally passively pollinating genus, or represent cases of more- or less-recent loss of active pollination in an actively pollinating genus. According to our observations, when most species of a species group pollinate actively, the few passively pollinating species have well-developed pollen pockets, as observed in *C. galili* (figure 1*f*), in *Pegoscapus mariae* pollinating *F. tuerkheimii* (Ramírez B. 1970), in *Eupristina sp.* pollinating *F. spathulifolia* and in *Platyscapa etiennei* (see SEM image in Berg & Wiebes (1992)) pollinating *F. densifolia*. This pattern suggests that rare passively pollinating species in mainly actively pollinating species groups represent relatively recent cases of loss of active pollination. Within such groups, reacquisition of active pollination would be very difficult to document.

Conversely, an absence of, or strong reduction in, pollen pockets (closed pockets or pockets necessitating SEM to reveal an opening) was observed in all exclusively passively pollinating genera or subgenera, namely *Tetrapus*, *Waterstoniella*, *Deilagaon* and *Blastophaga* subgenus *Blastophaga* (figure 2*b–d*). This suggests that these genera or subgenera either are of passively pollinating ancestry (no traces of pollen pockets) or they lost active pollination anciently (traces of pollen pockets). These genera or subgenera seem never to have evolved or re-evolved active pollination.

Finally, in the genus *Wiebesia*, many species have strongly reduced pollen pockets even though some species pollinate actively, and in genus *Pleistodontes*, some passively pollinating species have no pollen pockets or strongly reduced pockets, some have well-developed pockets and some pollinate actively. These genera require thorough investigation to map out trait evolution. The unique case of *F. macrophylla*, with a high anther-to-ovule ratio associated with active pollination by a species of *Pleistodontes*, needs biological investigation in the field. This may represent an ongoing transition between modes of pollination.

Hence, globally, comparing data on pollen-pocket reduction in passively pollinating species and the distri-bution of mode of pollination within and between fig-wasp genera, we have ample evidence for the frequent recent loss of active pollination in actively pollinating genera. Furthermore, in several mainly or exclusively passively pollinating genera we observed reduced pollen pockets indicating active-pollination ancestry. At the same time, we have no evidence for the recent acquisition of active pollination in passively pollinating species groups, but the genera *Wiebesia* and *Pleistodontes* are potential candidates. Hence, if we accept wasp genera to be valid monophyletic taxonomic entities, our results demonstrate multiple cases of the loss of active pollination and suggest few, maybe one, origins of active pollination. This could simply stem from the loss of a complex behaviour being much more easily achieved in evolutionary terms than the *de novo* acquisition of such a behaviour and the associated morphological traits. Mapping out our results on a molecular phylogeny of agaonid wasps has led to the suggestions that passive pollination is ancestral, that active pollination originated after the separation of *Tetrapus* from the lineage leading to other agaonid wasps and that active pollination has been lost independently several times in different wasp genera (Machado *et al.* 2001).

Most species are, however, actively pollinated. This situation could be an indicator of two long-term evolutionary processes: active pollination could be doomed to decrease in frequency within *Ficus* through the progressive appearance of passively pollinated lineages, or, alternatively, active pollination could be maintained over evolutionary time by actively pollinated species being less prone to extinction than passively pollinated ones.

From this perspective, the results in *Ficus* are analogous with the distribution of sex in living organisms. While the long-term benefits of sex are well explained by general theory, parthenogenesis would often be favoured by individual selection, if it were to appear. As in the case of passive pollination, sexual reproduction has been lost many times but this has mainly resulted in evolutionary dead ends (Maynard Smith 1978). As in the case of passive pollination in the section *Pharmacosycea* and in *Waterstoniella*-pollinated *Conosycea*, some asexual lineages have survived and diversified. Differential species survival is progressively becoming an acceptable explanation for the ubiquity of sex (Birky 1999; Brookfield 1999; Gouyon 1999). Hence, species selection may be a major driving factor for maintaining characters favouring species survival but these may be counter-selected by individual selection. Obviously, species selection can only work for species in which the appearance of mutations that can respond to this individual selection are very rare, or for species in which the conditions leading to disruptive selection occur rarely. Similarly, species selection may be an important force acting on the evolution of mutualisms, and may, in some cases, be more relevant to understanding them than the current emphasis on the role of selective conflicts on the evolutionary process.

REFERENCES

Anstett, M.-C., Hossaert-McKey, M. & Kjellberg, F. 1997 Figs and fig pollinators: evolutionary conflicts in a coevolved mutualism. *Trends Ecol. Evol.* **12**, 94–99.

Berg, C. C. 1989 Classification and distribution of *Ficus*. *Experientia* **45**, 605–611.

Berg, C. C. & Wiebes, J. T. 1992 African fig trees and fig wasps. *Nederlandse Akademie van Wetenschappen Verhandelingen Afdeling Natuurkund, Tweede Reeks* **89**, 1–298.

Berg, C. C., Vasquez Avila, M. & Kooy, F. 1984 *Ficus* species of Brazilian Amazonia and the Guianas. *Acta Amazonica* **14** (Suppl.), 159–194.

Birky Jr, C. W. 1999 An even broader perspective on sex and recombination. *J. Evol. Biol.* **12**, 1013–1016.

Bronstein, J. L. 1994 Conditional outcomes in mutualistic interactions. *Trends Ecol. Evol.* **9**, 214–217.

Brookfield, J. F. Y. 1999 Explanation and prediction and the maintenance of sexual reproduction. *J. Evol. Biol.* **12**, 1017–1019.

Compton, S. G., Holton, K. C., Rashbrook, V. K., Van Noort, S., Vincent, S. L. & Ware, A. B. 1991 Studies of *Ceratosolen galili*, a non-pollinating Agaonid wasp. *Biotropica* **23**, 188–194.

Corner, E. J. H. 1965 Check-list of *Ficus* in Asia and Australasia, with keys to identification. *Garden's Bull. (Singapore)* **21**, 1–186.

Corner, E. J. H. 1972 New taxa of *Ficus* (Moraceae). *Blumea* **20**, 427–432.

Figueiredo, R. A. & Sazima, M. 1997 Phenology and pollination ecology of three Brazilian fig species (Moraceae). *Botanica Acta* **110**, 73–78.

Frank, S. A. 1984 The behaviour and morphology of the fig wasps *Pegoscapus assuetus* and *P. jimenezi*: descriptions and suggested behavioral characters for phylogenetic studies. *Psyche* **91**, 289–308.

Galil, J. & Eisikowitch, D. 1969 Further studies on the pollination ecology of *Ficus sycomorus* L. (Hymenoptera, Chalcidoidea, Agaonidae). *Tijdschr. Entomol.* **112**, 1–13.

Galil, J. & Meiri, L. 1981 Number and structure of anthers in fig syconia in relation to behaviour of the pollen vectors. *New Phytol.* **88**, 83–87.

Galil, J. & Neeman, G. 1977 Pollen transfer and pollination in the common fig (*Ficus carica* L.). *New Phytol.* **79**, 163–171.

Gouyon, P.-H. 1999 Sex: a pluralist approach includes species selection. (One step beyond and it's good.) *J. Evol. Biol.* **12**, 1029–1030.

Herre, E. A. 1989 Coevolution and reproductive characteristics in 12 species of New World figs and their pollinator wasps. *Experientia* **45**, 637–647.

Herre, E. A., Knowlton, N., Mueller, U. G. & Rehner, S. A. 1999 The evolution of mutualisms: exploring the paths between conflict and cooperation. *Trends Ecol. Evol.* **14**, 49–52.

Janzen, D. H. 1979 How to be a fig. *A. Rev. Ecol. Syst.* **10**, 13–51.

Kerdelhué, C., Le Clainche, I. & Rasplus, J.-Y. 1999 Molecular phylogeny of the *Ceratosolen* species pollinating *Ficus* of the subgenus *Sycomorus sensu stricto*: biogeographical history and origins of the species-specificity breakdown cases. *Mol. Phylogenet. Evol.* **11**, 401–414.

Machado, C. A., Jousselin, E., Kjellberg, F., Compton, S. G. & Herre, E. A. 2001 Phylogenetic relationships, historical biogeography, and character evolution of fig pollinating wasps. *Proc. R. Soc. Lond* B **268**, 685–694.

Maynard Smith, J. 1978 *The evolution of sex*. Cambridge University Press.

Ramírez B., W. 1970 Taxonomic and biological studies of Neotropical fig wasps (Hymenoptera: Agaonidae). *Univ. Kansas Sci. Bull.* **49**, 1–44.

Weiblen, G. D. 2000 Phylogenetic relationships of functionally dioecious Ficus (Moraceae) based on ribosomal DNA sequences and morphology. *Am. J. Bot.* **87**, 1342–1357.

Wiebes, J. T. 1994 The Indo-Australian Agaoninae (pollinators of figs). *Nederlandse Akademie van Wetenschappen Verhandelingen Afdeling Natuurkunde., Tweede Reeks* **92**, 1–208.

Wiebes, J. T. 1995 The New World Agaoninae (pollinators of figs). *Nederlandse Akademie van Wetenschappen Verhandelingen Afdeling Natuurkunde, Tweede Reeks* **94**, 1–60.