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CONVERGENCE AND COEVOLUTION IN A MUTUALISM: EVIDENCE FROM A MOLECULAR PHYLOGENY OF *FICUS*

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Abstract.—The interaction between *Ficus* (Moraceae) and their pollinating wasps (Chalcidoidea: Agaonidae; more than 700 species-specific couples) is one of the most specialized mutualisms found in nature. Both partners of this interaction show extensive variation in their respective biology. Here we investigate *Ficus* life-history trait evolution and fig/fig wasp coadaptation in the context of a well-resolved molecular phylogeny. Mapping out variations in *Ficus* life-history traits on an independently derived phylogeny constructed from ribosomal DNA sequences (external and internal transcribed spacer) reveals several parallel transitions in *Ficus* growth habit and breeding system. Convergent trait evolution might explain the discrepancies between morphological analyses and our molecular reconstruction of the genus. Morphological characters probably correlate with growth habit and breeding system and could therefore be subject to convergent evolution. Furthermore, we reconstruct the evolution of *Ficus* inflorescence characters that are considered adaptations to the pollinators. Our phylogeny reveals convergences in ostiole shape, stigma morphology, and stamen:ovule ratio. Statistical tests taking into account the phylogenetic relationship of the species show that transitions in ostiole shape are correlated with variation in wasp pollinator head shape, and evolutionary changes in stigma morphology and stamen:ovule ratio correlate with changes in the pollination behavior of the associated wasp. These correlations provide evidence for reciprocal adaptations of morphological characters between these mutualistic partners that have interacted over a long evolutionary time. In light of previous ecological studies on mutualism, we discuss the adaptive significance of these correlations and what they can tell us about the coevolutionary process occurring between figs and their pollinators.

Key words.—Active pollination, coadaptation, comparative analysis, figs, parametric bootstrap, phylogeny.

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Coevolution can be defined as reciprocal changes in traits of interacting species (Janzen 1980) and occurs through interactions that range from parasitic to mutualistic associations. Under this definition, coevolution is detected as covariation between traits of interacting species. This can be achieved by mapping traits onto a phylogeny and investigating whether repeated evolution of similar traits is driven by characteristics of an interacting species. Such comparative studies reveal coadaptations and have been conducted for host-parasite interactions (Morand 1996; Sorci et al. 1997) but seldom on mutualisms (but see Brouat et al. 2001). The interaction between species of the genus *Ficus* (Moraceae) and their pollinating wasps (Agaonidae, Chalcidoidea, Hymenoptera) represents what is perhaps the most specialized case of pollination mutualisms and a model system for the study of coevolution. Each species of *Ficus* (Moraceae) is pollinated by one, or sometimes several, species of wasps that oviposit within its inflorescence (Berg 1989; Rasplus 1996). Because the reproductive cycles of mutualistic partners are linked, both partners should show reciprocal morphological adaptations if coevolution has occurred (Ramirez 1974; Janzen 1979; Herre 1989; Berg 1990). The diversity of the genus (more than 700 species) and the specificity of the interaction provide an ideal framework to explore the evolutionary trajectories of *Ficus* characters and to test existing hypotheses about fig/fig wasp coadaptations through comparative analyses. In addition, *Ficus* species exhibit extensive variation in their biology. For instance, they vary in growth forms, among species, from freestanding trees to root climbers, epiphytes, or even hemiepiphytes (hemiepiphytes germinate as epiphytes, but they grow roots that reach soil). *Ficus* breeding systems are also variable; half of the species is monoecious, whereas the other half is gynodioecious but functionally dioecious (Berg 1989). Variation in *Ficus* life history drastically changes the environment of fig wasps and also influences many associated fig morphological characters. It is thus important to establish the evolutionary trajectories of *Ficus* life-history traits in order to understand this interaction and to interpret character evolution in figs and fig wasps.

Fig Biology and Fig/Fig Wasp Interaction

Although they can exhibit very different growth forms, all *Ficus* species are recognized by their inflorescence, known as a fig (or syconium), which is a closed receptacle the inside of which is lined with numerous uniovulate female flowers and male flowers. When the fig is receptive, it emits volatiles that attract the specific wasps (Hossaert-McKey et al. 1994). Once a fig has been located, the wasps enter the closed receptacle via the ostiole, a slit formed by bracts situated at the apex of the fig. Within the fig cavity, agaonid wasps lay their eggs in some of the flowers, typically laying one egg per flower (Jousselin et al. 2001; Fig. 1), and pollinate the inflorescence in the process. Ovules that have received an egg are transformed into a gall in which wasp larvae complete their development. Ovules that have been fertilized by pollen

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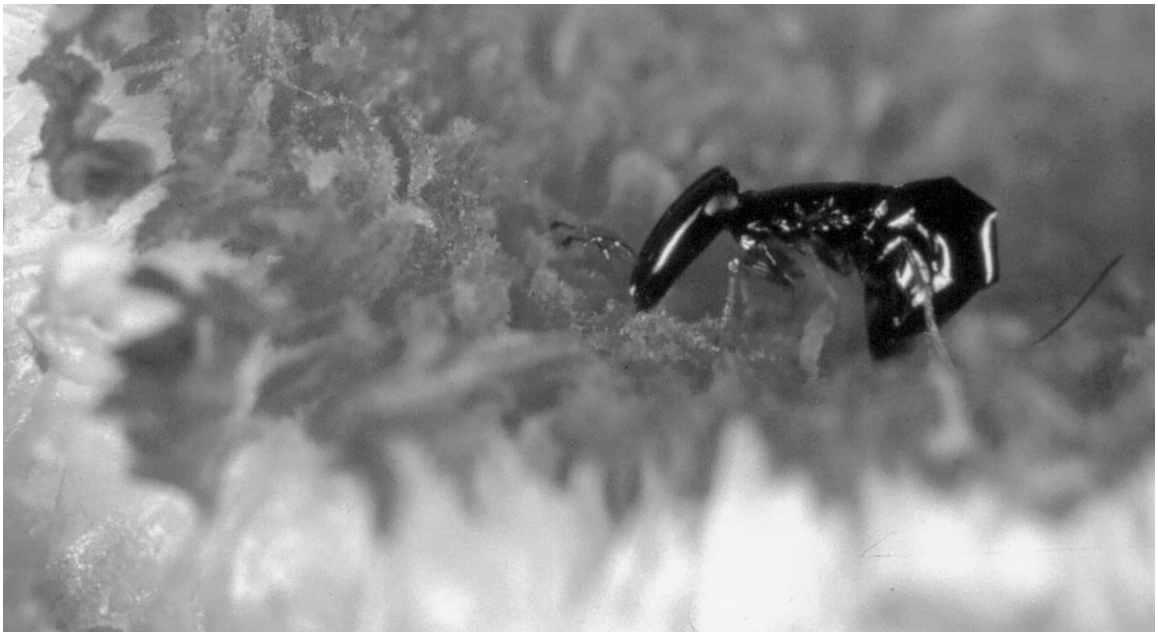

FIG. 1. Female of *Courtella armata*, the pollinator of *Ficus sansibarica* (section *Galoglychia*, monoecious), inside the fig cavity laying its eggs. *Courtella armata* is an active pollinator; in this fig species the stigmas form a cohesive structure known as a synstigma (photo J-Y. Rasplus).

carried in on the wasps but did not receive a wasp egg produce a seed (Verkerke 1989). Some weeks later, the adult offspring emerge into the fig cavity, the female wasps become loaded with pollen from male flowers and leave the ripe fig in search of a receptive fig in which to lay their eggs.

In monoecious *Ficus* species, seeds, wasps, and pollen are produced in the same fig. Thus each wasp offspring develops at the cost of a seed, creating a potential conflict with the host tree over ovule allocation to wasp or seed production (Janzen 1979; Herre and West 1997). In functionally dioecious *Ficus*, sexual functions are separated on different trees. Figs on female trees have female flowers that produce only seeds but do not produce either pollen or wasps. This is a typical case of pollination by deceit; wasps are attracted to receptive figs but cannot oviposit into flowers, probably due to ovipositor length constraints (Valdeyron and Lloyd 1979; Kjellberg et al. 1987; Weiblen 2001). Figs on male trees contain male flowers and many female flowers but the latter produce mainly wasps and very rarely seeds (Galil 1973; Valdeyron and Lloyd 1979; Jousselin and Kjellberg 2001). Hence male figs produce pollen, and pollen vectors, the wasps.

In addition, the way pollinators transport pollen varies among species. In about one third of *Ficus* species, pollination is passive (Kjellberg et al. 2001) and involves no obvious wasp adaptations aimed at transporting pollen (Galil and Neeman 1977). These figs produce abundant pollen grains that are released by anther dehiscence when the wasps emerge from their galls. Thus, the wasps get coated with pollen when they crawl between the stamens to exit their natal fig (Galil and Neeman 1977; Galil and Meiri 1981). In the remainder of the genus *Ficus*, pollination is active. Before leaving their natal fig, active pollinators seek out the anthers and load pollen into special thoracic structures known as pollen pockets. When inside a receptive fig, each time they oviposit, they pick out pollen from their pockets and deposit it on the stigmas (Galil and Eisikowitch 1969; Frank 1984; Greeff and Compton 1996).

Hypotheses about fig/fig wasp coadaptations are numerous. For instance, wasps that pollinate dioecious *Ficus* have short ovipositors relative to those pollinating monoecious *Ficus* (Ramirez 1974; Wiebes 1994; Weiblen 2001). Variation in pollination behavior of the wasps probably imposes very different selective pressures on the fig and should drive the evolution of floral traits. For example, passively pollinated *Ficus* species produce more pollen than actively pollinated *Ficus* species; this causes them to produce numerous anthers relative to their number of ovules (Galil and Meiri 1981; Kjellberg et al. 2001). It has also been suggested that mode of pollination selects for different stigma structure (Jousselin and Kjellberg 2001). In actively pollinated *Ficus* species, the stigmas of monoecious and female figs form a cohesive structure known as a synstigma (Verkerke 1989; Fig. 1). In passively pollinated figs, stigmas each project separately into the fig cavity. The shape of the fig ostiole also correlates with differences in pollinator characters (Ramirez 1974; Van Noort and Compton 1996). The arrangement of the ostiolar scales may be of two types. Either many of the bracts are interlocking forming a spiral passage into the fig cavity (spiral ostiole) or all the bracts (except sometimes the two or three external ones) are turned toward the inside of the fig (linear ostiole; Verkerke 1989). Wasps associated with hosts possessing linear ostioles have elongate, thin mandibular appendages whereas wasps on hosts with spiral ostioles have short appendages that are strongly fused to the mandible. The latter also have a lower length/width ratio of the head and have a soft zone on the frons, enabling longitudinal head folding.

Most studies concerning fig/pollinator trait correlations still lack a phylogenetic perspective. For instance, the difference in anther production between actively and passively pollinated species has been quantified for more than 100 *Ficus* species (Kjellberg et al. 2001), but all species were treated as independent datapoints. A robust fig phylogeny provides the template to statistically test evolutionary correlations between fig traits and pollinating wasp traits (Brooks and McLennan 1991; Harvey and Pagel 1991; Armbruster 1992; Lauder et al. 1993). A phylogeny allows a determination of how many times a given growth form and/or breeding system has evolved in the genus.

Two phylogenetic reconstructions of the genus *Ficus* are now available (Herre et al. 1996; Weiblen 2000). They comprise the reconstruction of relationships among a limited number of species by Herre et al. (1996) using *rbcL* chloroplast DNA, and a more extensive phylogeny reconstructed from a combined analysis of molecular (internal transcribed spacer; ITS) and morphological data by Weiblen (2000) that mainly focuses on dioecious *Ficus*. The aim of this study is to investigate the evolutionary trajectory of two *Ficus* life-history traits: growth form and breeding system, and to test several hypotheses about fig/fig wasp coadaptations in a phylogenetic context. To avoid using characters affected by selection linked to *Ficus* growth form, breeding system, or the ecology of the associated wasp, we chose to base our study on a phylogenetic reconstruction using molecular markers only. We also include some diversified sections of monoecious *Ficus* that were not included in the previous study. Because the phylogenetic study of *Ficus* based on ITS markers (Weiblen 2000) left some deep nodes unresolved, we reconstruct the phylogeny of *Ficus* using ITS and an additional marker, External Transcribed Spacer (ETS), which has been shown to be a useful complement to phylogenetic reconstruction using ITS (Baldwin and Markos 1998; Béna et al. 1998; Randal Linder et al. 2000). First, we use this phylogeny to map *Ficus* breeding system and growth habit and compare our results with previous phylogenetic reconstructions of *Ficus* and morphological classifications. Second, we reconstruct the evolution of several inflorescence characters that are thought to reflect adaptations to pollinators, namely ostiole shape, anther:ovule ratio; and stigma morphology. To test hypotheses about coadaptations, we conduct a formal comparative analysis to investigate whether transitions in these characters are correlated to differences in characters of the associated pollinators (Pagel 1994); that is, pollinator head shape and pollination behavior, respectively. In the final section we focus on the evolution of pollination behavior and correlated traits, and in light of previous ecological studies we propose hypotheses for the coevolutionary mechanisms that have shaped these traits.

Materials and Methods

Plant Material

We based our phylogenetic reconstruction on 41 species of *Ficus* scattered in the *Ficus* classification. All sections of the genus were represented by at least one species (except section *Leucogyne*, two species, and section *Sinosycidium*, one species). Furthermore, if a section was pollinated by more than one genus of wasps, we sampled representatives pollinated by each genus. For example, in section *Conosycea* we chose species pollinated by *Waterstoniella* as well as species pollinated by *Eupristina*. However, we did not obtain samples for *Ficus* species pollinated by genus *Deilagaon*, nor did we include figs in section *Galoglychia* pollinated by *Alfonsiella*, *Courtella*, and *Allotriozoon*; including them would have led to an overrepresentation of *Ficus* belonging to section *Galoglychia* in the phylogeny. Each sequence was taken from a single individual. Table 1 lists *Ficus* species, associated pollinator species, place of collection, voucher number, and database accession for all of the samples sequenced.

For each species included in the analysis, we examined morphological characters to check the assignment to section as well as the genus assignment of pollinating wasp. This allowed us to propose that *F. deltoidea* and *F. erecta* are pollinated by species belonging to the genus *Wiebesia* sensu Wiebes (1994) and not by the genus *Blastophaga* (for instance, female pollinators of both species have the longitudinal median suture on the mesotonum, which is typical of *Wiebesia* and absent in *Blastophaga* subgenus *Blastophaga*).

Four species belonging to different genera of Moraceae were chosen as outgroups: *Morus alba*, *Broussonetia papifera*, *Brossemum* sp., and *Artocarpus integer*. Our choice was guided by the phylogenetic reconstruction of Herre et al. (1996). *Ficus* ITS and ETS sequences, however, were too highly diverged to allow alignment with these other Moraceae. Analysis of the *rbcL* gene shows that *Ficus* section *Pharmacosycea* is the basal group of *Ficus* (Herre et al. 1996). Hence, species of section *Pharmacosycea* were designated as outgroup in the analysis.

In addition, the 46 ITS sequences obtained by Weiblen (2000) were retrieved from GenBank to allow a global analysis of all available ITS sequences.

DNA Polymerase Chain Reaction Amplification and Sequencing

Total DNA was extracted from 20–50 mg of fresh, dried, or frozen leaf tissue according to the cetyltrimethyl ammonium bromide protocol of Doyle and Doyle (1987). Double-stranded DNA of the complete ITS region were PCR-amplified using primers 75 and 92 (Desfeux et al. 1996). The ETS region was amplified using primers 18S-ETS and ETS-HEL-1 (Baldwin and Markos 1998). PCR amplifications were performed in a 25-μl volume containing 2 mM $MgCl_2$, 250 μM of each dNTP, 1 μM of each primer and one unit of Promega (Madison, WI) polymerase.

Following an activation step of 4 min at 94°C, the PCR mixture underwent 30 cycles of 1 min at 94°C, 1 min at 52°C, and 1 min at 72°C. To remove excess primers and DNTP after amplification, PCR products were gel-purified (QiaQuick, Qiagen, Valencia, CA). Sequencing was performed on both strands using the ABI Prism dye terminator cycle sequencing ready reaction kit (Perkin Elmer, Foster City, CA) in a 20-μl volume containing 20 ng of purified DNA, and 3.2 pmol of amplification primer. Sequencing reactions underwent 25 cycles of 30 sec at 96°C, 30 sec at 52°C, and 4 min at 60°C. PCR products were purified and sequenced with the ABI Prism dye terminator (Perkin Elmer).

TABLE 1. Species of *Ficus* used for phylogenetic analysis, associated pollinator genus, collection localities, accession numbers, and voucher specimen numbers (JYR, J.Y. Rasplus frozen leaves collection, Centre de Biologie et de Bestion des Populations; FK, F. Kjellberg herbarium).

Subgenus	Section	Species	Locality	GenBank accession numbers (ITS/ETS)	Voucher numbers
Pharmacosycea	*Pharmacosycea*	*F. insipida*	French Guyana	AY063592/AY063549	JYR 40
		F. glabrata	Panama	AY063593/AY063550	FK2000-9
		F. maxima	Panama	AY063595/AY063551	FK2000-8
		F. yoponensis	Panama	AY063594/AY063552	FK2000-14
	Oreosycea	*F. callosa*	India	AY063565/AY063526	
		F. racemigera	New Caledonia	AY063587/AY063554	
Urostigma	*Americana*	*F. nymphaeifolia*	French Guyana	AY063566/AY063527	
		F. schumacheri	French Guyana	AY063567/AY063528	JYR 37
	Conosycea	*F. subgelderi*	Brunei	AY063556/AY063517	FK 1998-49
		F. xylophylla	Brunei	AY063557/AY063518	JYR 32
		F. consociata	Brunei	AY063558/AY063519	JYR 25
		F. benjamina	Brunei	AY063559/AY063520	JYR 6
		F. microcarpa	Brunei	AY063560/AY063521	JYR 24
		F. binnendykii	Brunei	AY063561/AY063522	JYR 9
	Malvanthera	*F. pleurocarpa*	Australia	AY063568/AY063529	
		F. macrophylla	Australia	AY063571/AY063532	
		F. rubiginosa	Australia	AY063569/AY063530	
	Galoglychia	*F. glumosa*	South Africa	AY063562/AY063523	JYR 19
		F. cyathistipuloides		AY063563/AY063524	JYR 8
		F. lutea	Ivory Coast	AY063564/AY063525	JYR 16
	Stilpnophyllum	*F. elastica*	Ornamental	AY063555/AY063516	JYR 5
	Urostigma	*F. prolixa*	Tahiti	AY063581/AY063542	JYR 12
		F. religiosa	Lebanon	AY063582/AY063543	JYR 4
		F. salicifolia	South Africa	AY063586/AY063553	FK 1999-1
Sycomorus	*Sycomorus*	*F. mauritiana*	La Reunion	AY063570/AY0635311	JYR 41
		F. vallis-choudae	Tanzania	AY063574/AY063535	
		F. sycomorus	Tanzania	AY063575/AY063536	
		F. sur	Tanzania	AY063572/AY063533	
	Sycocarpus	*F. uncinata*	Brunei	AY063576/AY063537	JYR 30
		F. condensa	Brunei	AY063577/AY063538	FK 1998-103
	Adenosperma	*F. mollior*	Australia	AY063573/AY063534	
	Neomorphe	*F. variegata*	Brunei	AY063578/AY063539	FK 1996-40
Ficus	*Ficus*	*F. deltoidea borneensis*	Brunei	AY063579/AY063540	JYR 28
		F. erecta	Ornamental	AY063589	JYR 1
		F. aurata	Brunei	AY063590	FK 1998-81
		F. grossularioides	Brunei	AY063591/AY063548	JYR 2
	Rhizocladus	*F. pumila*	Ornamental	AY063580/AY063541	JYR 3
	Kalosyce	*F. punctata*	Brunei	AY063584/AY063545	FK 1997-23
	Sycidium	*F. parietalis*	Brunei	AY063583/AY063544	FK 1998-88
		F. lateriflora	La Reunion	AY063585/AY063546	JYR 44
		F. asperifolia	Gabon	AY063588/AY063547	JYR 7

Alignment and Phylogenetic Analysis

Sequences were verified by checking each sequence against its complement. They were aligned using the program ClustalW (Thompson et al. 1994), using ClustalW default options (open gap penalty = 10.0; extend gap penalty = 5.0, delay divergent = 40%; transitions: weighted). All alignments were checked by eye.

After excluding ambiguous positions, gaps of one to twelve nucleotides remained in the aligned sequences. In the phylogenetic analyses, single site gaps were treated as a new state. For gaps that were longer than one nucleotide, we coded the first site gap as a new state and coded all other sites ''missing data'' as suggested by Béna et al. (1998). In this way, longer gaps were counted as a single event. Excluding gaps from the analysis led to a similar topology but slightly less well-resolved phylogeny. Maximum parsimony analyses were conducted using PAUP* version 4.0b5 for Power Macintosh computers (Swofford 1999).

First, an analysis was conducted on all available ITS sequences: the sequences obtained by Weiblen (2000) and the ones obtained in this study. A total of 86 sequences were aligned and analyzed using maximum parsimony. Because dealing with so many sequences proved computer intensive, we used heuristic searches involving TBR branch swapping with only 100 random stepwise additions of taxa and Maxtrees set to 1000. For bootstrapping, we used heuristic searches of 100 replicates and 10 random taxon addition sequences and Maxtrees set to 1000.

Separate analyses were then performed on the ITS and ETS sequences obtained in this study. Heuristic searches were conducted involving TBR branch swapping with 1000 random stepwise additions of taxa. Nonparametric bootstrap resampling was used to estimate clade robustness (Felsenstein 1985). For bootstrapping, heuristic searches with 500 replicates and 10 random addition sequence replicates were conducted.

The congruence of the two datasets was then checked using the incongruence length difference (ILD) test (Farris et al. 1994). The ILD test compares the difference in the numbers of steps required by individual and combined analysis of the original partitions with the value obtained for a series of randomized partitions. The test was run with 1000 replicates and 50 random additions of taxa with all constant characters excluded (Cunningham 1997). To determine whether separate analysis of the two datasets yielded strongly supported (with a bootstrap value $>$ 50%) conflicting clades we compared how well each 50% bootstrap consensus trees fit the rival dataset using Templeton tests (Templeton 1993) as implemented in PAUP*. For example, a heuristic search with 1000 random taxon additions was conducted on ITS data to find the length of the most parsimonious (MP) trees compatible with the 50% bootstrap consensus ETS tree. The difference between tree lengths obtained through unconstrained and constrained searches was tested with the Wilcoxon sign-rank test. Subsequently, ITS and ETS datasets were combined into a single matrix for phylogenetic analysis.

Since a nonparametric bootstrap does not specifically test hypotheses about monophyly, parametric bootstrap tests (Huelsenbeck et al. 1996; Emerson et al. 2000) were used to test the monophyly of taxa whose position in the molecular phylogeny conflicted with morphological classification (i.e., Berg and Wiebes 1992; or Weiblen's cladistic analysis of morphological traits, Weiblen 2000). For each test, the clade to be tested was enforced as a topological constraint (null hypothesis) in a new heuristic search and the difference in lengths between the constrained and the unconstrained trees was calculated. The significance of this difference was tested through comparison of this difference to a distribution generated with 100 simulated sequence datasets. To create this dataset, we first analyzed our sequence data (ITS + ETS) with the program Modeltest (Posada and Crandall 1998). This program establishes the model of DNA evolution that best fits the data by comparing log-likelihood scores of different models (the scores of 56 models of base substitution are computed by PAUP* using a randomly chosen MP tree). Next, 100 sequence datasets were generated by simulation using (1) a MP tree constructed with PAUP* using ML optimization of branch length and with the constraint enforced (null hypothesis), and (2) the model (base frequencies, Ts: Tv ratio, proportion of invariable sites and gamma shape parameter) that best fitted our data selected by Modeltest. In this case, the model selected was the Rev (the general reversible model) with estimated substitution rates of: A–C = 1, A–G = 3.021, A–T = 0.797, G–C = 4.257, G–T = 1; base frequencies of: A = 0.2341, C = 0.2953, G = 0.299, T = 0.17; and proportions of invariant sites of 0.4967. Sequences were simulated using Seq-gen version 1.1 (Rambault and Grassly 1997). For each of the 100 simulated datasets, heuristic parsimony searches were carried out first with and then without the specified constraint. The resulting distribution of differences was then compared with the tree length difference between the empirical constrained and nonconstrained trees.

In addition, Decay indices were calculated using the program Autodecay version 4.0 (Eriksson 1998).

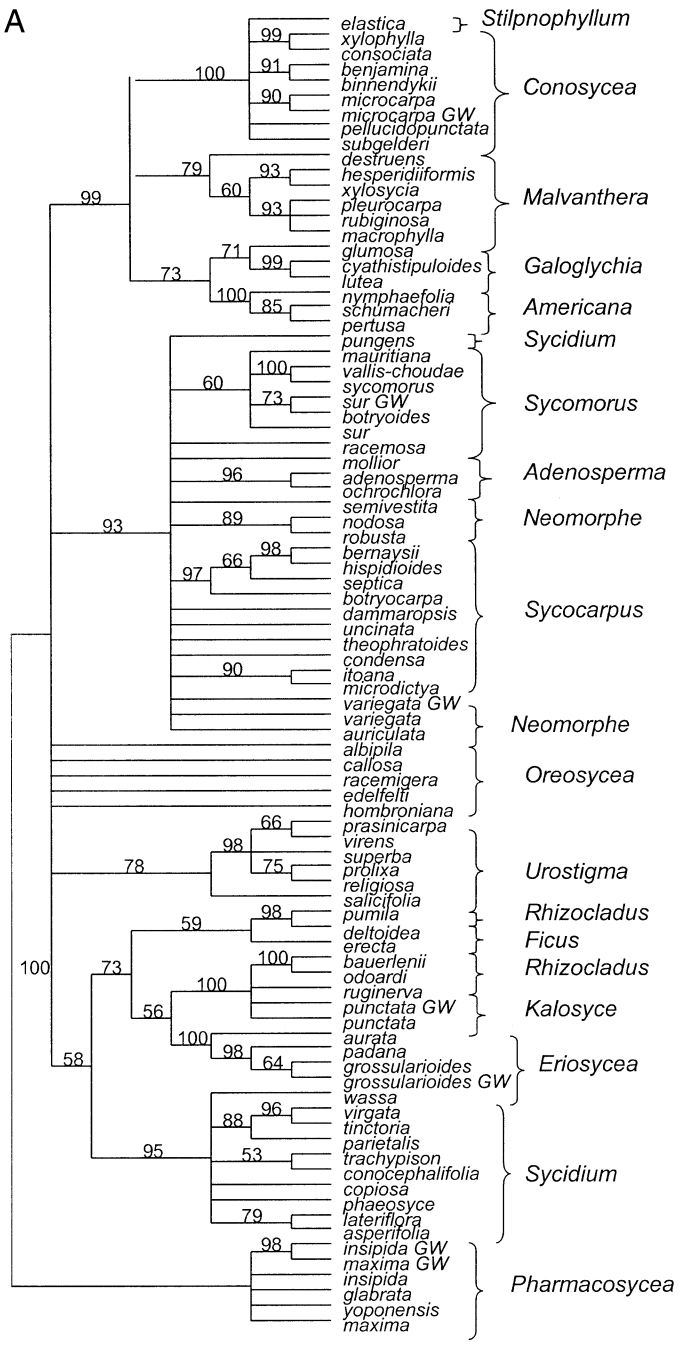

FIG. 2. (A) Strict consensus of equally parsimonious trees generated from a heuristic search conducted on all ITS sequences (tree length: 1087 steps, CI = 0.66). GW, sequences obtained in Weiblen 2000, retrieved from GenBank. (B) Association between pollinator genera and host figs. (C) Strict consensus of equally parsimonious trees generated from a heuristic search conducted on combined ITS and ETS data. The nonparametric bootstrap values (500 replicates) are given above each node and the decay values are given under each node.

Character Evolution

Ficus life-history traits

The distribution of breeding system (monoecious vs. dioecious) and growth form (terrestrial vs. hemiepiphytic)

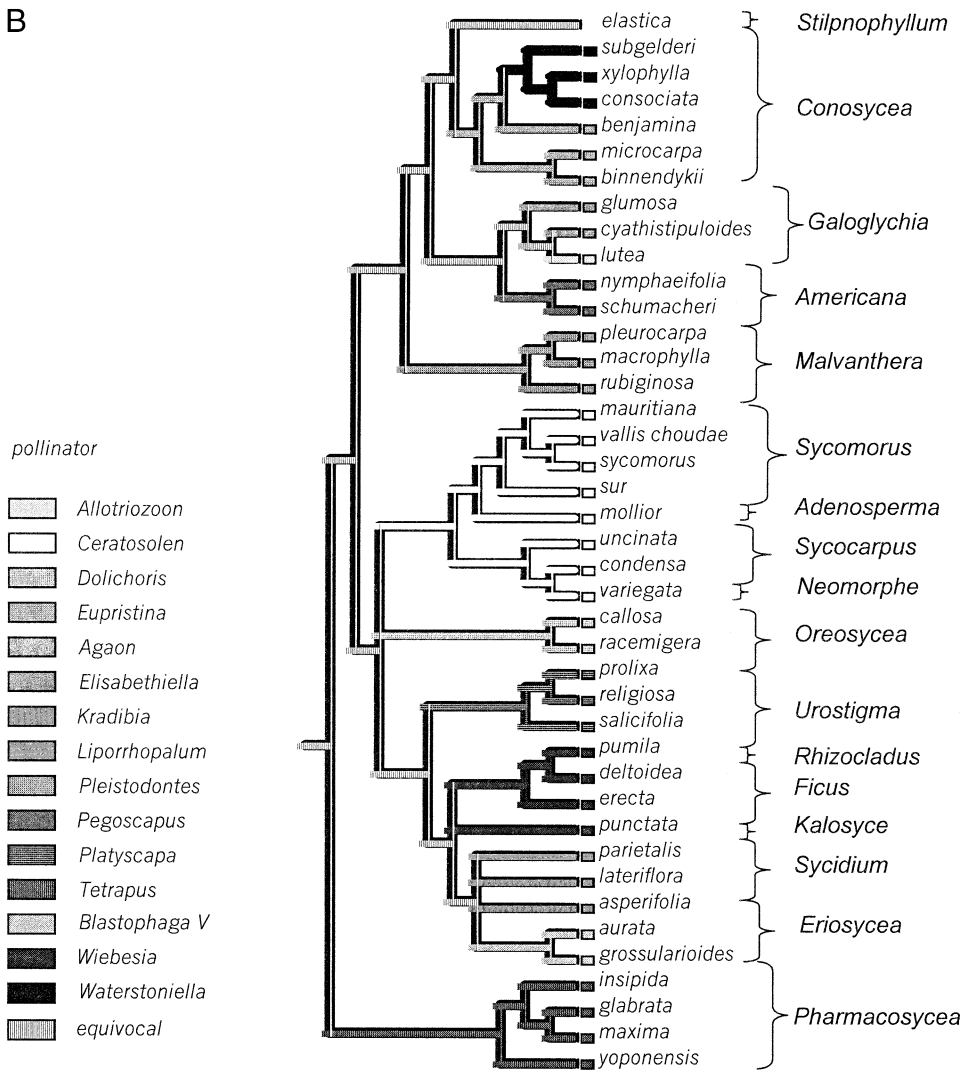

FIG. 2. Continued.

within genus *Ficus* was obtained from the literature (Corner 1965; Berg 1989) and confirmed through personal observations.

The evolutionary trajectories of these characters were inferred by mapping them on the strict consensus tree using the parsimony criterion as implemented in the program MacClade version 3.0 (Maddison and Maddison 1990). Polytomies were treated as uncertainties (soft polytomies).

Characters of the fig inflorescence that correlate with pollinators' traits

We reconstructed the evolution of the fig ostiole shape, anther:ovule ratio and stigma morphology on the fig phylogeny by mapping these characters on the strict consensus tree using the parsimony criterion (MacClade ver. 3.0; Maddison and Maddison 1990).

Ostiole shape and stigma morphology of the figs were based on Corner (1965) and Berg (1989) and independent evaluation of these traits by examination of our personal herbarium specimens or alcohol-preserved figs. Ostiole shape was treated as a two-state variable: (1) spiral (bracts interlocked and helicoidally arranged), and (2) linear (most bracts turned towards the inside of the fig cavity). Stigma morphology of monoecious and female figs was assigned two states: (1) platform-forming (all the stigmas are tightly packed and reach the same height in the fig cavity), and (2) non-platform-forming (stigmas are not in close contact with each other and they do not reach the same height in the fig cavity). The values for anther:ovule ratios (number of anthers:number ovules in a fig) were obtained from Kjellberg et al. (2001). Kjellberg et al. (2001) identified a highly significant difference in the anther:ovule ratio between actively and passively pollinated figs; with the exception of *F. macrophylla*, there was no overlap between these groups. Anther:ovule ratio was therefore treated as a qualitative variable with two states, low (< 0.15) and high (> 0.22).

The traits of the pollinators associated with the *Ficus* species included in our phylogeny were determined through the

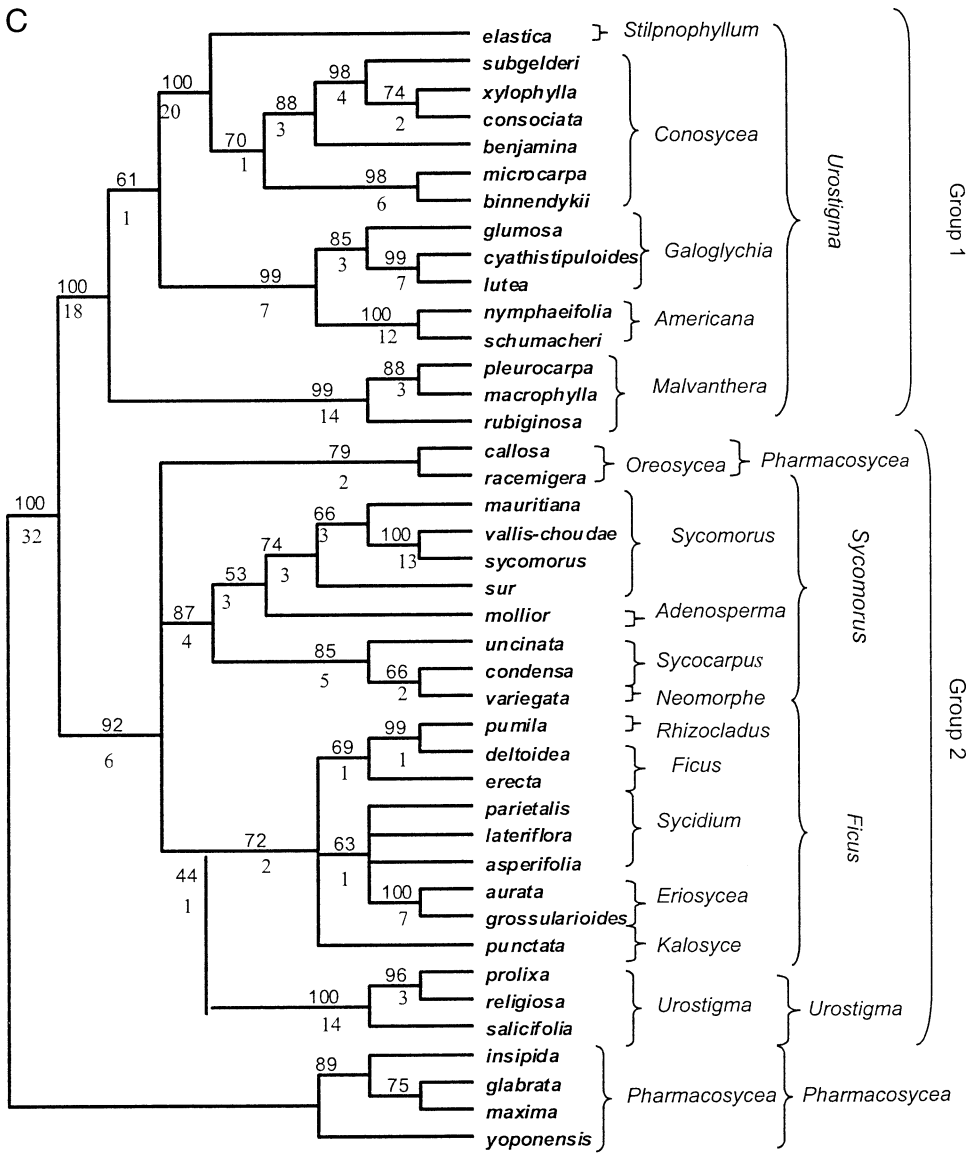

FIG. 2. Continued.

literature (Berg and Wiebes 1992, Kjellberg et al. 2001) as well as through our observations on various type specimens and on our personal collection of over 100 species. Head shape measurements were based on Berg and Wiebes (1992) and Wiebes (1994, 1995). Head shape was assigned two levels: (1) short: head as long as wide, length:width ratio ≤ 1, and (2) elongate: head longer than wide, length:width ratio > 1. Direct observation of pocket filling and emptying or presence of pollen within the thoracic pollen pockets were considered as evidence of active pollination, whereas lack of pollen pockets or lack of pollen within the pollen pockets upon emergence of agaonid wasps was considered as evidence for passive pollination (see Kjellberg et al. 2001).

Comparative Test

Our aim was to determine whether transitions in *Ficus* traits were associated with differences in traits of associated agaonid wasps. We thus conducted a formal test of correlated evolution (Pagel 1994) between fig characters and associated wasp characters. These correlations were investigated using a likelihood ratio (LR) test designed specifically for analyzing relationships between two binary characters in a phylogenetic context (Pagel 1994). This is achieved by comparing the fit of two models to the data mapped onto the MP tree in Figure 1 with branch lengths equal to the minimum number of changes required to draw the tree (Pagel 1994). For each pair of characters tested, two models of evolution are considered. In one model, two traits are allowed to evolve independently; in the other, they evolve in a correlated fashion. The hypothesis of correlated change is accepted if the dependent model fits the data better than the model of independent change. Significance is assessed through a likelihood ratio test. A Monte Carlo simulation is conducted to find the distribution of the LR under the null hypothesis (independent

change) in order to find the *P*-value of the LR observed for the real dataset (Pagel 1994). In our tests, wasp traits (i.e., head shape, pollination behavior) are considered to be the ''environmental'' variable; the independent change model assumes that shift in *Ficus* character states on the *Ficus* phylogeny are independent of associated pollinator traits and the ''dependent'' change model assumes that transitions in *Ficus* character states are dependent on pollinator traits.

Results

ITS-ETS Data and Phylogenetic Relationships in Ficus

ITS data and phylogenetic relationships in Ficus

After exclusion of 32 ambiguous positions, the aligned 86 ITS sequences were 722 bp in length. There were always differences between the sequences obtained from different individuals of the same species, but these were smaller than the differences between species belonging to different sections. The tree resulting from this analysis is largely unresolved due to the large number of samples relative to the length of the sequences (Fig. 2A). However it confirms the monophyly of subgenus *Ficus*, the monophyly of subgenus *Sycomorus*, and the monophyly of subgenus *Urostigma* excluding section *Urostigma*. *Ficus* sections in the *Urostigma* subgenus stand out as strong monophyletic entities.

ITS-ETS combined analysis

Complete sequences for ITS and ETS were obtained for 39 species. ETS fragments could not be amplified for *F. erecta* and *F. aurata*. We first analyzed the 41 ITS sequences and 39 ETS sequences separately. Aligned ITS sequences were 717 bp in length; after exclusion of 20 ambiguously aligned characters, there were 39 gaps and 149 characters were parsimony-informative. Aligned ETS sequences were 498 bp in length, there were 29 gaps, and 159 characters were parsimony-informative. The analysis of ITS sequences resulted in nine equally parsimonious trees of 523 steps (CI = 0.774), and the analysis of ETS sequences resulted in 24 equally parsimonious trees of 397 steps (CI = 0.70).

The ILD test detected significant incongruence between ITS and ETS data sets ($P = 0.001$). However, the ILD test does not distinguish whether incongruence between datasets results from different phylogenetic histories or different rates of evolution. It has also been shown that it is not a good measure of incongruence when datasets differ in size (Dowton and Austin 2002). The difference in tree topology seemed to be limited to poorly supported clades and terminal nodes. ITS data did not reject the ETS 50% bootstrap tree ($N = 46$, $Z = -1.85$, $P = 0.064$), similarly, ETS data did not reject the ITS 70% bootstrap tree ($N = 37$, $Z = -1.75$, $P = 0.085$). ETS sequences seem to evolve more rapidly than ITS sequences (Béna et al. 1998; Baldwin and Markos 1998), a feature which is sufficient to explain the lack of congruence detected by the ILD test.

The combined (ITS + ETS) phylogenetic analysis produced six equally parsimonious trees of 959 steps (CI = 0.71; RI = 0.85). The strict consensus of these trees was better resolved and better supported than trees obtained with either dataset analyzed separately. The genus was separated into three distinct clades (Fig. 2C). Following Herre et al. (1996), *Pharmacosycea* was designated as an outgroup. There were thus two monophyletic groups. Group 1 included all the sections traditionally included in subgenus *Urostigma* (monoecious hemiepiphytes), except section *Urostigma*. Group 2 consisted of monoecious and dioecious, mainly terrestrial species. It included subgenus *Sycomorus* (mainly dioecious) and subgenus *Ficus* (dioecious), as well as section *Oreosycea* (monoecious, terrestrial) and section *Urostigma* (monoecious, hemiepiphytic). Most *Ficus* sections were shown to be good monophyletic units except for the analyzed species of the related sections *Ficus* and *Rhizocladus*, and sections *Sycocarpus* and *Sycidium*. The mapping of pollinator association onto the tree suggests that each pollinator genus or subgenus was shown to pollinate a monophyletic group of *Ficus* except the monophyletic group of *Ficus* pollinated by *Waterstoniella* branching within *Eupristina*-pollinated *Ficus* (Fig. 2B). In subgenus *Ficus* all species pollinated by *Wiebesia* group together, suggesting that it may be necessary to revise the *Ficus* taxonomy based on the pollinators.

Parametric bootstrap

The main discrepancy between our reconstruction, classical taxonomy and Weiblen's (2000) combined molecular and morphological reconstruction was the placement of sections *Oreosycea* and *Urostigma* in group 2. The grouping of section *Urostigma* in Group 2 instead of Group 1 strongly contradicted inferences from morphological data. Although the node separating Group 1 from Group 2 was supported by a high nonparametric bootstrap value (92%), we further tested this branching using parametric bootstrapping. We also tested the position of section *Urostigma* as the sister group of subgenus *Ficus* using the same method. This position was weakly supported by nonparametric bootstraps (44%) and suggested polyphyly of dioecious *Ficus*.

Monophyly of subgenus Urostigma

We conducted heuristic searches enforcing the monophyly of subgenus *Urostigma*. The most parsimonious tree under the constraint of subgenus *Urostigma* monophyly was nine steps longer than the unconstrained tree. The greatest observed step-length difference between the null and alternative hypotheses generated from the simulated data was seven. The probability of observing a difference of nine steps was low enough ($P < 0.01$) to reject the null hypothesis that the subgenus *Urostigma* is monophyletic.

Monophyly of a clade constituted of subgenus Sycomorus and subgenus Ficus

To test whether section *Urostigma* constitutes the sister clade of subgenus *Ficus*, we conducted heuristic searches enforcing the monophyly of subgenus *Ficus* with subgenus *Sycomorus*. Constraining the two *Ficus* subgenera, comprising all dioecious *Ficus*, to form a monophyletic group gave the tree only two steps longer than the unconstrained tree. However, this corresponds to the greatest observed difference between the null and alternative hypotheses generated from the simulated data. According to the distribution of step-

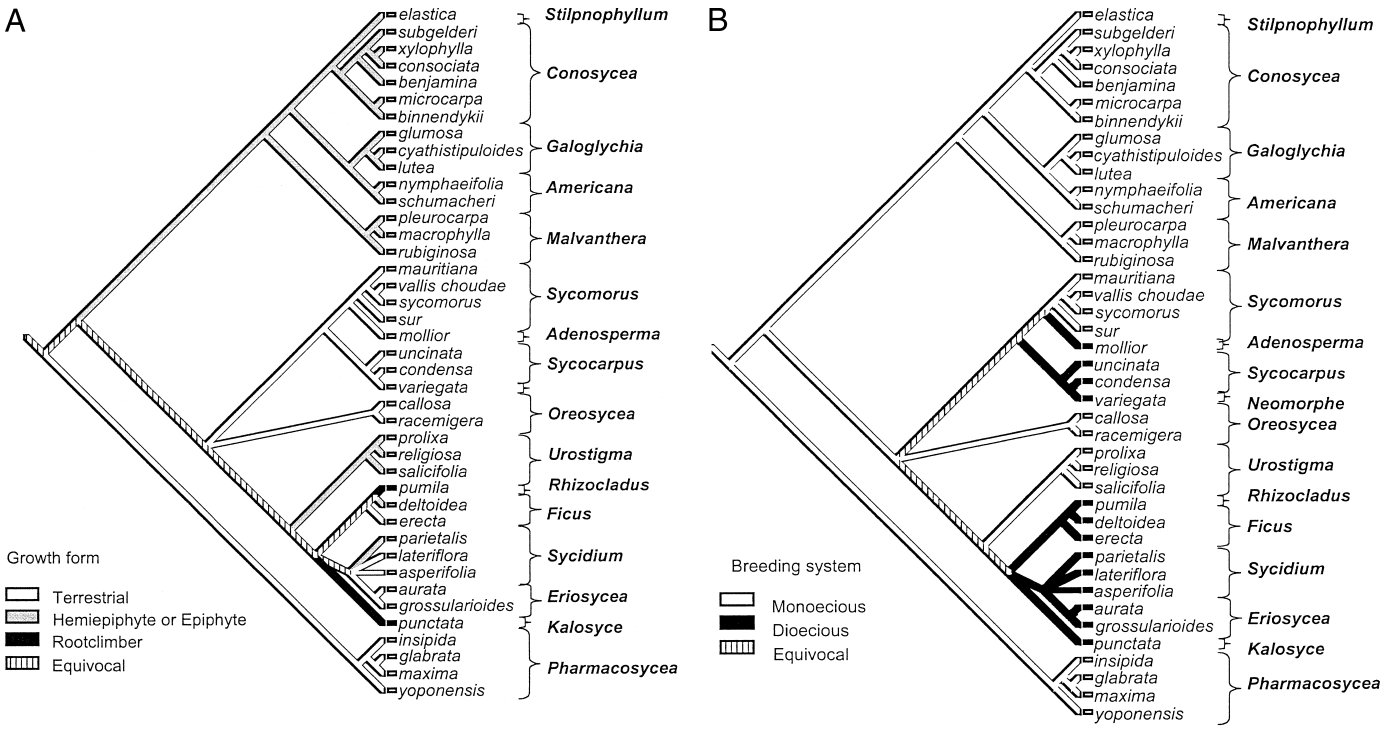

FIG. 3. Evolution of *Ficus* life-history traits. (A) Evolution of growth form. (B) Evolution of breeding system.

length differences generated by simulation, the probability of observing a two-step difference was $P = 0.05$, hence the monophyly of a clade including all species belonging to subgenera *Sycomorus* and *Ficus* can be rejected.

Character Evolution

Ficus life-history traits

The evolutionary trajectory of *Ficus* growth form is reconstructed in Figure 3A. Here the reconstruction is ambiguous. This figure suggests that there might have been repeated evolution of hemiepiphytism in the genus: once in the ancestor of Group 1, once in the ancestor of section *Urostigma*, and once within section *Sycidium*. Alternatively, hemiepiphytism in section *Urostigma*, *F. deltoidea*, and *F. parietalis* might be homologous and this growth habit has been lost four times. Hemiepiphytism could also be homologous between Groups 1 and 2, and have been lost independently five times.

Trait mapping onto the consensus tree under parsimony (Fig. 3B) gave monoecy as the primitive state. The reconstruction of this character is still ambiguous. If we assume that section *Urostigma* is correctly placed, dioecy could have arisen twice, once in the ancestor of subgenus *Sycomorus*, and once in the ancestor of the subgenus *Ficus*, with one reversal to monoecy in the ancestor of section *Sycomorus*. Alternatively, there could be a single acquisition of dioecy in the common ancestor of section *Urostigma*, subgenus *Ficus*, and subgenus *Sycomorus* with two reversals to monoecy (in section *Sycomorus* and in section *Urostigma*). These scenarios are dependent on the positions of section *Urostigma*: collapsing the uncertain node (bootstrap = 44%) linking section *Urostigma* and subgenus *Ficus* section would support a single acquisition of dioecy and only one reversal to monoecy.

Characters of the fig associated with pollinator traits

The distribution of ostiole shape is shown in Figure 4. Ostiole shape is linear in sections *Pharmacosycea*, *Malvanthera*, and *Galoglychia*. The most parsimonious trajectory for this character suggests that the primitive state constituted linear ostioles and that these evolved into spiral ostioles independently in the ancestors of Group 2, in the ancestors of section *Conosycea* and in the ancestors of section *Americana*. Alternatively, linear ostioles could have been lost in a common ancestor of section *Conosycea*, *Galoglychia*, and *Americana*, and then have been secondarily recovered in section *Galoglychia*. Alternatively, spiral ostioles could be primitive and have been lost three times independently. In any case, transition in ostiole type was associated with a transition in head shape of the associated pollinator (Fig. 4). We tested the significance of this association using a test of correlated evolution. The likelihood for the dependent change model between ostiole shape and pollinator head shape was greater than that for the independent change model. The Monte Carlo simulations showed that this difference was significant (Table 2). Hence ostiole shape and pollinator head shape are strongly correlated.

The anther:ovule ratio was determined for 36 of the 41 species included in our phylogeny. Figure 5a shows the evolutionary trajectory of this character. There have been at least six transitions between low and high anther:ovule ratio. For 29 of the 36 species, we had direct evidence (based on pollinator observations) for the mode of pollination of the as-

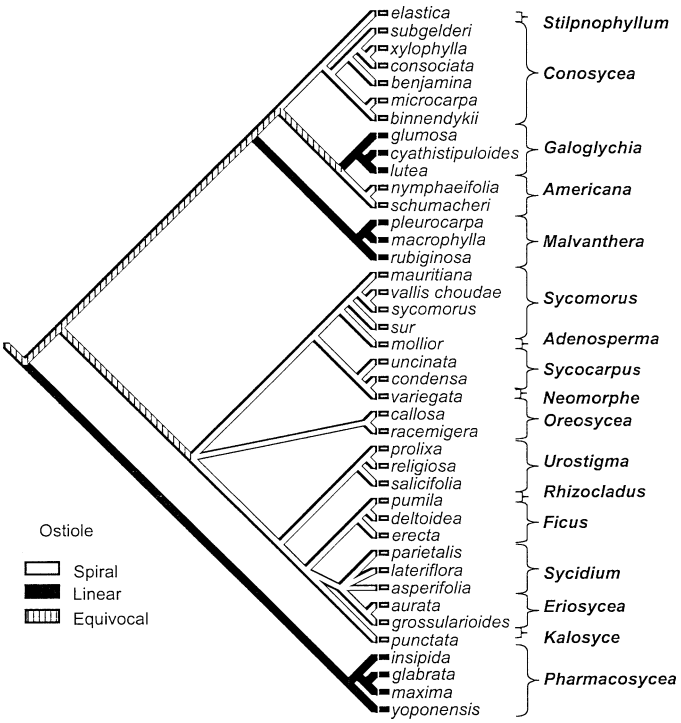

FIG. 4. Evolution of ostiole shape and associated pollinator head shape.

sociated agaonid wasp (Kjellberg et al. 2001; for *F. insipida* E. Jousselin, pers. obs.). There was an almost perfect correspondence between low anther:ovule ratio and active pollination, the only exception being the association between *F. macrophylla* (anther:ovule ratio = 0.53) and *Pleistodontes frogatti* (active, but covered with pollen). Because the test of correlated evolution implemented in Discrete (Pagel 1994) does not take into account missing data, the test was conducted on a smaller dataset consisting only of species for which we had values for anther:ovule ratio and mode of pollination. The test of correlated evolution showed a significant correlation between anther:ovule ratio and mode of pollination (Table 2).

Tightly packed stigmas in female and monoecious figs were associated with active pollination (Fig. 5b). According to Figure 5b, such stigma morphology has arisen once in the common ancestors of Groups 1 and 2 and was lost repeatedly in passively pollinated *Ficus* species. As in the previous analysis, species for which we did not have any information on stigma shape and/or direct observation of mode of pollination were excluded from the test of correlated evolution. The correlation test strongly supported an association between mode of pollination and the evolution of a stigmatic platform (Fig. 5b; Table 2). If the anther:ovule ratio was considered evidence for mode of pollination, the species that could be accounted for in the correlation test were more numerous (more than seven species) and this reinforced the strength of the association between mode of pollination and stigma cohesiveness (Table 2). The stigmas of male figs were always

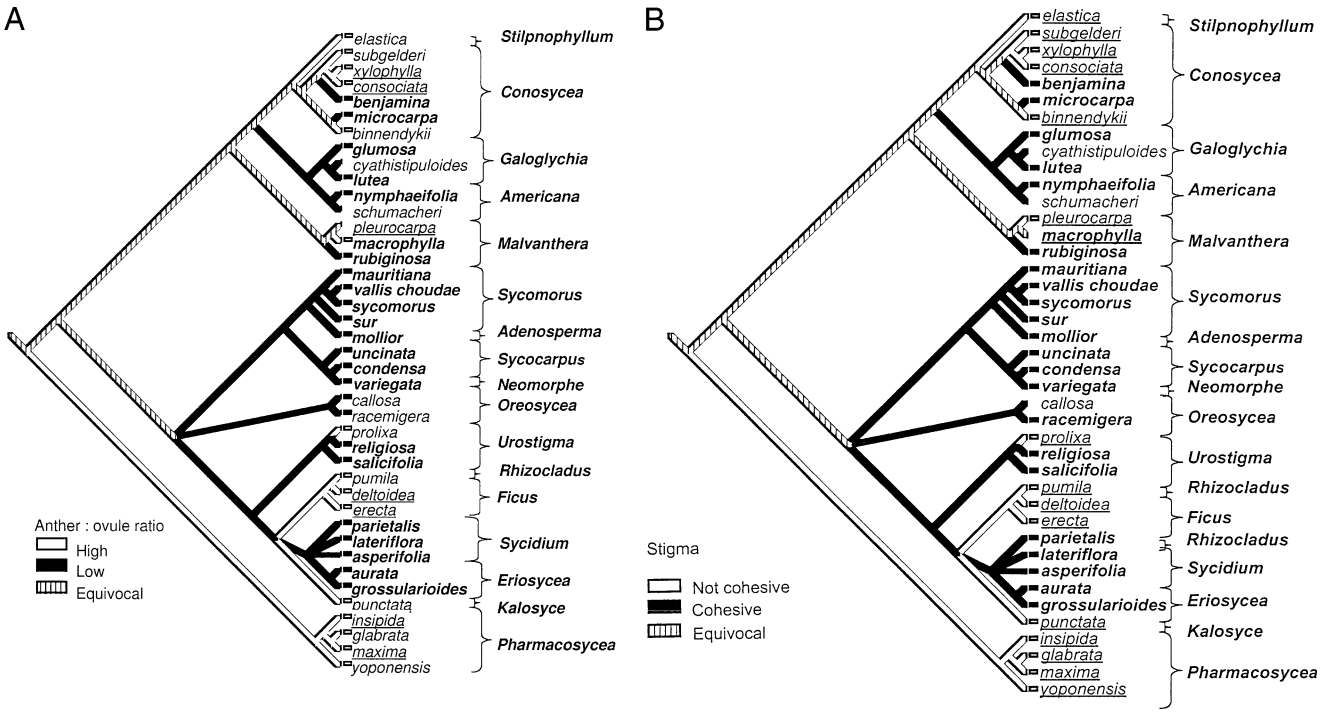

FIG. 5. Evolution of pollination mode and associated characters: (A) Evolution of anther:ovule ratio (species in bold are passively pollinated and underlined species are actively pollinated; there is no direct evidence of mode of pollination of associated agaonid wasps for other species; see Kjellberg et al. 2001). (B) Evolution of stigma morphology of monoecious *Ficus* and female figs of dioecious species (species in bold are passively pollinated and/or have high anther:ovule ratio; underlined species are actively pollinated or have low anther:ovule ratio; see Kjellberg et al. 2001). (C) Evolution of mode of pollination (inferred from direct observation and/or anther: ovule ratio).

tubular in actively pollinated species and tubular or bifid with short branches but always individualized in passively pollinated species.

Using a combination of the direct and indirect (anther:ovule ratio) evidence, we inferred the history of pollination mode on the *Ficus* phylogeny (Fig. 5c). The reconstruction suggested that active pollination arose once in the ancestor of Groups 1 and 2 and has been lost independently five times (twice in section *Conosycea*, in *Eupristina*-pollinated *Ficus* and in *Waterstoniella*-pollinated *Ficus*, once in section *Malvanthera*; once in section *Urostigma*; and once in subgenus *Ficus*).

Discussion

The phylogenetic reconstruction based on the combined analysis of ITS and ETS sequences agrees to a large degree with standard classifications (Berg 1989; Berg and Wiebes 1992) and previous molecular phylogenies (Herre et al. 1996; Weiblen 2000). However, there are a couple of major discrepancies with morphological taxonomy that confirm trends observed in previous molecular phylogenetic reconstructions. Our reconstruction supports the proposition that section *Oreosycea* does not belong to subgenus *Pharmacosycea*. Its position is ambiguous in the reconstruction based on ITS data alone (Weiblen 2000; Fig. 2a). The combined analysis of ITS and ETS sequences strongly suggests that it belongs to Group 2, the clade that includes all dioecious species. The combined analyses of ITS and ETS data also suggest that section *Urostigma* does not belong with the remainder of subgenus *Urostigma*: it branches in the clade including all dioecious *Ficus* species (Group 2) and this position is strongly supported by nonparametric bootstraps. This was also suggested by the molecular phylogenetic reconstruction (based on ITS data alone) of Weiblen but it was rejected when morphological data were included in the analysis (Weiblen 2000). The global analysis of all ITS sequences also fails to group section *Urostigma* with all the other species belonging to subgenus *Urostigma*. Furthermore, parametric bootstraps support the monophyly of a group formed by section *Urostigma* and subgenus *Ficus*.

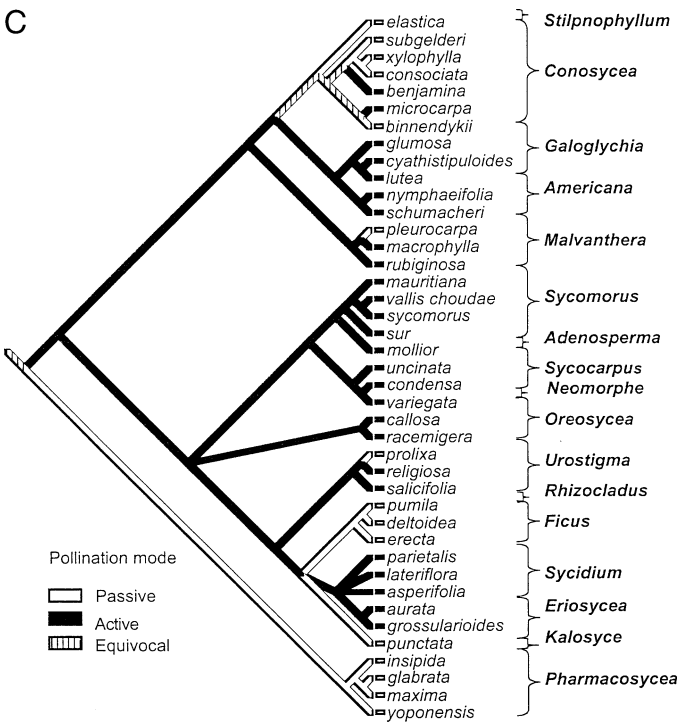

FIG. 5. Continued.

TABLE 2. Results of tests of correlated evolution between *Ficus* trait and associated pollinator trait. L(I), log-likelihood of independent model; L(D), log-likelihood of dependent change model; LR, likelihood ratio.

Ficus trait × associated pollinator trait	L(I)	L(D)	LR	*P*
Ostiole shape × pollinator head shape	−29.2	−8.3	20.8	<0.01
Anther/ovule × pollination mode	−25.2	−16.1	9.12	0.04
Stigma shape × pollination mode	−23.9	−11.4	12.58	<0.01
Stigma shape × (pollination mode or anther/ovule[1])	−30.2	−14.5	16.4	<0.01

[1] Indirect evidence for mode of pollination.

Breeding System and Mode of Life Evolution in Ficus

How can we interpret the discrepancy in the positioning of sections *Oreosycea* and *Urostigma* between our molecular phylogeny and the morphological analysis? If we carefully analyze the taxonomy of *Ficus* (Berg 1989; Berg and Wiebes 1992), it appears that species are grouped according to growth form and breeding system. Freestanding monoecious trees are almost all grouped in subgenus *Pharmacosycea*. The analysis of morphological characters by Weiblen positions them at the base of the tree as a paraphyletic sister group to all other species. All hemiepiphytic monoecious *Ficus* species are also grouped and constitute subgenus *Urostigma*. Dioecious *Ficus* species (subgenera *Ficus* and *Sycomorus*) and the monoecious section *Sycomorus* are grouped together. This grouping was also retrieved by the cladistic analysis of morphological traits by Weiblen (2000). Hence, the main consequence of the reconstruction based solely on molecular data, is the splitting of monoecious terrestrial species that do not belong to subgenus *Sycomorus* (i.e., sections *Pharmacosycea* and *Oreosycea*) into two clades, the splitting of monoecious hemiepiphytic species into two clades (Group 1 and section *Urostigma*) and the splitting of dioecious species into two clades (subgenus *Sycomorus* and subgenus *Ficus*). Thus, one possible explanation for the discrepancy between classical taxonomy and our molecular phylogeny is that characters used in the morphological classification are a result of convergent evolution driven by transitions in growth form and breeding system and hence do not reflect phylogenetic relationships accurately. It has previously been argued that numerous morphological characters in *Ficus* and their pollinating wasps are influenced by changes in breeding system and the ecology of the mutualism and thus yield incorrect estimates of the phylogeny (Herre et al. 1996; Machado et al. 2001). Similar growth habit in *Ficus* probably leads to similar vegetative morphology (Patiño et al. 1995). For example, hemiepiphytes have shiny coriaceous leaves that are

usually highly resistant to water stress, and generally produce large branches that shoot out projecting the plant into holes in the canopy. They also have a single secretion zone on the midrib at the lamina petiole junction. Terrestrial *Ficus* have thinner leaves that are more sensitive to water stress, and present two glands (often replaced by a nodal gland in section *Sycocarpus*), one on each side of the midrib. Such characters have been used to establish morphological classifications. We suggest that they represent adaptations to specific growth habits and are thus not necessarily homologous. In addition, in the discussion on the evolution of floral traits, we will show that similarities in floral traits reflect adaptive responses to breeding system and are also not good indicators of common ancestry.

Our reconstruction reveals multiple transitions in *Ficus* growth form and breeding system. The position of section *Urostigma* suggests that the transition from monoecy to dioecy occurred independently in subgenus *Ficus* and in subgenus *Sycomorus*. Nevertheless this proposition will need to be confirmed by better-resolved phylogenies using additional genes. As previously suggested by Berg (1989) and Weiblen (2000), there also seems to have been at least two reversals, maybe three, from dioecy towards monoecy, one giving section *Sycomorus*, and at least one, maybe two in section *Sycocarpus*, giving rise to *F. microdyctia* and *F. pritchardii* (Weiblen 2000). The latter are not shown in the combined analysis of ETS and ITS, because we could not sequence the ETS region for these two monoecious species. We also show that there could have been repeated independent evolution of hemiepiphytism in the genus. Furthermore, although not included in the phylogeny, one species of section *Pharmacosycea*, *F. crassiuscula*, is also hemiepiphytic. Hence, hemiepiphytism could have evolved four times independently within *Ficus*. Alternatively, hemiepiphytism may have evolved only once and been lost repeatedly.

We cannot yet tell what factors promoted such lability in *Ficus* growth form and breeding system. Considering the scarcity of hemiepiphytism among trees (Putz and Holbrook 1986), the possibility that hemiepiphytism has evolved four times in *Ficus* is quite surprising. Along the same lines, a terrestrial tree like *F. carica* (section *Ficus*) can regularly be seen growing on a host tree (F. Kjellberg, pers. obs.), the roots descending within the hollow trunk of the host, suggesting either an intrinsic capacity of *Ficus* in general to develop as hemiepiphytes, or traces in *F. carica* of hemiepiphytic ancestry. The selective pressures that favored changes in the *Ficus* breeding system are subject to debate (Herre 1989; Kjellberg and Maurice 1989; Kerdelhué and Rasplus 1996; Machado et al. 2001). Putative explanations comprise conflicts of interest between partners, selective pressures exerted by nonmutualistic wasps (Kerdelhué and Rasplus 1996) or environmental factors such as seasonality (Kjellberg and Maurice 1989). Our study, by positioning the monoecious section *Urostigma* within a mainly dioecious group of species provides a basis for further investigation aimed at identifying ecological variables that are linked to these changes and exploring their consequences on the evolution of the interaction.

Ficus/Agaonid Correlated Traits

Mapping *Ficus* inflorescence characters onto our phylogeny reveals covariation between traits of *Ficus* and traits of their associated pollinators. We identified several transitions in ostiole type within the *Ficus* phylogeny that are significantly correlated with differences in pollinator head shape. Furthermore, within *Pleistodontes*, *P. rigisamos* has an atypical rigid mandibular appendage (Wiebes 1991) intermediate between that of wasps associated with linear ostioles and that of wasps associated with spiral ostioles. A posteriori, we observed that its host, *F. destruens* (a species belonging to section *Malvanthera*), has a spiral ostiole. The closest relative of *P. rigisamos*, *P. imperialis* (Lopez-Vaamonde et al. 2001), has a typical mandibular appendage and its host fig *F. rubiginosa* presents a linear ostiole. Hence, there is an obvious causal relationship between ostiole shape and agaonid wasp head shape. What is the adaptive significance of this association? The ostiole is commonly interpreted as a morphological filter that limits the entry of nonadapted wasps into the fig cavity. The match between pollinator head shape and ostiole structure may have evolved as one of the factors that maintain the specificity of the interaction. Interestingly, this wasp adaptive syndrome has been used to define two tribes within Agaonidae, the Blastophagini, associated with spiral ostioles and the Agaonini, associated with linear ostioles (Wiebes 1994). Our study, similarly to Weiblen (2001), suggests that such a classification is confounded by convergent evolution.

Our reconstruction also reveals repeated independent evolution of common *Ficus* floral traits; that is, stigma structure and anther:ovule ratio, and these were associated with differences in agaonid wasp pollination behavior. Passively pollinated species invariably present high anther:ovule ratio and noncohesive stigmas, whereas, except for *F. macrophylla*, actively pollinated species present low anther:ovule ratio and a stigmatic platform in monoecious and female figs. Are there common selective advantages of the floral traits that characterize passive and active pollination? In passively pollinated *Ficus*, figs must ensure the coating of the pollinator's body with pollen. During this process, high pollen production by the associated *Ficus* increases the quantity of pollen dispersed by the wasp and hence might be favored by selection (Galil and Neeman 1977; Kjellberg et al. 2001). The irregular stigmatic surface of passively pollinated *Ficus* species probably favors pollen deposition on the stigmas, by brushing the wasp's body. In actively pollinated species, the selective pressures acting on *Ficus* floral traits are strikingly different. The process of active pollination is more efficient than passive pollination in terms of amount of pollen loaded on the wasp relative to the pollen produced. Figs can probably optimize their male function by decreasing their investment in pollen and allocating more resource to another component of the male function: wasp production. As for stigma structure, its adaptive significance can be understood in light of studies investigating the function of active pollination for the wasps. It has been proposed that active pollination has evolved as a way for the wasps to fertilize the flowers into which they oviposit (Verkerke 1989; Jousselin and Kjellberg 2001; Jousselin et al. 2003). In this context, the tightly packed stigmas and their cohesiveness in female and monoecious figs could be a counteradaptation allowing the tree to equalize the chances of successful pollination of all flowers. When stigmas are cohesive, pollen tubes can grow from the stigma of

one flower to the ovule of another, increasing seed production when pollen is not evenly distributed (Verkerke 1989; Jousselin and Kjellberg 2001). This is a phenomenon similar to functional syncarpy that might evolve under conditions of pollen limitation (Renner et al. 1997; Armbruster et al. 2002). Conversely, the separated tubular stigmas of male figs could be an adaptation favoring precise pollen deposition by agaonid wasps (only flowers receiving an egg should be fertilized to avoid seed formation; Jousselin and Kjellberg 2001). However, there is a major difference between female and monoecious figs. In female figs stigmas are tubular and stacked alongside each other whereas in monoecious figs the stigmas present one or two elongate branches that interlace with the branches of other stigmas. We suggest that the tubular stigmas of female figs result from male-female developmental correlations. Hence the stigma morphology shared by actively pollinated dioecious *Ficus* is strongly constrained by selective pressures linked with breeding system and mode of pollination and is thus not a good indicator of common ancestry.

Adaptation or Coadaptation?

We provide strong evidence for correlations between traits of *Ficus* and their pollinating wasps. Are these correlations evidence for coevolution between the partners of the mutualism? Coevolution is defined as the evolution of one species in response to selection exerted by a second one followed by the evolution of the other species (Janzen 1980). Hence, these correlations represent the product of coevolution (i.e., reciprocal adaptive responses) to the extent that evolutionary transitions in *Ficus* traits correspond to transitions in agaonid wasp traits. It is still difficult to conduct a formal test of congruence between our phylogeny and the published molecular phylogenies of agaonid wasps (see Machado et al. 2001; Weiblen 2001) due to differences in taxon samplings; that is, wasp genera associated with some of the *Ficus* sections in our reconstruction were not always included in the agaonid phylogeny. Furthermore, deep nodes in the agaonid wasp phylogenies are still poorly resolved. However, mapping out associated wasps on the molecular phylogeny of *Ficus* shows that each wasp genus pollinates a well-defined phylogenetic entity of *Ficus* (Fig. 2c). This supports the hypothesis that cocladogenesis is the predominant pattern. Furthermore, a visual comparison of our phylogeny to published molecular reconstructions of the agaonid wasps suggests that although host shifts have occurred, both phylogenies are parallel to a large extent (Machado et al. 2001), and comparison of the phylogenies of the *Ficus* subgenus *Sycomorus* and the pollinating wasp genus *Ceratosolen* also supports a cocladogenesis hypothesis (Weiblen and Bush 2002). Hence, it is likely that character transitions observed in the fig phylogeny do not simply reflect adaptations of *Ficus* in response to pollinator host shifts. Finally, we have evidence that transitions in characters linked to mode of pollination in the *Ficus* phylogeny reflect transitions in mode of pollination in the phylogeny of fig-pollinating wasps. The molecular phylogeny of agaonid wasps confirms that losses of active pollination behavior occurred independently within pollinator genera *Waterstoniella*, *Pleistodontes*, *Blastophaga*, and *Platyscapa* (Machado et al. 2001). Similarly, according to the molecular phylogeny of agaonid wasps, pollinators of sections in which spiral ostioles have evolved (e.g., *Pegoscapus* and *Waterstoniella*) are not sister groups (Machado et al. 2001), which suggests independent losses of elongate head and independent modification of mandibular appendages. Hence, our correlations do not correspond to a single change in the wasp phylogeny. They do represent reciprocal adaptive changes between *Ficus* and their pollinating fig wasps and represent evidence for the coevolutionary process occurring between the partners of the mutualism.

Can Phylogenetic Inquiry Be Used to Elaborate Coevolutionary Scenarios?

Our study shows that *Ficus* and agaonid wasp adaptations respond to each other over evolutionary time. This is well illustrated by the evolution of pollination mode and correlated *Ficus* traits. However, because of the generally strict association between the host's and the wasp's traits, the sequence of events leading to transitions from one stable state to another cannot be inferred from the phylogeny. Our phylogenetic reconstruction gave passive pollination as the ancestral state in the genus *Ficus*; active pollination and correlated fig traits would have arisen once and been lost repeatedly. These multiple losses probably reflect conflicts of interests between the mutualistic partners (Machado et al. 2001). What are the evolutionary events leading to the losses of active pollination behavior and fig associated traits? In light of ecological studies exploring these conflicts, we can propose two evolutionary scenarios that are compatible with the evolutionary history of fig floral traits.

We hypothesize that agaonid wasps start losing pollination behavior if the cost of pollination behavior becomes higher than the resulting benefits. If we further assume that active pollination behavior is aimed at the pollination of flowers into which wasps lay their eggs, such a possibility appears likely. In fig species in which pollen is limiting; for example, few foundresses visit figs and/or few pollen grains are transported per foundress relative to the number of flowers contained in an inflorescence, because pollen is diverted toward any flower through the stigmatic surface, a large proportion of the wasp eggs are laid in nonfertilized flowers (for data on *F. microcarpa* and *F. salicifolia*, see Jousselin et al. 2003). Agaonid wasps could thus be selected to increase their capacity to develop in nonfertilized flowers and the benefit of active pollination might diminish to the point of allowing loss of the behavior. Such a loss would be followed by an increase in pollen production in the host tree, ensuring the passive loading of pollen on the wasp, and reversion toward irregular stigmatic surfaces, ensuring passive pollen deposition by allowing the stigmas to come into contact with the wasp body.

Alternatively, we hypothesize that an increase in anther: ovule ratio precedes the loss of active pollination by the wasp. When pollination is active, the service rendered by the wasps may in some cases be less beneficial to the fig, either because the quantity of pollen transported is low or because active pollination behavior results in the selective fertilization of flowers in which the wasp lays an egg. This conflict over the fig male function (i.e., pollen export) might constitute a se-

lective pressure favoring an increase in pollen production in the host fig. This increase would result in pollen being transported passively on the wasp body in addition to the pollen transported actively. This scenario may reflect the situation of *F. macrophylla*/*P. frogatti*, in which an active pollinator is associated with a *Ficus* species that produces high quantities of pollen. Passive pollen transport might then lower the cost threshold of loss of active pollination behavior: if *Ficus* traits ensure that pollen is transported passively, active pollen transport might become redundant and its costs might become higher than its benefit.

Hence, despite abundant ecological information, it is impossible at this time to establish the evolutionary sequence leading to the losses of active pollination and correlated traits. This would require the finding of several transitional cases such as the case of *F. macrophylla* and *P. frogatti*. Nevertheless, it is likely that correlated traits of figs and agaonid wasps characterizing the two modes of pollination represent a coevolutionary arms race, reflecting the conflicts of interest inherent in mutualistic interactions.

Conclusion

Our molecular phylogeny of *Ficus* reveals repeated evolution of similar breeding systems and modes of life in the genus. This probably led to convergent evolution of vegetative and floral characters, which could wrongly be interpreted as reflecting common ancestry. Our study also reveals the parallel evolution of several fig/agaonid wasp coadaptations. Though probably ubiquitous, coevolution has rarely been demonstrated. Measuring it in natural populations is a difficult task, as it requires identification of reciprocal targets of phenotypic selection in species that interact with each other (e.g., Clayton et al. 1999). Such studies are impossible when the coevolved traits under study are rapidly fixed, eliminating the variation necessary for intraspecific studies. We show that coevolution can be investigated by detecting correlations between transitions in traits in the phylogenies of species that are associated. The *Ficus*/fig wasp system is ideal for such studies because numerous simple fig and fig wasp characters have been quantified through taxonomical studies. Elaborating coevolutionary scenarios, however, requires thorough ecological studies aimed at understanding how conflicts between interactors shape the evolution of the partners. We exemplify such an approach with our study on the evolution of agaonid wasp pollination behavior and *Ficus* coevolved traits.

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