Journal of Biogeography (1996) **23**, 521–530

Molecular phylogenies of figs and their pollinator wasps

EDWARD ALLEN HERRE[1]*, C. A. MACHADO[1,2], E. BERMINGHAM[1], J. D. NASON[3], D. M. WINDSOR[1], S. S. MCCAFFERTY[1], W. VAN HOUTEN[4], and K. BACHMANN[5] [1]*Smithsonian Tropical Research Institute, Apartado 2072, Balboa, Republic of Panama or S.T.R.I., unit 0948, APO AA 34002-0948, U.S.A.;* [2]*Department of Ecology and Evolutionary Biology, University of California, Irvine, California 92717, U.S.A.;* [3]*Department of Botany, University of Georgia, Athens, Georgia, 30602, U.S.A.;* [4]*Department of Agronomy, Purdue University, West Lafayette, Indiana 47906, U.S.A. and* [5]*Hugo de Vries Laboratory, University of Amsterdam, Kruislaan 318, 1098 SM, Amsterdam, the Netherlands*

Abstract. We collected and analysed nucleotide sequence and protein electrophoretic data in order to estimate phylogenies of figs and fig-pollinating wasps at several taxonomic scales. The relatively conserved chloroplast gene coding rbCl allowed the estimation of the taxonomic position of *Ficus* relative to other genera within the Moraceae. Further, in conjunction with chloroplast tRNA spacer genes, rbcL sequences allowed the partial resolution of the phylogenetic associations of fig species from different parts of the world with representatives from all the recognized subgenera of *Ficus*. The phylogeny of the corresponding wasp species that pollinate most of those taxa was estimated using mitochondrial COI–COII and 12S ribosomal genes. At a fine scale, the phylogenies of species within two subgenera of figs growing in Panama (*Urostigma*, and Pharmacosycea) were estimated by using protein electrophoretic data. The phylogeny of the corresponding pollinator wasp species was estimated using COII sequence data. Although we need to extend the taxa sampled and augment the molecular database, the host and pollinator phylogenies show a high degree of congruence and the results support the predominance of strict-sense co-evolution between figs and their pollinator wasps at both global and fine scales.

Key words. Moraceae, *Ficus*, chloroplast DNA, co-evolution, mitochondrial DNA, mutualism.

INTRODUCTION

Figs (*Ficus* spp., Moraceae) and the minute wasps that pollinate them (Agaoninae, Agaonidae, Chalcidoidae, Hymenoptera) (Bouček, 1988, 1993) collectively represent perhaps the most extremely co-specific pollination mutualism known (Corner, 1940; Ramirez, 1970a,b, 1974; Janzen, 1979; Wiebes, 1979; Berg, 1989a). The fig is completely dependent upon the wasp for the pollination of its flowers and, the resulting production of seeds and fruits. The wasp, for its part, is completely dependent upon the fig for the production of seeds which it uses as sites to complete its life cycle (Corner, 1940; Galil & Eisikowitch, 1968; Ramirez, 1969, 1970b; Herre, 1989, this issue; Bronstein, 1992; Compton, 1993). Furthermore, fig fruits constitute a large portion of the diet of a wide range of tropical vertebrate frugivores (e.g. many species of birds, monkeys and bats), and thus the population sizes of these animals also depend on the fig's pollination by the wasp (Janzen, 1979; Milton *et al.*, 1982; McKey, 1989; Windsor *et al.*, 1989; Kalko *et al.*, this issue).

Figs comprise one of the most diverse genera of flowering plants with over 700 described species currently divided among four recognized subgenera. These four subgenera are further subdivided into sections and subsections, some of which show very interesting disjunctions in their geographical distributions (Corner, 1958, Berg, 1989a). The division into subgenera largely reflects the fact that some figs are monoecious while others are functionally dioecious (Corner, 1940, 1958, 1985; Wiebes, 1979; Berg, 1984, 1989a). In monoecious figs, individual fruit perform both female and male function, producing both viable seeds as well as pollen and the wasps that transport it to other trees (Janzen, 1979; Herre, 1989). In dioecious figs, individual trees produce fruits that are specialized to produce either viable seeds, or pollen plus wasps (Corner, 1940, 1958; Berg, 1984; Kjellberg *et al.*, 1987; Patel, Hossaert-McKey & McKey, 1993).

There are over twenty recognized genera of pollinator wasps (Wiebes, 1979, 1982). Generally, each fig species is thought to be pollinated by a fig-specific wasp species, and fig species belonging to any given subsection of the figs are pollinated by wasp species belonging to one or a few genera (Ramirez, 1970a, 1974; Wiebes, 1979; Berg, 1989a). Although different authors have slightly different ideas regarding the precise degree of congruence of wasp and fig phylogenies, the general correspondance of pollinators and hosts at higher taxonomic levels is widely accepted (Ramirez, 1974; Wiebes, 1979, 1982; Corner, 1985; Berg, 1989a). If generally true, this pattern would suggest evolutionary association by common descent and strict co-evolution of

* Corresponding author

fig and wasp species. Indeed, many authors consider figs and fig wasps to represent one of the few good examples of strict-sense co-evolution between plants and animals (Ramirez, 1970a,b, 1974; Janzen, 1979; Mitter & Brooks, 1983; Brooks, 1988; but see Wiebes, 1979, 1982; Miller, 1987; Berg, 1989a; Schemske 1983).

There are, however, patterns of fig/wasp association which suggest that novel host fig species are sometimes colonized by wasp pollinators that subsequently displace the resident pollinator species. Thus, instead of strict co-evolution between all figs and their wasps, there is evidence for at least some level of evolutionary association through colonization. For example, given some existing phylogenetic reconstructions based on morphological characters, different subsections of Asian figs in the subgenus Urostigma do not show congurence with their wasp pollinators (Wiebes, 1979; Berg, 1989a). In this group, different fig species which are grouped together are nonetheless pollinated by species of wasps recognized to be in different genera. Similarly, different species of wasps that are recognized to be members of the same genus pollinate fig species placed in different Urostigma subsections. More generally, wasps that have been divided into two different subfamilies, the Agaoninae and the Blastophaginae, do not associate congruently with the fig subgenera, Pharmacosycea and Urostigma (Wiebes, 1979; Berg, 1989a).

In fact, events that might lead to such phylogenetic 'mismatches' have been observed. For example, there are cases in which two distinct wasp species are known to pollinate a single fig species (Ramirez 1970a,b, 1974; Wiebes, 1979, 1982; Berg, 1989a). Indeed, Compton (1990) reported that an isolated tree of *F. lutea* received wasps that were not even of the same genus as its "normal" pollinator. Further, M. Hossaert (pers. comm.) reports several cases in which the fruit of fig species that have been introduced into Florida have been colonized by the wrong species of fig wasp. These observations indicate that colonization events do happen. The unanswered questions are: under normal circumstances how frequently do wasps colonize novel hosts, and how important have these colonization events been in the historical association of figs and their pollinating wasps?

Existing phylogenies of both figs and fig wasps are based on morphological criteria (Ramirez, 1970a,b, 1974; Wiebes, 1979, 1982; Corner, 1985; Berg, 1989). However, many of the characters used to classify the figs and the wasps are also those that are intimately involved in the interactions between the two groups. For example, number of flowers, size of style, form of stigma, ostiolar characteristics and size of seed are all fig characters that are likely to influence or have been shown to influence the wasp's reproductive success in a profound manner (Janzen, 1979; Herre, 1989; Verkerke, 1989; Bronstein, 1992). By the same token the conformation of the wasp's head, the form of the mandibles, the presence or absence of corbiculae (structure where pollen is carried in some species) and the length of the ovipositor are all characters that appear to be influenced by the mechanics of the wasp's interaction with the fig (Janzen, 1979; Herre, 1989; Bronstein, 1992; Compton, 1993; van Noort, this issue; Kjellberg, this issue).

The fact that, overall, there is a good match of character states across the fig and the wasp species is strongly suggestive, at the very least, of function. However, characters that are not affected by selection that is associated with the details of the interaction would provide a much more powerful and independent basis to establish the evolutionary relationships among the species. Certainly, the study of the evolutionary relationships of the characters that are themselves intimately involved in the fig and wasp interactions requires a phylogeny based on independent characters.

Nucleic acid sequences have been extensively and successfully used to estimate phylogenies from many organisms (Avise, 1986; Hillis, 1987; Miller, 1987; Moritz *et al.*, 1987; Kocher *et al.*, 1989; Golenberg *et al.*, 1990; Hafner & Nadler, 1990, Bermingham & Lessios, 1993; Page, 1993a,b), and almost certainly provide such useful characters for the case of the various fig and wasp species. Most molecular characters, particularly synonymous nucleic acid base substitutions, are independent of morphological characteristics. Other advantages of the use of molecular information include the possibility of rapidly obtaining phylogenetically informative characters, and of secure comparisons across homologous molecules. Although the metronomic quality of even taxon-specific molecular clocks is controversial (Bermingham & Lessios 1993), when evolutionary rate constancy can be demonstrated it permits even more precise examination of hypotheses of co-evolution and co-speciation (Page, 1993a,b).

The work presented here utilizes molecular data to reconstruct the phylogeny of species belonging to: (i) the major genera of Moraceae (including the figs), (ii) the different subgenera of the figs, (iii) some corresponding genera of pollinators and (iv) different species of neotropical figs and their respective pollinators. We used sequences from chloroplast DNA (rbcL, tRNA spacer) and protein electrophoretic data to infer relationships among the plant species. The relationships among the pollinating wasps were inferred using the cytochrome oxidase subunits I and II (COI–COII), and 12S rRNA coding regions of the mitochondrial genome.

MATERIALS AND METHODS

Collections, plants

Material representing species from the major genera of Moraceae was obtained directly from the field or from botanical collections (see Table 1). The *Ficus* species were chosen in order to represent each of the recognized subgenera of figs, with particular attention given to sections in which there were reported to be apparent 'mismatches' with the pollinators (Wiebes, 1982; Berg, 1989; see Table 1). Material from the Panamanian fig species was collected on Barro Colorado Island (BCI).

Nucleotide sequences already published were utilized for the case of *Magnolia* (Golenberg *et al.*, 1990). The unpublished rbcL sequence from *Morus alba* was obtained directly from E. M. Golenberg (Wayne State University, U.S.A.). Fig material from different parts of the world was obtained with the cooperation of several researchers

TABLE 1. List of the species from which rbcL (all) and tRNA spacer (*Ficus*) sequences were collected. Sources and collection sites (when known) of non-Panamanian species are indicated. The classification scheme of *Ficus* subgenera and sections follows Berg (1989a).

Taxon	Source	Collection site
Moraceae		
Artocarpus altisis	Summit Garden, Panama	Asia
Morus alba	Golenberg, E.	
Cecropia insignis	Panama	
Brosimum alicastrum	Panama	
Dorstenia contrajerva	Panama	
Dorstenia psilurus	Utrecht Botanical Gardens	Africa
Poulsenia armata	Panama	
Ficus		
subgenus: Ficus, section: Ficus		
F. carica	Las Cascadas, Panama	Mediterranean
F. deltoidea	Utrecht Botanical Gardens	
F. pumila	Utrecht Botanical Gardens	
subgenus: Ficus, section: Sycidium		
F. asperifolia	Bergen Botanical Gardens	
subgenus: Sycomorus, section: Sycomorus		
F. sycomorus	USDA, Miami, Fl.	Africa
F. racemosa	Summit Garden	Australia
subgenus: Urostigma, section: Americana		
F. trigonata	Panama	
F. pertusa	Panama	
section: Malvanthera		
F. macrophylla	USDA, Miami, Fl.	Australia
section: Galoglychia		
F. natalensis	USDA, Miami, Fl.	South Africa
subgenus: Pharmacosycea, section Pharmacosycea		
F. yoponensis	Panama	Panama
F. maxima	Panama	
F. insipida	Panama	
Pharmacosycea, section: Oreosycea (Old World)		
F. callosa	USDA, Miami, Fl.	
F. dicranostyla	Bergen Botanical Garden, Norway	

(C. C. Berg, S. G. Compton, D. McKey) (see Table 1). Material from the Panamanian fig species was collected in Barro Colorado Island (BCI).

Collections, wasps

Whenever possible, wasps were collected from the same individual fig trees ultimately used in our analyses of fig phylogenies. Many of the Old World specimens were obtained from ethanol preserved collections (J. Cook, L. Chou, S. G. Compton, F. Kjellberg, J. T. Wiebes). Panamanian wasps were collected in the vicinity of the Panama Canal from the same fig species that naturally occur there. Pollinator wasps were obtained from single foundress broods and single fruits in order to maximize the probability of obtaining single mitochondrial lineages (see Machado *et al.*, this issue for a more detailed information). In the case of all Panamanian species, wasps were collected from at least two separate fruit or trees of the same species in order to provide a within-species cross check. Further, care was taken to select fruit in which there were no nematode or mite infections, in order to reduce the possibility of contamination of the DNA samples (see Poinar & Herre, 1991; Herre, 1993). Many of the Panamanian wasp species we included in this study are in the process of being described (Wiebes, 1995) and we will refer to them on the basis of its host fig.

We have included the following species of pollinators (subfamily Agaoninae, *sensu* Bouček, 1988, 1993) from six different genera: *Elisabethiella baijnatii* (ex. *F. burtt-davyii*), *E. stuckenbergi* (ex. *F. thonningii*), *Ceratosolen capensis* (ex *F. sur*), *Blastophaga psenes* (ex. *F. carica*), *Pleistodontes froggatti* (ex. *F. macrophylla*), *Pegoscapus silvestrii* (ex. *F. pertusa*), *P. hoffmeyerii* (ex. *F. obtusifolia*), *P. gemellus* (ex. *F. popenoei*), *P. sp.* (ex. *F. trigonata*), *P. sp. lopesi* (ex. *F. near trigonata*), *Tetrapus ecuadoranus* (ex. *F. yoponensis*), *T. americanus* (ex. *F. maxima*) and *T. costaricanus* (ex. *F. insipida*).

DNA extractions

Plant genomic DNA was extracted either from fresh or dried leaves using protocols described by Doyle & Doyle (1990). One complete leaf was used on each case. Fig wasp DNA was extracted from ethanol preserved specimens or from newborn individuals. Three to ten individual wasps were ground in 100 µl of 2 × CTAB extraction buffer. The ground insects were then diluted to 400 µl using 2 × CTAB to which 20 µl of proteinase K solution (20 mg/ml) was added. The reaction mix was incubated overnight at 55°C with constant agitation. The homogenate was extracted once with equal volumes of equilibrated phenol (pH 8.0), phenol-chloroform-isoamyl alcohol (25:24:1), and chloroform-isoamyl alcohol (24:1). The supernatant was removed and the DNA precipitated with two volumes of isopropyl alcohol. After 12 hours at minus 20°C, the solution was centrifuged at 14,000 r.p.m. for 30 min. The resulting pellet was washed once with 500 µl of 70% ethanol, centrifuged at 14,000 r.p.m. for 15 minutes and dried under vacuum. The dried DNA pellet was resuspended in 100 µl of TE buffer and stored at minus 20°C.

DNA protocols, plants

Relationships among genera within the Moraceae were determined using a segment (approx. 800 bp) of the rbcL gene encoding the large subunit of ribulose-1,5-biphosphate carboxylase. For determining the relationships among the species representing the different subgenera of figs, the rbcL sequences were augmented with non-coding sequences from two regions of the chloroplast genome (Taberlet *et al.*, 1991): (i) the tRNA leucine (UAA) intron (approx. 600 bp) and (ii) the intergenic spacer between the tRNA leucine (UAA) 3′ exon and tRNA phenylalanine (GAA) (approx. 400 bp). The sequences were amplified using the polymerase chain

reaction (Saiki *et al.*, 1988). Thirty-five cycles of amplification were performed with a temperature profile of 1 min at 94°C, 1 min at 37–45°C, and 2 min at 72°C. We used combinations of the following primers that were provided by G. Zurawski (DNAX Research Institute, Palo Alto, California):

Z-234
(5′CGTTACAAAGGACGATGCTACCACATCGA),
Z-674
(5′TTTATAAATCACAAGCCGAAACTGGTGAAATC),
Z-895
(5′GCAGTTATTGATAGACAGAAAAATCATGGT),
Z-895R
(5′ACCATGATTCTTCTGCCTATCAATAACTGC),
Z-1020R
(5′ATCATCGCGCAATAAATCAACAAAACCTAAAGT),
Z-1204R
(6′CCCTAAGGGTGTCCTAAAGTTTCTCCACC).

Primers with an 'R' after the name indicate the antisense strand. Region (i) of the non-coding region was amplified using primers: c (5′CGAAATCGGTAGACGCTACG) and d (5′GGGATAGAGGGACTTGAAC). Region (ii) was amplified using primers: e (5′GGTTCAAGTCCCTCTATCCC) and f (5′ATTTGAACTGGTGACACGAG) (Taberlet *et al.*, 1991) Amplification conditions were similar to those mentioned above, but the annealing temperatures were raised to 55°C.

DNA protocols, wasps

For inferring relationships among species representing the six genera of pollinating wasps considered in this study we collected sequences from the III domain of the small ribosomal mitochondrial subunit (12S rRNA) (Simon *et al.*, 1989), known to evolve at relatively slow rates and hence useful for determining relationships at higher taxonomic levels. For studying relationships at the intrageneric level (e.g. within the Panamanian *Pegoscapus* and *Tetrapus*), we used sequences from the region spanning the last 250 bp of the cytochrome oxidase subunit I gene and the first 300 bp of the cytochrome oxidase subunit II gene (Beckenbach, Wei & Liu, 1993).

We amplified 350 bp of the 12S region using the primers 12Sa (5′TAGGATTAGATACCCTATTA) (Simon *et al.*, 1989) and 12Sb2 (5′AAGAGCGACGGGCGAT) (modified after Simon *et al.*, 1989). The COI–COII region was amplified using primers S2792 (5′ATACCTCGACGTTATTCAGA) and A3389 (5′TCATAAGTTCATTATCATTG) provided by R. Harrison (Cornell University). The PCR amplifications were initiated with a denaturing step of 3 min at 94°C followed by 30–35 cycles with a 94°C step for 45 seconds, and annealing step at 42–50°C for 1 min, and an extension step at 72°C for 2 minutes. Reamplification and sequencing protocols are described in more detail in Machado *et al.* (this issue).

Protein electrophoresis

Fourteen loci were assayed in order to determine the relationships among the seven Panamanian species of figs considered in this part of the study (three from subgenus Pharmacosycea (section Pharmacosycea) and four from subgenus *Urostigma* (section Americana)). Detailed protocols are described in Nason *et al.* (this issue).

Phylogenetic analyses, nucleotide sequences

Where possible, sequences were read from both strands. This was not possible in the chloroplast spacer region in which sequencing was only successful in one direction (primers c and e). However, for the remainder of the sequences there was generally 50–90% of overlap. For inferring the relationships inside Moraceae, rbcL sequences were analysed. Previously published nucleotide sequences of *Magnolia macrophylla* (Golenberg *et al.*, 1990) were used as outgroup. Both fossil and morphological evidence supports this assumption. The rbcL sequence from *Morus alba* was obtained directly from E. Golenberg (Wayne State University). Given that the initial analysis of the Moraceae species put figs from the section Pharmacosycea outside from the rest of the other figs (this topology was also confirmed using *Poulsenia armata* as outgroup), we used sequences from *F. maxima* as outgroup for resolving the relationships inside the rest of the genus *Ficus*. For this, we combined rbCl and chloroplast tRNA spacer data.

For the phylogenetic analysis of the sequences of pollinating wasps, 12S and COI–COII sequences from non-pollinating (parasitic) wasps *Idarnes* (from *F. obtusifolia*) and *Critogaster* (from *F. glabrata*) were used as outgroups (see Machado *et al.*, this issue). Molecular and morphological analyses support this assumption (Machado *et al.*, this issue; Boucek, 1988, 1993). It was not possible to obtain complete sequences of the entire COI–COII region for all species included in this study. For that reason we only analysed the amino acid sequences coded for by the first 276 bp of the COII gene in addition to the 12S sequences to estimate the relationships among the different genera.

Initial alignments of the COI–COII sequences were performed by eye and the program MacVector (International Biotechnologies, Inc Version 4.1). 12S sequences of the pollinator wasps were aligned using CLUSTAL V (Higgins & Sharp, 1988). Final alignments were adjusted by eye. A maximum parsimony analysis of the aligned sequences was undertaken using the program PAUP (Version 3.1; Swofford, 1991). Only phylogenetically informative sites were considered in the analysis. 12S sequences were analysed considering gaps as missing data. The most parsimonious trees were determined using the exhaustive search option in PAUP when the total number of taxa was less than ten. The branch and bound algorithm was used when the number of taxa was greater than ten. The consistency index (CI) was calculated for each tree (Kluge & Ferris, 1969). The shortest tree was analysed using the program MacClade (Version 3.0; Maddison & Maddison, 1992) to examine unambiguous and transversional changes supporting each node. The reliability of each node was determined using bootstrap resampling procedures (Felsenstein, 1985). For the intergeneric comparisons, we obtained a strict consensus tree of the

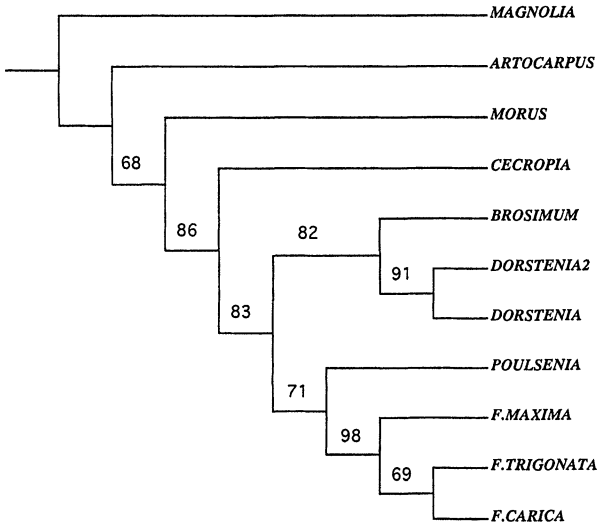

FIG. 1. The most parsimonious tree for the rbcL sequences from species of Moraceae generated by the Branch and Bound algorithm of PAUP. Seventy-six steps; CI=0.632. Numbers above the branches are bootstrap values from 500 replications.

most parsimonious COII and 12S trees using the program COMPONENT (Version 2.0; Page, 1993a,b).

Isozyme data

Phylogenies were inferred for seven Panamanian *Ficus* species, three in the subgenus Pharmacosycea (freestanding figs) and four in the subgenus Urostigma, (hemiepiphytic or strangling figs) which occur on BCI (see Table 1). These species maintain substantial variation in gene frequency in a number of isozyme loci (see Nason *et al.*, this issue). This variation was assayed to obtain estimates of Nei's D (Nei, 1987) which were used in conjunction with the unweighted pair-group method with arithmetic mean (UPGMA) to infer evolutionary relationships within each subgenera. Estimates of Nei's D value and UPGMA analysis were carried out with the program BIOSYS-1 (Swofford & Selander, 1981).

RESULTS

The placement of *Ficus* within Moraceae

The results of the cladistic analyses for the rbcL data are shown in Fig. 1. Using *Magnolia magnophylla* as an outgroup, *Artocarpus* appears to be an outgroup to all of the rest of the Moraceae that were considered in this study. Interestingly, the results strongly support the placement of *Cecropia* within a monophyletic Moraceae. This result is not consistent with the family status given to the Cecropiaceae (Berg, 1978, 1989b; but see Humphries & Blackmore, 1989). These analyses also support *Poulsenia* as being the taxon most closely related to the figs (see Humphries & Blackmore, 1989; Berg, 1989b) (Fig. 1).

The organization of fig subgenera and their associated genera of pollinators

Figs.

The rbcL region sequenced showed very few variable loci (fourteen) among the *Ficus* taxa that were surveyed. This lack of variability restricted our ability to clearly resolve many of the phylogenetic relationships within *Ficus*. Nonetheless, based on the analyses mentioned above, as well as analyses of the two non-coding chloroplast spacer regions, the New World Pharmacosycea (represented in this study by *F. maxima*, *F. yoponensis* and *F. insipida*) appear to be a clear outgroup to all of the rest of the figs (Table 1, Figs 1 and 2). Interestingly, based on several sets of morphological characters, the New World Pharmacosycea are considered to be the most primitive group of figs (Corner, 1985; Berg, 1989a). Further, previous phylogenetic reconstructions have united the Old World Oreosycea (represented by both *Ficus callosa* and *F. dicranostyla* (Table 1)) with the New World Pharmacosycea, as two sections within the subgenus Pharmacosycea (Corner, 1958, 1985; Berg, 1989a). Instead, these analyses suggest that the Oreosycea are much more closely related to the subgenus Urostigma (Table 1, Fig. 2).

The analyses also suggest that species of the subgenus Urostigma (including the species from the New World section, Americana) are not particularly closely related to the New World Pharmacosycea, as had been thought (Ramirez, 1974; Wiebes, 1982; Corner, 1985; Berg, 1989a). Instead, they are more closely related to the subgenera Ficus (represented by *F. carica*, *F. pumila* and *F. deltoidea*) and Sycomorus (represented in this study by *F. sycomorus* and *F. racemosa*) (Table 1, Fig. 2). Interestingly, unlike the pantropical Urostigma, the subgenus Ficus and the subgenus Sycomorus are both found only in the Old World (Berg, 1989a). Further, all members of Urostigma are monoecious, whereas all members of the subgenus Ficus and several members of the subgenus Sycomorus are dioecious (Wiebes, 1979; Berg, 1984, 1989a). Therefore, the simple monoecious/dioecious split that has been at the base of most classifications of figs does not seem to be supported. Incomplete sequence data from *F. asperifolia* (subgenus Ficus, section Sycidium) places it closest to the Sycomorus group (see Berg, 1984, 1989a).

Pollinators.

Using non-pollinator (parasitic) fig wasps (e.g. *Idarnes*, *Critogaster*) as an outgroup (see Bouček, 1988, 1993; Machado, *et al.*, this issue), analyses of 12S sequence data indicate that the pollinators form a clear monophyletic group. Using both COII and 12S nucleotide data to analyse relationships within the pollinators, the species from the genera of wasps *Pegoscapus*, *Elisabethiella*, *Pleistodontes* (all pollinators of host figs from the subgenus Urostigma) form a clear monophyletic group (Table 1, Fig. 2). Interestingly, these three genera had been assigned to different subfamilies owing to their very different morphologies (Wiebes, 1982). Moreover, members of the genus, *Pegoscapus*, are restricted to the New World, whereas the other two genera are only found in the Old World,

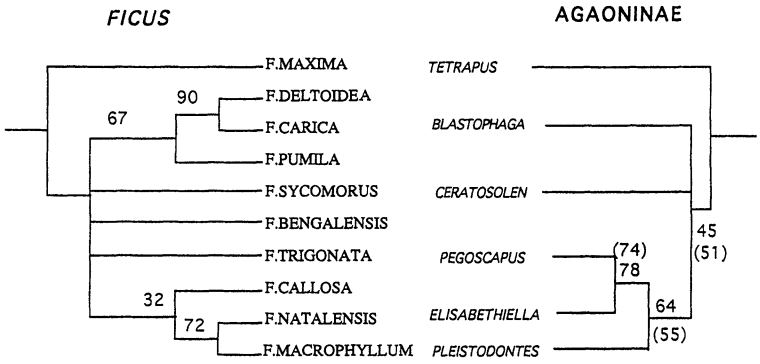

FIG. 2. Molecular phylogenies of *Ficus* and the Agaoninae. Names of fig hosts correspond to the names of their respective genera of pollinator. The *Ficus* phylogeny was reconstructed using rbcL and tRNA spacer sequences. Consensus of ten most parsimonious trees of fourteen steps, CI = 0.632. Numbers on branches are bootstrap values from 500 replications. The phylogeny of the pollinating wasps is the strict consensus of the most parsimonious trees obtained from the analysis of the COII amino acid sequence (92 amino acids) and the 12S region (185 bp). COII: 111 steps, CI = 0.775. 12S: 108 steps, CI = 0.657. Bootstrap values for 12S and for COII (in parentheses) are showed for the best supported branches.

mirroring the distributions of their figs (Wiebes, 1982; Berg, 1989a). Unfortunately, we were not able to obtain sequence data from the samples of *Dolichoris* wasps, pollinators of the Oreosycea, for which rbcL data suggests are allied with the Urostigma (see above).

Despite an abundance of variable loci in the COII and 12S regions, the relationships among wasps of the remaining genera, *Tetrapus* (pollinators of the New World Pharmacosycea), *Ceratosolen* (pollinators of the subgenus Sycomorus) and *Blastophaga* (pollinators of the Ficus section of the subgenus Ficus), were less clear. Bootstrap values supporting the *Tetrapus* species as an outgroup to the rest (the pattern found in their host figs) are low for both genomic regions (45 and 51 for 12S and COII, respectively). However, a structural difference in the mitochondrial genome unites *Tetrapus* with the non-pollinators (e.g. *Idarnes*) and separates them both from the other pollinator wasps. Specifically, all species of pollinator wasps except for *Tetrapus* possess an A–T rich intergenic sequence of variable length between the COI and Leucine t-RNA (UUR) genes. *Tetrapus*, along with the parasitic fig wasps, lack this insertion. This is a significant observation given that the mitochondrial genomes of the majority of metazoans studied lack such non-coding sequences (Crozier & Crozier, 1993). These observations lead us to hypothesize that *Tetrapus* is the sister taxon to all other pollinating wasps. Indeed, if the phylogenetic position of *Tetrapus* suggested by the lack of this insertion is supported, the basal sister group relationships of figs and wasps would be congruent (Fig. 2).

Relationships of pollinators and host figs at a fine scale

Seven species of Panamanian figs (four belonging to Urostigma and three belonging to Pharmacosycea) were collected along with their pollinators. In addition to the rbcL, chloroplast tRNA spacer regions and protein data, ITS (internal transcribed spacer) sequences were obtained for all four Urostigma but only one species of the Pharmacosycea (not shown). The split between the three fig species belonging to the Pharmacosycea and the four belonging to the Urostigma was supported by all datasets. The phylogenetic relationship of the seven species of Panamanian figs was estimated using protein electrophoretic patterns (Fig. 3). ITS was available for the whole group of Urostigma, where it gave consistent, although weak (few informative bases) support to the relationships suggested by the protein data. The phylogeny of the Panamanian pollinator wasps was estimated using the data from the COI–COII region (Fig. 3) and was found to be congruent with the phylogeny of the host figs estimated by using the protein data (Fig. 3). Here, wasps sampled from two or more fruit (usually from different individual trees) from the same host fig species showed identical sequences.

DISCUSSION

Despite the fact that rbcL is very conserved, the level of resolution reached with this molecular data was sufficient to determine the relationships among the genera comprising the Moraceae. The results of the cladistic analyses strongly suggest that the Moraceae (including *Morus*, *Ficus*, and *Cecropia*) constitute a monophyletic group (Fig. 1). This view is not consistent with some morphology-based cladograms of the Moraceae (Berg, 1978, 1989b). The molecular results presented here suggest that some of the morphological characters that have previously been used to propose relationships within the Moraceae could be homoplasic, Interestingly, the analyses identify *Artocarpus*, which has a fossil record tracing back to Cretaceous strata from Greenland, as an outgroup to the rest of the genera (see Berg, 1989b; Collinson, 1989; Humphries & Blackmore, 1989).

Additionally, the pattern of relationships among the figs that is suggested by the rbcL and spacer data is different from previous morphology-based phylogenies (Ramirez, 1970a,b, 1974; Corner, 1985; Berg, 1989a). For example, the Old World Oreosycea does not appear to be a sister group of Pharmacosycea as has been proposed. If the pattern

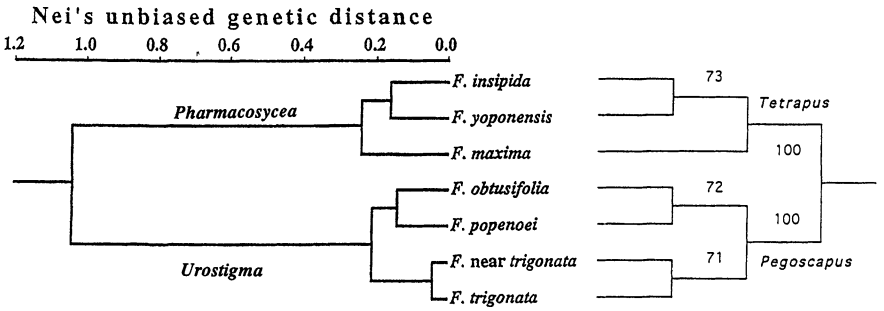

FIG. 3. Comparison of the phylogenies of seven species of Panamanian figs and their pollinator wasps. The phylogeny of the figs is a UPGMA tree based on estimates of Nei's genetic distance (D) between seven species representing the Pharmacosycea (freestanding figs) and Urostigma (strangling figs) subgenera. The cophenetic correlation for this tree is $r=0.93$. The phylogeny of the wasps is the most parsimonious tree that results from the analysis of 492 nucleotides from the COI–COII mitochondrial region. The tree has 313 steps, and CI = 0.684. The tree was rooted using the sequences from one *Idarnes* and *Critogaster* wasps. Bootstrap values from 500 replications are shown over each branch.

suggested by the molecular information is correct, then it appears that the morphological characters that have been considered to unite the Oreosycea and the Pharmacosycea are likely to present a case of convergence. Indeed, at least some Oreosycea species show morphological similarities to some Urostigma species (Berg, 1989a).

Further, there is no simple monoecious–dioecious split among the taxa (Ramirez, 1974; Corner, 1985; Berg, 1989a). The monoecious New World Pharmacosycea appear to be very distinct genetically from all the other groups of figs, which include both monoecious (e.g. all Urostigma, all Oreosycea and some Sycomorus) and dioecious (all Ficus and some Sycomorus) species. Partial rbcL sequence data place the dioecious *F. asperifolia* (section Sycidium, subgenus Ficus, *sensu* Berg, 1989a) with the subgenus Sycomorus. Interestingly, the same genus of pollinator wasp (*Ceratosolen*) is associated with at least some host fig species assigned to both groups. Overall, these analyses suggest that either dioecy has evolved twice (in the subgenus *Ficus*, as well as in the subgenus Sycomorus plus Sycidium) or been lost once (in the Sycomorus group) (see Berg, 1984, 1989a; Corner, 1985). Nevertheless, there were very few variable loci in the rbcL and chloroplast spacer regions. Beyond the clear separation of the monoecious New World Pharmacosycea from all other groups of figs, and these data need to be augmented in order to clearly resolve the patterns of evolution of monoecy and dioecy among the rest (Ficus, Sycomorus, Sycidium, Urostigma and Oreosycea).

These results also give some insights concerning the historical biogeography of the group. It is generally thought that many components of the tropical floras of South America and Africa share a common Gondwanaland origin and that the members of shared families have been separated since the Cretaceous (Coetzee, 1990; Gentry, 1990; Romero, 1990; but see Maisey, 1990). Nevertheless, the results here shown combined with observations of the fossil record of figs (Collinson, 1989) suggest that instead of a simple Cretaceous separation scenario, figs 'colonized' the New World twice. The analyses of the molecular data suggest an ancient separation in the lineages leading to the New World Pharmacosycea and the rest of the figs, followed by a 'reinvasion' (or, perhaps, 'colonization') of the New World by what became Urostigma (Americana). Interestingly, the Americana group has apparently undergone a tremendous amount of radiation and speciation (*ca.* 120 species) relative to the New World Pharmacosycea (*ca.* twenty species). This is reflected in the much greater diversity in growth form and fruit shape, colour and size found in the Urostigma Americana (Herre, 1989, this issue; Berg, 1989a, pers. comm.).

As is the case with the figs, portions of the phylogenetic relationships among the wasp genera suggested by the molecular data are also not well resolved. Unlike the case with the rbcL and spacer regions from the fig chloroplast in which there are few variable loci, the sequences from the 12S and COII regions of the wasp mitochondria show an abudance of variable loci. Nonetheless, analyses of these regions do not clearly resolve the relationships among *Tetrapus* (pollinators of New World Pharmacosycea), *Ceratosolen* (pollinators of Sycomorus) and *Blastophaga* (pollinators of Ficus). It is a structural change in the mitochondrial genome that suggests that *Tetrapus* is an outgroup to the others. Nonetheless, there is sufficient resolution to point out clear disagreements with previous phylogenetic reconstructions of the pollinators' wasp taxa. For example, in some phylogenetic reconstuctions that were based on morphological similarities, *Tetrapus*, *Pleistodontes* and *Elisabethiella* have been joined, while *Pegoscapus* was placed in a separate subfamily (Wiebes, 1982). This view is not consistent with the pattern suggested by the molecular data. One possibility is that many of the morphological characters used in previous attempts at phylogenetic reconstruction of the wasps may be relatively evolutionarily flexible. In line with this possibility, van Noort (this issue) has shown that even very distantly related pollinator and parasite taxa may converge on very similar head morphologies.

Despite the problems of resolution in both the genera of wasps and subgenera of the figs, at a course (high) taxonomic level, the phylogenetic match of the fig and wasp groups suggests that strict sense co-evolution predominates (see Fig. 2). The rbcL data place the New World Pharmacosycea clearly outside the rest of the figs, while *Tetrapus* (their pollinators) are placed outside the rest of the pollinators by

a rearrangement of the mitochondrial genome. The rbcL and chloroplast spacer data further join the different species within the subgenus and section Ficus and suggest the joining of most of the sections of the subgenus, Urostigma. Similarly, the 12S and COII data clearly join the corresponding pollinators of the Urostigma (*Pegoscapus*, *Elisabethiella* and *Pleistodontes*).

Unlike the other wasp genera in which active pollination behaviour has been reported, *Blastophaga* and *Tetrapus* both pollinate passively (Frank, 1984; Berg, 1989a; Kjellberg, this issue). It is tempting to speculate that the passive pollination behaviour that the wasps from these genera exhibit represents a primitive condition uniting a natural group from which the other wasp genera are derived. Such a relationship, combined with the hypothesis of strict-sense co-evolution, would predict that the members of the subgenus Ficus are most closely related to the New World Pharmacosycea. Ongoing studies are attempting to clarify the relationships of the subgenus Ficus with respect to the other groups of figs (e.g. Sycomorus). Similar work is ongoing with respect to the placement of their associated genera of pollinators (*Blastophaga*, *Kradibia*, *Ceratosolen*, etc.). However, if the separation of most of the lineages leading to presently recognized genera and subgenera of the wasps and the figs occurred roughly simultaneously, or over a relatively short period of time, phylogenetic structure at these levels would be very difficult to detect. Indeed, in the case of both the figs and the wasps, most variation appears in the branches of the phylogenetic reconstructions, consistent with a rapid separation of lineages followed by extended periods of isolation.

Nonetheless, the general pattern of co-evolution suggested at the level of subgenera, genera and sections also appears to hold at a fine level, among closely related species of host figs and pollinators within their respective genera (Fig. 3). Further, with few exceptions, this co-evolutionary pattern seems to predominate across at least four sets of ecologically associated taxa: host figs, pollinator wasps, parasitic wasps and wasp-parasitizing nematodes (Poinar & Herre, 1991; Herre, 1993; Machado *et al.*, this issue, Machado *et al.*, in prep., Herre *et al.*, in prep.). The mechanism underlying this pattern is almost certainly based on chemical attractants released by the fig. Indeed, it appears that both pollinator and parasitic wasps are attracted by the same chemicals (van Noort, Ware & Compton, 1989; Ware *et al.*, 1993; Hossaert-McKey, Gibernau & Frey, 1994).

All these findings raise the question of mechanisms of speciation in figs and their associated taxa. Figs are an ancient, speciose group, with high levels of electrophoretically detectable genetic variability (Berg, 1989a; Collinson, 1989; Nason *et al.*, this issue). Interestingly, despite relatively low adult densities, the number of individuals that constitutes a breeding population and the area that breeding populations of figs occupy is the largest for any species of plant known (see Nason *et al.*, this issue). Both direct and indirect evidence indicates that the minute wasps are very effective long-distance pollinators (Compton, 1990, 1993; Nason *et al.*, this issue). Effective long-distance gene flow would thus appear to make speciation more difficult. However, as mentioned above, colonization events are known (e.g. Compton, 1990), and it is at least possible that they could lead to speciation in both the figs and the wasps involved.

In all cases, wasps that were sampled from two or more fruits (or from fruits collected from two or more individual trees) from a single host fig species showed identical, or nearly identical, sequences. This finding supports the proposition of pollinator specificity to host fig species. However, in order to estimate the nature frequency of 'mistakes', deeper within-species sampling is needed, preferably across several species. Ideally, wasps should be sampled from large numbers of fruit of a given tree, from several different trees in a population, and from trees representing a large number of distinct populations across the geographical range of the fig species (see Nason *et al.*, this issue, for estimates of the area occupied by a population). In conjunction with phylogenetic reconstructions, determining 'mistake rate' is essential for understanding the factors that influence speciation and radiation of both the figs and the pollinators, as well as all of the other associated taxa.

Colonization events (in evolutionary time) of novel host figs by different pollinator species could lead to the birth of new fig lineages which would, in the long or short term, attract (or breed) their own specific wasp pollinator. Importantly, when a colonization event occurs, then the resulting hybrid should inherit chloroplast DNA solely from the maternal lineage, whereas the nuclear DNA should be a mixture of both donors. Such a scenario would allow reticulate evolution to take place, and opens the possibility that genetic lineages may only partially reflect phylogenetic associations. Nonetheless, it should be possible to detect such events. Both for increasing the resolution of the existing phylogenetic relationships and for detecting possible colonization events during the evolution of the figs, identifying nuclear and chloroplast genes with appropriate levels of sequence variation is a very important task. Further work will clarify the relative importance of the strict-sense co-speciation that these preliminary findings suggest predominates, and of colonization events during the evolutionary history of the fig–wasp mutualism.

ACKNOWLEDGMENTS

We thank Stuart West, Steve Compton, Finn Kjellberg, Lien Chou, James Cook, C. C. Berg, D. McKey, M. Hossaert-McKey and J. T. Wiebes for providing us with collections of wasps and plant material from the Old World tropics. G. Urawski provided rbcL primers. R. Harrison provided COI and COII primers. E. M. Golenberg provided unpublished rbcL sequences for *Morus alba*. Discussions with C. C. Berg, J. T. Wiebes, S. G. Compton, M. Hossaert and F. Kjellberg were particularly useful. Elisabeth Kalko suggested many improvements for the final manuscript. This work was supported by a Smithsonian Molecular and Physiological Post-Doctoral Fellowship, two Smithsonian Scholarly Studies Awards, a NATO Post-Doctoral Fellowship and the Smithsonian Molecular Apprenticeship Program.

REFERENCES

Avise, J.C. (1986) Mitochondrial DNA and the evolutionary genetics of higher animals. *Phil. Trans. R. Soc. Lond. B* **312,** 325–342.

Beckenbach, A.T., Wei, Y.W. & Liu, H. (1993) Relationships of the *Drosophila obscura* species group, inferred from mitochondrial cytochrome oxidase II sequences. *Mol. Biol. Evol.* **10,** 619–634.

Berg, C.C. (1978) Cecropiaceae, a new family of the Urticales. *Taxon*, **27,** 39–44.

Berg, C.C. (1984) Floral differentiation and dioecism in *Ficus* (Moraceae). *Minisymposium: Figs and fig insects*, pp. 15–25. (ed. by F. Kjellberg & G. Valdeyrons). CNRS, Montpellier, France.

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

Berg, C.C. (1989b) Systematics and phylogeny of the Urticales. *Evolution, systematics, and fossil history of the Hamamelidae, Vol. 2: 'Higher' Hamamelidae* (ed. by P. R. Crane and S. Blackmore) pp. 193–220. Clarendon Press, Oxford.

Bermingham, E. & Lessios, H.A. (1993) Rate variation of protein and mitochondrial DNA evolution as revealed by sea urchins separated by the Isthmus of Panama. *Proc. Natl. Acad. Sci., U.S.A.* **90,** 2734–2738.

Bronstein, J.L. (1992) Seed predators as mutualists: ecology and evolution of the fig/pollinator interaction. *Insect–plant interactions*. (ed. by E. Bernys). CRC Press, Boca Raton.

Brooks, D.R. (1988) Macroevolutionary comparisons of host and parasite phylogenies. *Ann. Rev. Ecol. Syst.* **19,** 235–259.

Bouček, Z. (1988) 6. Family Agaonidae. *Australasian Chalcidoidea (Hymenoptera). A biosystematic revision of genera of fourteen families, with a reclassification of species*, pp. 156–209. CABI, Wallingford, U.K.

Bouček, Z. (1993) The genera of chalcidoid wasps from *Ficus* fruit in the new world. *J. Nat. Hist.* **27,** 173–217.

Coetzee, J.A. (1990) The Paleofloras of Africa. *Biological relationships between Africa and South America. 37th Annual Systematics Symposium*. Missouri Botanical Garden, MI.

Collinson, M.E. (1989) The fossil record of the Moraceae. *Evolution, systematics, and fossil history of the Hamamelidae, Volume 2: 'Higher' Hamamelidae*, pp. 319–339. (ed. by P. R. Crane and S. Blackmore). Clarendon Press, Oxford.

Compton, S.G. (1990) A collapse of host specificity in some South African fig wasps. *South Afr. J. Sci.* **86,** 39–40.

Compton, S.G. (1993) One way to be a fig. *Afric. Entomol.* **1,** 151–158.

Corner, E.J.H. (1940) *Wayside Trees of Malaya.* Singapore Government Printing Office, Singapore.

Corner, E.J.H. (1958) An introduction to the distribution of *Ficus*. *Reinwardtia*, **4,** 325–355.

Corner, E.J.H. (1985) Ficus (Moraceae) and Hymenoptera (Chalcidoidea): figs and their pollinators. *Biol. J. Linn. Soc.* **25,** 187–195.

Crozier, R.H. & Crozier, Y.C. (1993) The mitochondrial genome of the honey-bee, Apis mellifera: complete sequence and genome organization. *Genetics*, **133,** 97–117.

Doyle, J.J. & Doyle, J.L. (1990) Isolation of plant DNA from fresh tissue. *Focus*, **12,** 13–15.

Felsenstein, J. (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*, **39,** 783–791.

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

Galil, J. & Eisikowitch, D. (1968) Flowering cycles and fruit types of *Ficus sycomorus* in Israel. *New Phytol.* **67,** 745–758.

Gentry, A.H. (1990) Diversity and floristic composition in lowland tropical forest in Africa and South America. *Biological relationships between Africa and South America. 37th Annual Systematics Symposium*. Missouri Botanical Garden, MI.

Golenberg, E.M., Giannasi, D.E., Clegg, M.T., Smiley, C.J., Durbin, M., Henderson, D. & Zurawski, G. (1990) Chloroplast DNA sequence from a Miocene Magnolia species. *Nature*, **344,** 656–658.

Hafner, M.S. & Nadler, S.A. (1990) Cospeciation in host-parasite assemblages: comparative analysis of rates of evolution and timing of cospeciation events. *Syst. Zool.* **39,** 192–204.

Herre, E.A. (1989) Coevolution of reproductive characteristics in twelve species of new world figs and their pollinator wasps. *Experientia*, **45,** 637–647.

Herre, E.A. (1993) Population structure and the evolution of virulence in nematode parasites of fig wasps. *Science*, **259,** 1442–1445.

Hillis, D.M. (1987) Molecular versus morphological approaches to systematics. *Ann. Rev. Ecol. Syst.* **18,** 23–42.

Hossaert-McKey, M., Gibernau, M. & Frey, J.E. (1994) Chemosensory attraction of fig wasps to substances produced by receptive figs. *Ent. Exp. Appl.* **70,** 185–191.

Humphries, C.J. & Blackmore, S. (1989) A review of the classification of the Moraceae. *Evolution, systematics, and fossil history of the Hamamelidae, Volume 2: 'Higher' Hamamelidae*, pp. 267–277. (ed. by P. R. Crane and S. Blackmore) Clarendon Press, Oxford.

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

Kjellberg, F., Gouyon, P.-H., Ibrahim, M. & Valdeyron, G. (1987) The stability of the symbiosis between dioecious figs and their pollinators: a study of *Ficus carica L.* and *Blastophaga psenes L. Evolution*, **41,** 693–704.

Kluge, A.G. & Ferris, J.S. (1969) Quantitative phyletics and the evolution of Anurans. *Syst. Zool.* **18,** 1–32.

Kocher, T.D., Thomas, W.K., Meyer, A., Pääbo, S.V., Villablanca, F.X. & Wilson, A.C. (1989) Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proc. Natl. Acad. Sci. U.S.A.* **86,** 6196–6200.

Maddison, W.P. & Maddison, D.R. (1992) *MacClade: analysis of phylogeny and character evolution*, Version 3.0. Sinauer Associates, Sunderland, MA.

Maisey, J.G. (1990) A final Gondwanaland biota? *Biological relationships between Africa and South America. 37th Annual Systematics Symposium*. Missouri Botanical Garden, MI.

McKey, D. (1989) Population biology of figs: Applications for conservation. *Experientia*, **45,** 661–673.

Miller, J.S. (1987) Host–plant relationships in the Papilionidae (Lepidoptera): parallel cladogenesis or colonization? *Cladistics*, **3,** 105–120.

Milton, K., Windsor, D.M., Morrison, D.W. & Estribi, M.A. (1982) Fruiting phenologies of two neotropical *Ficus* species. *Ecology*, **63,** 752–762.

Mitter, C. & Brooks, D.R. (1983) Phylogenetic aspects of coevolution. *Coevolution* (ed. by D. J. Futuyma and M. Slatkin). Sinauer, Sunderland, MA.

Moritz, T.E. *et al.* (1987) Evolution of animal mitochondrial DNA: relevance for population biology and systematics. *Ann. Rev. Ecol. Syst.* **18,** 269–292.

Nei, M. (1987) *Molecular Evolutionary Genetics.* Columbia University Press, New York.

Page, R.D.M. (1993a) *COMPONENT*, Version 2.0. Natural History Museum, London.

Page, R.D.M. (1993b) Maps between trees and cladistic analysis of historical associations among genes, organisms, and areas: the problem of multiple lineages. *Syst. Biol.* **42,** 77–84.

Patel, A., Hossaert-McKey, M. & McKey, D. (1993) *Ficus*-pollinator research in India: Past, present, and future. *Curr. Sci.* **65,** 243–253.

Poinar Jr, G.O. & Herre, E. A. (1991) Speciation and adaptive radiation in the fig wasp nematode, *Parasitodiplogaster* (Diplogasteridae: Rhabditida), in Panama. *Rev. Nematol.* **14,** 361–374.

Ramirez B.W. (1969) Fig wasps: mechanism of pollen transfer. *Science*, **163,** 580–581.

Ramirez, B.W. (1970a) Host specificity of fig wasps (Agaonidae). *Evolution*, **24,** 681–691.

Ramirez, B.W. (1970b) Taxonomic and biological studies of neotropical fig wasps (Hymenoptera: Agaonidae). *University of Kansas Sci. Bull.* **46,** 1–44.

Ramirez, B.W. (1974) Specificity of Agaonidae: the coevolution of Ficus and its pollinators. PhD thesis, University of Kansas.

Romero, E.J. (1990) Paleofloras of South America. *Biological relationships between Africa and South America. 37th Annual Systematics Symposium.* Missouri Botanical Garden, MI.

Saiki, R.K., Gelfand, D.H., Stoffel, S., Scharf, S.J., Higuchi, R. Horn, G.T., Mullis, K.B. & Ehrlich, H.A. (1988) Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science*, **239,** 487–491.

Schemske, D.W. (1983) Limits to specialization and coevolution in plant–animal mutualisms. *Coevolution* (ed. by Nitecki, M. H.) University of Chicago Press, Chicago, London.

Simon, C., Pääbo, S. V., Kocher, T.D. & Wilson, A.C. (1989) Evolution of mitochondrial ribosomal RNA in insects as shown by the polymerase chain reaction. *Molecular evolution. UCLA symposia on molecular and cellular biology.* New series, vol. 122 (ed. by M. Clegg and S. Clark), pp. 235–244. Alan R. Liss Inc., New York.

Swofford, D. (1991) *PAUP: phylogenetic analysis using parsimony. Version 3.1.* Illinois Natural History Survey, Champaign, Illinois.

Swofford, D.L. & Selander, R.B. (1981) BIOSYS-1: A FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. *J. Hered.* **72,** 281–283.

Taberlet, P., Gielly, L., Pautou, G. & Bouvet, J. (1991) Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Mol. Biol.* **17,** 1105–1109.

Van Noort, S., Ware, A.B. & Compton, S.G. (1989) Pollinator-specific volatile attractants released from the figs of *Ficus burtt-davyi. Sud-Afrikaanse Tydskr. vir Wetensap.* **85,** 323–324.

Verkerke, W. (1989) Structure and function of the fig. *Experientia*, **45,** 612–621.

Ware, A.B., Perry, T.K., Compton, S.G. & Van Noort, S. (1993) Fig volatiles: their role in attracting pollinators and maintaining pollinator specificity. *Plant Syst. Evol.* **186,** 147–156.

Wiebes, J.T. (1979) Co-evolution of figs and their insect pollinators. *Ann. Rev. Ecol. Syst.* **10,** 1–12.

Wiebes, J.T. (1982) The phylogeny of the agaonidae (Hymenoptera, Chalcidoidea). *Neth. J. Zool.* **32,** 395–411.

Wiebes, J.T. (1995) Agaonidae (Hymenoptera, Chalcidoidea) and Ficus (Moraceae): fig wasps and their figs, XV (Meso-American *Pegoscapus*) *Proc. Kon. Ned. Akad. v. Wetenschappen*, **98**, 167–83.

Windsor, D.M., Morrison, D.W., Estribi, M.A. & de Leon, B. (1989) Phenology of fruit and leaf production by 'strangler' figs on Barro Colorado Island, Panama. *Experientia.* **45,** 647–653.

Note added in proof:

Additional sequencing suggests a close affinity between the pollinator genera, *Ceratosolen* and *Kradibia*, consistent with the placement of their host sections (Sycomorus and Sycidium, respectively) together. Further, extensive sampling within the pollinator genus, *Ceratosolen*, has shown that the pollinator of *Ficus sycomorus* (*C. arabicus*) and its Cuckoo (*C. galili*) are not sister taxa and are in fact only distantly related within the genus.