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PHYLOGENY AND ECOLOGY OF DIOECIOUS FIG POLLINATION

A thesis presented

by

GEORGE DANIEL WEIBLEN

to

THE DEPARTMENT OF ORGANISMIC AND EVOLUTIONARY BIOLOGY

in partial fulfillment of the requirements
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PHYLOGENY AND ECOLOGY OF DIOECIOUS FIG POLLINATION

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Michael J. Donoghue (Thesis advisor)

ABSTRACT

The evolution of mutualistic interactions between the dioecious figs (Ficus subg. Ficus, Moraceae) and their pollinating wasps (Hymenoptera: Agaonidae) was examined using comparative methods. Fig species are either monoecious or gynodioecious depending on the arrangement of unisexual florets within the specialized inflorescence or syconium. Due to complex interactions with pollinators (Agaoninae), the gynodioecious species are functionally dioecious. In Chapter 1, the evolutionary relationships of dioecious figs were examined through phylogenetic analyses based on the internal transcribed spacer region of nuclear ribosomal DNA (nrDNA) and morphology. Chapter 2 describes a parallel study of the pollinators of dioecious figs using mitochondrial DNA (mtDNA) sequences and morphology. Separate and combined analyses suggest that neither dioecious figs nor their pollinators are monophyletic. However, fig/pollinator associations were largely congruent with phylogeny and support a revised classification of Ficus.

Comparative analyses in Chapter 3 examined aspects of coevolution including the cospeciation of interacting lineages and the coadaptation of interacting traits. Pararell phylogenies and correlated rates of evolution in fig nrDNA and pollinator mtDNA sequences support a history of cospeciation. Reconstructions of breeding system evolution indicated that dioecy evolved once or twice with at least two reversals to monoecy in a dioecious lineage. Changes in pollinator ovipositor length were correlated with changes in fig breeding system. The correlated evolution of fig style lengths and pollinator ovipositors suggests a role for coadaptation in the regulation of resource conflicts between mutualists.

Chapter 4 summarizes ecological studies in New Guinea, examining the impact of non-pollinating fig wasps on the mutualism and suggesting a new hypothesis for the origin and maintenance of dioecious fig pollination. Chapter 5 presents an argument that fig pollination is an extreme case of coevolution in plant/insect interactions. The associations of herbivores in a range of insect guilds were poorly correlated with host phylogeny, compared to the associations of specialized fig wasps. Most Ficus herbivores in New Guinea, including leaf chewing and sap sucking insects, are oligophagous and their patterns of association are not explained by host phylogeny, suggesting that other factors play an important role in shaping interactions between plants and insects in general.

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CHAPTER 1

Splitting the fig: Phylogenetic relationships of dioecious Ficus based on ITS sequences and morphology

"I have been rent, like the morning toast, by two forces splitting biology into macro-molecules and macro-organisms, and I do not know how this rift may be spanned. I cannot conceive what energy level, chemical bond, or carbon-grouping can decide whether it is insect-pollination or curiosity that will be inherited. But the pendulum has swung. The young botanist...models molecules and chromosomes, and works very largely in vitro. Nevertheless, if biology is not to stand still, the pendulum will return and its amplitude will be the strength of those who have put their trust in the macrocosm." E. J. H. (Corner 1963), p. 1000

Introduction

The genus Ficus (Moraceae) includes some 750 species of woody plants occurring in most tropical and subtropical forests around the world (Berg 1989). These species of trees, shrubs, climbers and hemi-epiphytic stranglers are recognized by their specialized inflorescence and pollination syndrome (Janzen 1979b, Berg 1990b). Resembling a fleshy fruit in outward appearance, the fig is an enlarged receptacle enclosing hundreds of unisexual flowers accessible only by a tight, bract-filled opening or ostiole. The enclosed inflorescence, or syconium, protects the flowers against most parasites except for diminutive insects capable of entering through the ostiole (Berg 1990a). The interior of the inflorescence is the location of an obligate mutualism with pollinating seed predators, fig wasps in the family Agaonidae of parasitic Hymenoptera (Chalcidoidea). Interactions between figs and fig wasps are among the best known examples of reproductive interdependence between plants and their pollinators (Bronstein 1992). In addition, fig wasps are specialized to the extent that unique pollinator species are associated with most fig species (Ramirez 1970, Wiebes 1979a); but see (Rasplus 1994, Michaloud et al. 1996, Kerdelhue et al. 1997).

The intertwined life cycles of figs and pollinators, together with their extreme specificity, are the basis for much speculation on the nature and extent of coevolution involved (Ramirez 1974, Janzen 1980, Wiebes 1987, Thompson 1994a). Have figs and their pollinators cospeciated? What floral adaptations, if any, prevent pollinators from preying on all the seeds in figs? Dioecious figs have generated interest in regard to the second question, due to the apparent conflicts with pollinators in regard to seed resources (Grafen and Godfray 1991). Dioecious figs also provide a framework in which to

examine the two major components of coevolution: patterns of speciation and reciprocal adaptations (Thomson 1994). Until the present, our knowledge of coevolution in dioecious figs has been shaped by taxonomy (Wiebes 1963b, Wiebes 1979a, Corner 1985), ecology (Galil 1973, Kjellberg et al. 1987a, Corlett 1993, Weiblen et al. 1995, Patel and McKey 1998), behavior (Hossaert-McKey et al. 1994, Ware and Compton 1994b), and anatomy (Verkerke 1987, Beck and Lord 1988a, Verkerke 1989). Phylogenetic studies are limited and the relationships of the dioecious species have not been examined in detail (Yokoyama 1995, Herre et al. 1996). This chapter provides an analysis of phylogenetic relationships in dioecious figs based on DNA sequences and morphology.

Life cycles of dioecious figs and their pollinators

Morphologically, figs are monoecious or gynodioecious according to the arrangement of the unisexual florets within the syconium (Figure 1). Due to complex interactions with pollinating fig wasps, however, the gynodioecious species are functionally dioecious (Berg 1989, Weiblen et al. 1995). Inside the protogynous syconia, female fig wasps actively pollinate heterostylous florets while ovipositing in a fraction of fig ovaries. The fate of the ovaries in dioecious species is determined by the interaction of pollinator ovipositors and style lengths in two types of figs (Ganeshaiah et al. 1995). Seed figs contain only long-styled pistillate florets that are fertilized and unharmed (Galil 1973). Gall figs contain staminate florets and short-styled pistillate florets, enabling pollinators to deposit their eggs in close proximity to fig embryos. Gall figs are functionally staminate because fig wasp larvae consume all of the developing seed (Weiblen et al.

1995). The release of pollen from staminate florets in gall figs coincides with eclosure and mating of the fig wasps. Flightless males chew an exit from the syconium and the winged females escape in search of receptive figs in which to complete their life cycle. The ecology of dioecious fig pollination is described in greater detail in Chapter 4.

Classification of dioecious figs

Ficus is the largest genus in the Moraceae, a mostly tropical woody plant family recognized by the presence of latex, stipules, and paired inflorescences with unisexual flowers (Corner 1962a). As the only member of the tribe Ficeae (Rohwer 1993), the genus has long been noted for its distinctive reproductive morphology (Berg 1989). In the last century, Ficus was split into several genera (Gasparrini 1844, Miquel 1862) that became the basis for a subgeneric classification after the genus was reunited (Miquel 1867b, Miquel 1867a). Miquel classified the dioecious species in four subgenera (Covellia, Erythrogyne, Eusyce and Synoecia) based on microscopic floral characters. Almost a century later, Corner (1965) united the dioecious figs under one of four subgenera in his reclassification of the genus (Table 1). Although Miquel (1867a) and King (1887a) recognized striking similarities between some monoecious and dioecious species, Corner (1960b) split them on the basis of breeding system alone. Corner (1965) went on to recognize subgenera based on breeding system, growth form, and inflorescence position in the following scheme: (A) subg. Urostigma including monoecious stranglers with axillary figs, (B) subg. Pharmacosycea including monoecious trees with axillary figs, (C) subg. Sycomorus including monoecious trees with cauliflorous figs, and (D) dioecious subg. Ficus including trees, banyans, and climbers

with cauliflorous and axillary figs. A reclassification grouping some monoecious and dioecious species based on pollinator associations was later rejected (Ramirez 1977, Corner 1985). However, local floras (Corner 1970b, Berg and Wiebes 1992) have commented on the striking similarities between species with different breeding systems. This chapter presents a phylogenetic analysis of subg. Ficus and their relatives, examining classification, pollinator relations, and breeding system evolution in dioecious figs.

Geographical distribution of dioecious figs

Ficus can be found in all three tropical regions (Figure 2A) but the majority of species occur in Asia, the Indo-Papuan Islands and Australia. The dioecious species are restricted to the old world tropics. Malesia is the center of diversity for dioecious figs in terms of species richness and endemism (Figure 2B). Most dioecious species occur here, comprising an estimated 343 species out of the 503 species in the region (68%; Berg 1989). In addition, five of the eight sections in subg. Ficus are centered in Malesia (Table 1). Dioecious sect. Adenosperma, for instance, is restricted to New Guinea, Australia and the Solomon Islands (Corner 1958). However, some dioecious species are widespread, such as F. variegata, which is distributed from eastern India to New Guinea and from northern Australia to southern Japan (Corner 1965). Dioecious coastal species, such as F. tinctoria, have even wider distributions on remote islands in the Pacific and Indian Oceans (Corner 1958). Geographical range in most dioecious species is more restricted. For example, at least 11 species of sect. Sycocarpus are found only in New Guinea (Corner 1958). It is also noteworthy that the regional species richness of Ficus

reaches its peak not in the continental masses of Asia and Australia, but in the complex of islands stretching from the Solomons in the east to Borneo in the west. Species within this region were the focus of sampling for phylogenetic analysis.

Objectives of phylogenetic analysis

The main objective of this study was to test the monophyly of the dioecious figs (subg. Ficus) with reference to the monoecious figs (subg. Pharmacosycea, Sycomorus and Urostigma). There were three additional objectives to the study. These included: (B) identification of major shifts in breeding system, such as changes from monoecy to dioecy and from dioecy to monoecy, (C) reconstruction of phylogenetic relationships for comparison with pollinator phylogeny (Chapter 2) and tests of coevolutionary hypotheses (Chapter 3), and (D) reconstruction of phylogenetic relationships in a local assemblage of dioecious figs for comparison with sympatric fig wasp assemblages (Chapter 4) and comparison with the associations of insect herbivores in general (Chapter 5).

The primary source of characters for phylogeny reconstruction was the internal transcribed spacer (ITS) region of nuclear ribosomal DNA. The ITS region has proven useful for resolving phylogenetic relationships at lower taxonomic levels in plants due to its high interspecific variability (Baldwin et al. 1995). However, the limitations of ITS and other genes in reconstructing Ficus phylogeny were also explored (*see ITS heterogeneity and chloroplast genes*). As a supplement to ITS sequences, morphological characters for Ficus were analyzed separately and in combination.

Issues in phylogenetic analysis

Whether or not to combine morphological and molecular data sets in a single analysis has been a subject of considerable debate in the recent systematic literature (Bull et al. 1993, de Queiroz et al. 1995, Huelsenbeck et al. 1996). Different analytical approaches to this problem are based on principles of total evidence, separate analysis, and conditional combination (Huelsenbeck et al. 1996). According to the principle of total evidence, systematists have argued that inferences based on all the available data are more likely to be correct than inferences based on a subset of the data (Barrett et al. 1991). This is only the case if different sources of data share the same underlying phylogenetic history, an assumption of the total evidence approach. However, conflicting species phylogenies are often inferred from different gene regions and morphological characters (Swofford 1991, Doyle 1992). Sources of conflict between data sets can result from either systematic error or from data sets not sharing the same phylogenetic history (de Queiroz et al. 1995). Systematic error results from the failure of phylogenetic reconstruction methods to make accurate assumptions about the evolutionary processes affecting character change. Maximum parsimony, for example, may fail when multiple characters exhibit correlated patterns of homoplasy (Felsenstein 1978). Molecular data with unequal base composition, codon usage, or differences between synonymous and non-synonymous substitutions may also converge on the wrong tree (Naylor and Brown 1998). Furthermore, sampling characters from independent data sets, such as morphology and molecules, increases the sampling variance and the chance of obtaining an inaccurate result (de Queiroz 1993). On the other hand, data sets may also differ with respect to their underlying phylogenetic history. In the case of molecular data, phylogenies inferred from different genes may conflict with each other or

with species phylogeny due to lineage sorting (Maddison 1997) or lateral transfer (McDade 1992, Sang et al. 1997).

Separate analyses have the advantage of highlighting points of conflict, without indicating whether systematic errors or different histories are responsible for incongruent phylogenies. If incongruence is due to sampling or random errors in phylogeny estimation, then a combined analysis may provide the best estimate of phylogeny (de Queiroz et al. 1995). A conditional approach favors combined analyses in the event of "weak" incongruence while favoring separate analyses in the event of "strong" incongruence (Huelsenbeck et al. 1996). Different statistical methods have been developed for estimating the extent of incongruence (Kishino and Hasegawa 1989, Templeton 1993, Farris et al. 1994).

When morphology and molecules are not significantly incongruent, their combination can recover phylogenetic signal that is hidden in separate analyses (Barrett et al. 1991). Practical and theoretical considerations on separate versus combined analysis of ITS and morphological data sets for Ficus are explored using several approaches (see *Tests of congruence*). In Ficus, it has been suggested that morphology may yield incorrect estimates of phylogeny because of convergent evolution in reproductive traits; however previous studies did not specifically test this proposition (Herre et al. 1996). With regard to the question of breeding system evolution, the issue of including characters of interest in phylogeny reconstruction (de Queiroz 1996) was examined using sensitivity analysis (Donoghue and Ackerly 1996).

Materials and Methods

Taxon sampling

The evolutionary relationships of dioecious figs were examined through phylogenetic analyses of 46 species (Table 2). Sampling was limited to representatives of the major taxonomic divisions of Ficus (subgenera and sections). The choice of ITS and morphology as sources of characters did not permit the inclusion of other Moraceae as outgroups in the phylogenetic analysis, due to difficulties associated with sequence alignments and the assessment of homology (see Results: *ITS heterogeneity and chloroplast genes*). Evidence from the chloroplast gene rbcL (Herre et al. 1996) and morphology (Berg 1989a) suggests that the neotropical sect. Pharmacosycea is a sister group to the rest of Ficus. Two representatives of sect. Pharmacosycea were designated as outgroups. Sampling of the monoecious subgenera also included 15 species representing the sections Oreosycea, Urostigma, Conosycea, Malvanthera, Americana, and Sycomorus. In addition, twenty nine species comprising eight percent of dioecious subg. Ficus were sampled. At least two representatives of each dioecious section were sampled, in addition to the species included in ecological studies (Chapter 4 and 5). Four field trips were made to tropical lowland forests in Malesia between 1995 and 1997 to obtain collections of fertile plant specimens and DNA. Sampling localities included sites in Australia, Indonesia, Papua New Guinea and the Solomon Islands.

Nuclear ribosomal DNA

Sources of DNA included leaves preserved in silica gel in the field, herbarium specimens less than 10 years old, and fresh leaves harvested from cultivated plants. Voucher specimens for all DNA sources are deposited at the Harvard University Herbaria (A; Appendix 1).

Genomic DNA was extracted from 10-20 mg of dried leaves (30-50 mg when fresh). The protocol of Doyle and Doyle (1987) was modified to avoid problems associated with DNA isolation from leaves containing latex. Leaves were ground in liquid N_2 and incubated at 60° C in a 400 µL solution of 2X CTAB buffer with 4% polyvinyl pyrrolidone (mol. wt. 40000) and 0.8 µL ß-mercapto-ethanol. After 1 hr, samples were centrifuged for 5 min and the aqueous supernatant was twice extracted with 400 µL of phenol:chloroform:isoamyl alcohol (25:24:1). The supernatant was extracted a third time with chloroform:isoamyl alcohol (24:1). DNA extracts were cleaned with a GENECLEAN II® kit (BIO 101 Inc.), serially diluted, and amplified with a PCR reagent system (Gibco BRL Inc.).

Primers ITS4 and ITS5 (White et al. 1990) were used for amplification of the region including the two internal transcribed spacers and the 5.8S subunit of nuclear ribosomal DNA. The thermal conditions for amplification included: (A) denaturation at 96° C (2 min); (B) 2 cycles of denaturation at 94°C (30 s), annealing at 40° C (30 s) and extension at 72° C (60 s); (C) 35 cycles as in (B) but with annealing at 55° C (30 s); and (D) final extension at 72° C (4 min). PCR products were quantified on 0.4% agarose gels using a Low DNA Mass™ ladder (Gibco BRL Inc.) and single bands were purified with a QIAquick™ PCR purification kit (QIAGEN® Inc.). PCR products were cycle sequenced in both directions using primers ITS2, ITS3, ITS 4 and ITS5 (White et al. 1990). ITS2 and ITS3 sequencing primers were redesigned for Ficus (5'-GCATCGATGAAGAACGTAGC-3' and 5'-GGAAGGAGAAGTCGTAACAAGG-3', respectively). Sequences were collected using Long Ranger™ polyacrylamide gels (FMC Bioproducts Inc.), a 377 PRISM™ sequencer (Applied Biosystem Inc.), and DNA Sequencing Analysis software version 2.1.1 (Applied Biosystem Inc.). Chromatograms were edited with Sequencher™ software (Gene Codes

Inc.) and aligned manually (Appendix 2). Matrices were also deposited in TreeBASE (http: www.herbaria.harvard.edu/treebase). Thirty-three ambiguous positions corresponding to 4.3% of the aligned sequences were excluded from analysis (i.e. positions 283-286, 465-483 and 529-547). Thirty-four gaps remained in the aligned sequence following the exclusion of these ambiguous sites. Nineteen autapomorphic indels were treated as missing data. The presence or absence of fifteen remaining indels was coded in a supplemental set of characters (Appendix 2), but all indel positions were excluded from analyses of the aligned sequences.

ITS heterogeneity and chloroplast genes

Molecular cloning examined heterogeneity among ITS paralogues in dioecious figs. ITS heterogeneity within species was explored because the inclusion of divergent paralogues and pseudogenes in phylogenetic analysis has the potential to yield inaccurate estimates of species phylogeny (Buckler et al. 1997). PCR products from five species were cloned and sequenced for comparison with the results of direct sequencing. In addition, multiple ITS clones from F. nodosa and F. variegata were sequenced to look for the presence of heterologous ITS copies within species. ITS PCR products were ligated and transformed using the pGEM®-T Easy Vector System (Promega Corp.). Transformed cells were screened with ampicillin and recombinant plasmid DNA was isolated using the Wizard® Plus Miniprep DNA purification system (Promega Corp.).

Two chloroplast gene regions were amplified and sequenced as potential sources of additional characters for phylogenetic studies of dioecious figs. ndhF, a single copy chloroplast gene encoding one subunit of NADH dehydrogenase, has been useful in phylogenetic studies of plant families and genera (Olmstead and Sweere 1994). ndhF was

amplified and sequenced for three Ficus species according to protocols in Ferguson (1998). Non-coding regions of chloroplast DNA have been useful in elucidating phylogenetic relationships at lower taxonomic levels, due to higher rates of nucleotide substitution than in coding regions (Taberlet 1991). The trnL intron, located in the region encoding the leucine (UAA) transfer RNA, was amplified according to protocols in Taberlet (1991) and sequenced for 16 Ficus species.

Morphology

Sixty-four discrete morphological characters were selected from the taxonomic literature (Corner 1933, Corner 1955, Corner 1958, Corner 1960b, Corner 1960a, Corner 1961, Corner 1965, Corner 1967, Corner 1969, Corner 1970a, Corner 1970b, Corner 1976, Corner 1978) and by examination of living plants and more than 800 herbarium collections. Representative vouchers for morphological study are listed in Appendix 1. Sixty-one characters with two to five states were potentially informative in phylogenetic analysis (Appendix 3). Reproductive characters included the position and structure of the syconium, inflorescence bracts and unisexual florets (Figure 1). Vegetative characters included branching architecture, ptyxis, phyllotaxis, and leaf venation. The position of epidermal glands, hairs, and cystoliths were also a major source of characters and states. Scoring of morphological characters is listed in Appendix 4. Ten out of 64 characters were not applicable to some taxa and were treated as missing data (-), which can be problematical in phylogenetic analysis (Maddison 1994). Approximately 4.7% of the matrix consisted of non-applicable character states while <0.5% and 1.4% of the matrix consisted of unscored (?) and polymorphic (&) character states, respectively.

Phylogenetic analyses

Phylogenetic analyses were performed with PAUP* version 4.0b1 for Power Macintosh computers (Swofford 1998). Under the optimality criterion of parsimony, heuristic searches were conducted according to PAUP* default settings, except that 1000 random addition sequence replicates were used with MAXTREES were set to increase without limit. All characters were unordered and weighted equally. Uninformative characters were excluded from all analyses. Bootstrap resampling (Felsenstein 1985a) and decay analyses (Bremer 1988, Donoghue et al. 1992) were used to estimate clade robustness. Bootstrapping involved heuristic searches with 10,000 replicates and a random addition sequence with N = 1. In the case of ITS and combined analyses, the option to save multiple equally parsimonious trees per replicate was disabled to reduce the search times on Power Macintosh 7300 and Macintosh G3 computers. Decay analyses were performed using the program Autodecay version 2.9.5 (T. Eriksson) with 10 random addition sequence replicates per heuristic search.

Tests of congruence

The issue of combining morphological and molecular datasets (Swofford 1991, Donoghue and Sanderson 1992, Larson 1994, Huelsenbeck et al. 1996) was explored using two statistical methods (Mason-Gamer and Kellogg 1996). The incongruence length difference (ILD) test estimates congruence based on data partitions (Farris et al. 1994, Swofford 1998). The ILD test measures observed incongruence in two data partitions (i.e. morphology and ITS) against a distribution of incongruence measures

taken from random partitions of the combined data sets. This test was performed using the "partition homogeneity" option in PAUP*. Search options included 100 partition replicates with 10 random addition sequence replicates per partition and MAXTREES was set to increase without limit. All parsimony-informative characters (241) were equally weighted and unordered in the combined analyses (Cunningham 1997b, Cunningham 1997a).

Another statistical test of congruence, based on the comparison of rival trees, was also implemented. Templeton's test considers the conflict between trees generated by separate analyses with the possibility of taking into account the strength of support for nodes in rival trees (Templeton 1993, Larson 1994). Each data set was analyzed to find the most parsimonious trees compatible with constraint trees from the rival data set. For example, morphological data were analyzed to find the shortest trees compatible with the shortest trees from a separate analysis of the ITS data. Constraint trees from the rival data set included the strict consensus tree, bootstrap consensus trees (50%, 70% and 90%), and a most parsimonious tree selected at random. Constrained search parameters were 100 random addition sequence replicates with MAXTREES set to increase without limit. Most parsimonious trees from the constrained and unconstrained searches were selected at random and compared using a non-parametric sum of signed ranks test (Wilcoxon) under the "Tree scores" option in PAUP*. It was not possible to apply tests of congruence based on maximum likelihood to compare morphological and ITS data sets (Kishino and Hasegawa 1989).

In addition to statistical measures of conflict, comparisons were made between consensus trees and bootstrap values from the separate analyses. The strict consensus of

the shortest ITS and morphological trees highlighted cases of complete agreement (but see Barrett et al. 1991). Comparisons of bootstrap values between conflicting nodes in the separate analyses were used to identify points of weak and strong incongruence between the results from morphological and ITS data. Molecular and morphological data were also analyzed in combination. All 241 potentially informative characters were unordered and assigned equal weight. The evolution of morphological characters was reconstructed under parsimony using MacClade and one of the shortest combined trees selected at random (Maddison and Maddison 1992). A list of apomorphies for major clades was generated using PAUP*.

Results

Nuclear ribosomal DNA

Amplification of ITS from Ficus yielded single bands with the exception of F. racemosa, which produced two bands. Gel purification the two bands showed that the longer fragment aligned to other Ficus while the shorter fragment was most similar to ITS sequences from pathogenic fungi (Euascomycetes). This putative fungal sequence was excluded from further analysis. Cloning results from four species agreed with the results of direct sequencing at 99% of nucleotide positions. Results from cloning and direct sequencing differed from each other at three to seven positions in the aligned sequence and no two clones from the same plant differed by more than five and eight positions of the aligned sequence (~1%) in F. nodosa and F. variegata, respectively. The location of nucleotide differences among ten clones from each species was scattered such that clones could not be grouped below the level of species. This kind of heterogeneity was suggestive of random errors by DNA polymerase during the cycle sequencing reactions possibly induced by high GC-content in the ITS region. Overall, the results of cloning

and direct sequencing suggest that ITS heterogeneity did not pose a major problem for phylogeny reconstruction in Ficus.

Manually aligned ITS sequences for 46 species were 761 bp in length including 33 positions with ambiguous alignment. Parsimony analyses of ITS alone were based on 643 bp excluding the ambiguous positions and indel positions coded as binary characters. One hundred and sixty-five nucleotide positions (25.6%) were potentially informative. In addition, fifteen out of 35 indels were potentially informative. Analysis of the 180 characters combined found a single island of 208 most parsimonious trees of 453 steps (CI = 0.55). The strict consensus was congruent with the bootstrap consensus at 29 of 31 nodes with >50% support (Figure 3). Two clades with bootstrap values less than 60% did not appear in the strict consensus but are shown in Figure 3. A clade with F. pungens as sister to subsect. Sycocarpus and a clade with F. septica as sister to the rest of subsect. Sycocarpus were compatible with 158 and 50 out of 216 most parsimonious trees, respectively.

In agreement with results from rbcL (Herre et al. 1996), the neotropical and paleotropical sections of subg. Pharmacosycea did not form a clade (Figure 3) and there was marginal support from ITS for the paraphyly of sect. Oreosycea. Monoecious subg. Urostigma was not monophyletic due to the position of sect. Urostigma as sister to a dioecious clade but support for this relationship was weak. Subgenus Ficus was polyphyletic and divided into two highly supported clades. One entirely dioecious clade included the well-supported and monophyletic sects. Ficus, Kalosyce, Rhizocladus and Sycidium, excluding F. pungens. The other clade included dioecious sects. Adenosperma, Neomorphe, Sycocarpus, F. pungens, and monoecious subg. Sycomorus.

Relationships within this clade were not well resolved, although monophyly of sect. Adenosperma, subsect. Sycocarpus and subg. Sycomorus were each highly supported. In addition, the derivation of monoecious subg. Sycomorus within dioecious subg. Ficus received strong bootstrap support.

ITS substitutions and the position of indels were also reconstructed on one of the shortest trees under parsimony (Figure 4). The largest genetic difference, according to branch lengths, was between the neotropical sect. Pharmacosycea and the rest of the genus. Some species had numerous apomorphies (i.e. 17 in F. albipila). However, species within Ficus, Kalosyce, Rhizocladus, and Urostigma clades had fewer than 4 substitutions in terminal branches and ITS did not contain sufficient nucleotide variation to adequately resolve phylogenetic relationships within these sections. On the other hand, relationships among sections were often supported by multiple substitutions (e.g. Kalosyce and Rhizocladus). The phylogenetic distribution of indels also provided support for major clades of dioecious figs. For example, two indels were shared by species in a dioecious clade including subg. Sycomorus. A pair of indels supported sect. Sycidium while another pair supported a Ficus-Kalosyce-Rhizocladus clade. Single indels also characterized sects. Kalosyce and Rhizocladus and subsect. Sycocarpus.

Two chloroplast gene regions showed similar levels of phylogenetic information. Interspecific variation in trnL sequences from 18 species representing all Ficus subgenera was very low. Out of 498 aligned bases, unambiguous nucleotide substitutions were detected at seven positions (1%) and only one of these was potentially informative. There were three autapomorphic indels and a four bp insertion shared by F. odoardi, F. punctata and F. ruginerva (sects. Kalosyce and Rhizocladus). Also, ndhF was partially

sequenced for F. microcarpa (subg. Urostigma) and two dioecious species (F. copiosa and F. wassa). The three species together showed only 13 nucleotide substitutions out of 1202 aligned positions (1%). F. copiosa and F. wassa, closely related, were distinguished by six substitutions out of 2150 positions (0.3%). Due to the scarcity of potentially informative characters in Ficus chloroplast genes, phylogenetic analyses were limited to ITS sequences and morphology.

Morphology

The morphological data alone yielded six most parsimonious trees of 339 steps (CI = 0.47). (Figure 5). The strict consensus was congruent with the bootstrap consensus at 20 out of 21 nodes with >50% support. (Figure 5). A clade representing neotropical sect. Pharmacosycea with 57%, shown in Figure 5, was not present in the strict consensus due to the position of F. albipila as sister to F. insipida in the most parsimonious trees. Morphological analysis indicated that subg. Ficus was not monophyletic and that monoecious subg. Sycomorus was derived within a paraphyletic sect. Neomorphe. The dioecious figs including subg. Sycomorus were sister to monoecious subg. Urostigma. These three subgenera were derived within a paraphyletic subg. Pharmacosycea. However, morphological support for subgeneric relationships was relatively weak, as indicated by low bootstrap values at deep nodes compared to shallow nodes. Monoecious subg. Urostigma had a bootstrap value of 64% but support for the paraphyly of sect. Oreosycea was lacking. The monophyly of monoecious sects. Conosycea, Malvanthera, and Urostigma was upheld in the morphological analysis, in contrast to dioecious sects. Sycidium, Sycocarpus, and Neomorphe, which were polyphyletic or paraphyletic.

However, highly supported clades of dioecious figs included sects. Adenosperma, Ficus, Kalosyce and Rhizocladus.

Conflict and congruence

Comparing morphological and ITS consensus trees (Figures 3 and 5), 18 of 45 nodes were in absolute agreement. Nineteen nodes in the ITS tree were not recovered in the analysis of morphology alone. Similarly, 21 nodes in the morphological tree were not present in the ITS consensus. However, most conflicting nodes were weakly supported (<50% bootstrap support) in one analysis or the other and most of nodes with >50% support were congruent with the rival consensus tree. For example, 18 of 21 nodes in the morphological consensus having >50% support were in agreement with the ITS consensus. Although ten nodes with >50% support in the ITS consensus were not present in the morphological consensus, none were contradicted by bootstrap values >60% in the morphological analysis.

An incongruence length difference (ILD) test based on 100 replicates found that the sum of tree lengths from separate analyses of ITS and morphological data sets was significantly less than the sum of tree lengths taken from random partitions of the combined data sets (Figure 6A). This result suggests significant conflict between the morphological and ITS data sets. However, the interpretation of this result is ambiguous because the ILD test does not distinguish among alternative hypotheses for conflict (i.e. whether conflict results from different phylogenetic histories or systematic error in one or both data sets). As a global test of incongruence, the ILD test does not identify particular conflicts that might suggest different histories for the data sets. The observation that separate analyses yielding highly similar topologies often fail the ILD test has questioned the sensitivity of the test (i.e.

Soltis et al. 1998). Significant incongruence does not, at face value, provide strong evidence of two data sets not sharing the same phylogenetic history, and therefore, the ILD test alone was not decisive with respect to whether or not to combine data sets in a single analysis.

An ILD test for incongruence between ITS nucleotide substitutions and indels was not statistically significant (Figure 6B). The indel partition had very few characters (15), and consequently, the number of equally parsimonious trees exceeded the memory allocated to PAUP* in some replicates. The most parsimonious trees may not have been found in these cases and, as a result, the ILD null distribution could be marginally skewed in the direction of longer trees. Although a skewed distribution could lead to falsely rejecting the null hypothesis of data congruence (type I error), this is not the case for ITS nucleotide substitutions and indels, where no significant difference was found.

Results of Templeton tests for incongruence are summarized in Table 3. ITS sequence data strongly rejected the shortest morphological trees. Similarly, the morphological data rejected the shortest ITS trees. However, taking into account the relative support for clades in the separate analyses had a strong impact on the results of these tests. For example, ITS sequences marginally rejected the morphology-based 50% bootstrap consensus and morphological data significantly rejected the ITS 50% bootstrap consensus. However, neither the ITS or morphological datasets rejected their rival 70% or 90% bootstrap consensus trees. Morphology did not reject the combined data consensus tree (Figure 7), and although ITS marginally rejected the combined tree, this result was not statistically significant after correcting for multiple tests (Dunn-Sidak correction; Sokal and Rohlf 1981). It appeared, therefore, that statistically significant conflict between ITS and morphology was limited to weakly supported nodes in the separate analyses. Based on direct

comparisons of bootstrap values and statistical tests of conflict, there appeared to be no strong incongruence between ITS and morphology, however, local tests of incongruence ought to be explored in the future.

Combined analyses

The combined analysis recovered eight most parsimonious trees of 747 steps (CI = 0.50) on two tree islands (Maddison 1991). The combined analysis recovered eight most parsimonious trees of 747 steps (CI = 0.50) on two tree islands. The strict consensus was congruent with the bootstrap consensus at all but three of 30 nodes with >50% bootstap values (Figure 7). A weakly supported clade (54%) including sect. Neomorphe, subsect. Sycocarpus and subg. Sycomorus was recovered in three of the eight trees (Figure 7). Seven trees from one island showed the sister relationship of F. nodosa and F. robusta that was recovered in the bootstrap consensus. In addition, a clade including subg. Ficus plus subg. Sycomorus was recovered in the bootstrap consensus (63%) but not in the strict consensus due to a difference between tree islands. Furthermore, sect. Oreosycea was not monophyletic in any of the most parsimonious trees but the precise relationships of species in sect. Oreosycea differed between tree islands. The island of seven trees supported a mostly dioecious clade with F. albipila as its sister group (as shown in Figure 8). A single most parsimonious tree on the other island placed F. albipila as sister to a dioecious clade including sects. Ficus, Kalosyce, Rhizocladus and Sycidium (Figure 9). In this tree, F. edelfeltii plus F. hombroniana were sister to a clade including subg. Urostigma and the other mostly dioecious clade (sects. Adenosperma, Neomorphe, Sycocarpus and monoecious subg. Sycomorus).

The combined analysis supported the monophyly of subg. Sycomorus and Urostigma but subg. Ficus and Pharmacosycea were either polyphyletic or paraphyletic, depending on the tree island. Separate and combined analyses agreed on the derivation of monoecious subg. Sycomorus within a clade of dioecious figs. Also in agreement with results from rbcL (Herre et al. 1996), neotropical sect. Pharmacosycea and paleotropical sect. Oreosycea did not form a clade. Within monoecious subg. Urostigma, there was strong support for the monophyly of the Indo-Australian sects. Conosycea, Malvanthera and Urostigma. Two strongly supported clades containing dioecious figs were also recovered in the combined analysis but it was not entirely clear whether these were sister groups (Figure 7). The first of these clades was entirely dioecious and included sects. Ficus, Kalosyce, Rhizocladus and Sycidium (excluding F. pungens). Bootstrap and decay values strongly supported the monophyly of each of these sections and the exclusion of F. pungens from Sycidium. Relationships within Sycidium were resolved but mostly not supported by high bootstrap values. Sections Kalosyce and Rhizocladus were strongly supported sister groups and this clade was sister to sect. Ficus.

A second major clade of mostly dioecious figs, including F. pungens, sects. Adenosperma, Neomorphe, Sycocarpus, and monoecious subg. Sycomorus, had high bootstrap support (89%). However, basal relationships within this clade were not well resolved in the combined analysis. Although sect. Sycocarpus was clearly not monophyletic, relationships within the section were mostly unresolved. Section Neomorphe was not monophyletic due to the highly supported relationship of F. semivestita to sect. Adenosperma. Members of sect. Neomorphe (excluding F. semivestita) belong to a well-supported clade including monoecious subg. Sycomorus. The sister relationship between

dioecious F. itoana and monoecious F. microdictya also received strong support in the combined analysis.

Discussion

ITS and dioecious fig phylogeny

ITS proved to be a useful tool for the molecular systematics of dioecious figs due to moderate levels of interspecific sequence variation and low levels of intraspecific heterogeneity. Ancestral polymorphisms reported for ITS in some plant species (cf. Wendel et al. 1995, Campbell et al. 1997) were not detected in Ficus. Divergent ITS paralogues appear to be common in lineages having a history of hybridization and polyploidy (Buckler et al. 1997). However, natural hybridization and polyploidy are rare in Ficus (see Chapter 4 on hybridization; also Storey 1975). Cytology has been examined in over 100 species and the great majority are diploid (2N = 26), with the notable exception of a sterile triploid (3N = 39) cultivar of F. elastica Roxb. (Löve 1969, Hans 1972, Meera and Gill 1974, Ohri and Khoshoo 1986). Tetraploids (2n = 52) have been observed in F. cordata (sect. Urostigma) and members of sect. Galoglychia including F. glumosa Delile and F. stuhlmannii Warb (Condit 1964). Additional reports of diploid and tetraploid cytotypes in F. insipida (sect. Pharmacosycea), F. pseudopalma Blanco. (sect. Ficus), and F. thonningii Bl. (sect. Galoglychia) are unconfirmed.

Although the ITS region was phylogenetically informative, the ability to resolve relationships within Ficus was limited. Ficus ITS sequences were highly diverged from other Moraceae and alignment across other genera was not meaningful due to the presence of overlapping indels. Alignment of ITS sequences within Ficus was

straightforward but the rooting of trees had to be based on the results of previous molecular and morphological studies (Herre et al. 1996; Berg 1989a,b). The choice of neotropical sect. Pharmacosycea as an outgroup was corroborated by the 5.8S nuclear ribosomal subunit located between the ITS1 and ITS2 spacers. A phylogenetic analysis of 5.8S sequences from Antiaropsis, Dorstenia, Milicia and ten Ficus species (G. Weiblen, unpublished) was consistent with results from rbcL in placing sect. Pharmacosycea as a sister group to the rest of Ficus (Herre et al. 1996).

Fossils also provide indirect evidence for the ancient origin of sect. Pharmacosycea. Tertiary fossil figs from Europe are among the oldest known Moraceae, dating from the Lower Eocene (~50 mya; Collinson 1989). Although these fossils have not been assigned to extant subgenera, an early fossil fig wasp was assigned to Tetrapus, a genus that pollinates extant sect. Pharmacosycea (Brues 1910). Tetrapus mayri and fossil figs from the Florissant shale in Colorado, dating from the Lower to Middle Miocene, indicate that pollination by Tetrapus evolved no less than ~20 mya (Brues 1910, Cockerell 1910). However, Wiebes (1995) suggested that a fossil fig wasp from Dominican amber (15-40 mya; Poinar 1993) might be a species of Pegoscapus, which pollinates extant sect. Americana in subg. Urostigma.

Chloroplast DNA sequences from the gene ndhF and the trnL intron did not show sufficient nucleotide variation to reconstruct phylogenetic relationships in dioecious figs. Chloroplast genes appear to be more useful in reconstructing phylogenetic relationships within Moraceae (e.g. Herre et al. 1996) and within plant families in general (Olmstead and Palmer 1992, Bogler and Simpson 1995, Clark et al. 1995, French et al. 1995, Ferguson 1998). Additional phylogenetic studies on the relationships between Ficus and

other Moraceae genera using chloroplast genes may provide insights on the origins of fig pollination and the fig inflorescence. However, ITS sequences support the conclusion from rbcL that the new and old world representatives of subg. Pharmacosycea are not sister taxa.

Low levels of sequence variability among closely related species also limit the utility of ITS for resolving phylogenetic relationships within most sections (Figure 4). For example, nearly identical ITS sequences were obtained from closely related taxa, such as F. odoardi and F. bauerlenii (sect. Rhizocladus) or F. bernaysii and F. hispidioides (sect. Sycocarpus). Additional nuclear gene regions are needed to corroborate results based on ITS and morphology. Candidates for future phylogenetic analyses include genes for soluble starch synthase (waxy; Tanaka et al. 1995) and alcohol dehydrogenase (ADH; Sang et al. 1997), which might also be used to explore phylogenetic relationships closer to the species level.

Tests of incongruence

Significant incongruence was detected between the morphological and ITS data sets by both the ILD (Farris et. al. 1994) and Templeton tests (Table 3; Templeton 1993, Larson 1994). However, it was not clear whether statistically significant conflict, as measured by these tests, represented "strong" incongruence (i.e. that different data sets do not share the same history; Cunningham 1997a). Incongruence may also be attributed to systematic error and global tests do not differentiate between alternative sources of incongruence (Mason-Gamer and Kellogg 1996). Templeton tests are potentially more

informative in this regard because they can also consider levels of support for rival clades in separate analyses.

Results of the Templeton test were highly sensitive to the choice of rival constraint trees (Table 3). If weakly supported clades were included in rival constraint trees, the data significantly rejected the null hypothesis that random errors in phylogeny estimation account for length differences in rival trees. However, the null hypothesis was not rejected if only strongly supported clades were included. This was true for both the ITS and morphological data sets when the rival constraint trees were limited to clades with >70% or >90% bootstrap support. The arbitrary level of bootstrap support considered "strong" seemed reasonable based on empirical studies of phylogenetic accuracy (Hillis and Bull 1993).

The overall results for Ficus provided no decisive evidence of strongly supported incongruence between data sets, although instances of local incongruence deserve further consideration. For example, ITS and morphological analyses differed with regard to the monophyly of subg. Urostigma. Mophological and combined analyses placed all the monoecious stranglers in a clade with 65% and 64% bootstrap support, respectively (Figure 5). On the other hand, ITS showed sect. Urostigma to be the sister group to a dioecious clade with 59% support (Figure 3). Decay analysis for ITS indicated that five additional steps are required to contradict the placement of sect. Urostigma apart from the other monoecious stranglers. However, reciprocal Templeton tests of local incongruence were not significant (P = 0.20 and P = 0.37 for ITS and morphological data, respectively). Another local conflict involved a clade including F. botryocarpa, F. hispidioides and F. septica with 58% bootstrap support in the morphological analysis versus a rival clade

including F. bernaysii, F. botryocarpa and F. hispidioides with 54% bootstrap support in the ITS analysis. Both clades were present in the combined most parsimonious trees and support from the separate analyses was relatively weak (<60%). These two cases of local conflict are not very suggestive of different phylogenetic histories in the ITS and morphological data sets. In future exploration of these data sets, it might be possible to minimize the effects of systematic error through changes of character weighting under parsimony or alterations of rate parameters under maximum likelihood (de Queiroz et al. 1995, Huelsenbeck et al. 1996).

Comparisons of bootstrap values as measures of relative clade support suggest that the results of combined analyses are better supported than either of the separate analyses. Compared to ITS, bootstrap values for 15 nodes increased in the combined analysis while support for six nodes decreased. Bootstrap support for 21 nodes increased in the combined analysis compared to the separate morphological analysis and none decreased. In the absence of evidence for strong incongruence, the combined data provided the best-supported estimate of dioecious fig phylogeny. Similar conclusions have been reached in studies of other plant groups (Manos 1997, Kelley 1998; Soltis et al. 1998) but also see Mason-Gamer and Kellogg (1996). Classification, breeding system evolution, and associations with pollinators will be discussed in terms of the combined analyses (Figures 7-8). However, it is important to caution against the misinterpretation of inferences based on the conditional combination approach. Increased bootstrap support in the combined analyses may not imply increased phylogenetic accuracy and this possibility needs further attention (Sanderson 1995). Inferences from the combined

phylogeny should be regarded as preliminary until corroborated by analyses of additional genes and taxa.

Classification of dioecious figs

The combination of ITS and morphological characters, in general, provide a robust estimate of phylogeny for the dioecious figs that sheds some light on the accepted classification (Corner 1965) and proposed alternatives (Ramirez 1977; Berg 1989a). Although some groups appear to be monophyletic, the dioecious figs are not (Figure 7). In general, Corner's subgeneric classification (Table 1) is not supported by the result of ITS, morphology, or combined analyses (Figures 3, 5 and 7). Pharmacosycea is not monophyletic and the neotropical and paleotropical groups do not appear to be sister taxa in spite of their morphological similarity. Based on the combined analysis, Urostigma may be monophyletic but separate analyses conflicted in this regard. ITS and combined analyses strongly support the existence of two major clades of dioecious figs derived within paraphyletic sect. Oreosycea. Whether the dioecious clades are sister taxa, however, is unclear from the results of the combined analyses.

Separate and combined analyses indicate that monoecious Sycomorus is monophyletic and nested in a clade of dioecious Ficus. The close relationship of monoecious Sycomorus and dioecious Ficus was first noted by Miquel (1867) and later formalized by King (1887a), but Corner (1960b) split these taxa on the sole basis of breeding system (see *breeding system evolution*). The mostly dioecious clade recovered in the phylogenetic analysis corresponds to subg. Sycomorus sensu Ramirez (1977), including sect. Adenosperma, Neomorphe, Sycocarpus, Sycomorus and Ceratosolen-

pollinated Sycidium. Within this clade, basal relationships are not well resolved but sect. Sycocarpus appears to be paraphyletic. Phylogenetic analyses support the view of Berg (1989a) that sect. Adenosperma and sect. Sycocarpus are closely related. Also in agreement with Berg (1989a), a clade including sect. Neomorphe, subsect. Sycocarpus and subg. Sycomorus is well supported in the ITS and combined analyses.

One entirely dioecious clade has no parallel in Corner's (1965) classification, but instead corresponds to subg. Ficus sensu Ramirez (1977), including sects. Ficus, Kalosyce, Rhizocladus and Sycidum but excluding all Ceratosolen-pollinated species (see *congruence with pollinator classification*). Within this clade two distinct lineages were recognized by Berg (1989a), one including sect. Sycidium and the other sects. Ficus, Kalosyce plus Rhizocladus. The combined phylogenetic analysis also strongly supports the monophyly of each of these sections, excluding Ceratosolen-pollinated Sycidium, which Ramirez (1977) transfered into a revised subg. Sycomorus. In general, the combined phylogenetic analysis supports the modifications of Corner's scheme proposed by Ramirez (1977) and Berg (1989a). Morphological apomorphies for clades recovered in the combined analysis are discussed in terms of one most parsimonious tree selected at random (Figure 8).

Morphological apomorphies

A clade including F. edelfeltii, F. hombroniana and subg. Urostigma is marked by shifts from syconia with ostiolar to dispersed staminate florets and from paraxial to abaxial cystoliths in leaves. The monoecious stranglers in subg. Urostigma are further characterized by syconia with three apical bracts, pistillate florets with undivided styles, the absence of spongy pith in twigs, and a solitary leaf gland at the base of the midrib.

Within the monoecious strangling clade, sect. Urostigma has staminate florets in the ostiolar position and deciduous growth. Sessile syconia and a red pistillate perianth are apomorphies for sect. Conosycea. Within subg. Urostigma, sect. Malvanthera is most distinct; having seed-producing florets with inferior ovaries, sclereids in the receptacle, staminate florets with elongate pedicels, and unilocular anthers.

F. albipila and the mostly dioecious clade share apomorphies including semicordate leaves and pubescence on the twigs, petiole, lamina, peduncle and the exterior and interior of the syconium. The mostly dioecious clade is characterized by cauliflory, syconia with sclereids, persistent basal bracts, simple styles, plicate ptyxis and leaves with serrate margins. One of two subclades, including sects. Adenosperma, Neomorphe, Sycocarpus and monoecious subg. Sycomorus, is marked by shifts to syconia with glabrous peduncles, a fluid-filled lumen during the interfloral phase, a funnelform stigma in short-styled florets, red ovaries, and a fused staminate perianth. Large cauliflorous trees with buttresses and deciduous growth in sect. Neomorphe and subg. Sycomorus are also characterized by pubescent syconia, pistillate tepals fused at the base and staminate florets containing a pistillode. A reversal to monoecy in subg. Sycomorus is accompanied by the occurrence of syconia on elongate leafless branches, pubescent basal bracts, staminate tepals fused at the base, pubescent stipules and cuneate leaf bases. Section Neomorphe excluding F. semivestita may be a sister group to monoecious Sycomorus, having caducous basal bracts, mucronate anthers, scalariform tertiary venation, and ostiolar staminodes in seed figs. Dioecious figs pollinated by Ceratosolen subg. Strepitus (see *pollinator classification*) may belong to a clade distinguished from other members of sect. Sycocarpus by having white ovaries and entire leaves.

The position of F. pungens as sister to subsect. Sycocarpus is supported by having pistillate florets with setose styles, twigs with a waxy gland below the node, and scalariform tertiary venation. Within the dioecious clade, subsect. Sycocarpus is

characterized by syconia with pustules and transverse ridges, staminate florets with elongate pedicels, yellow latex, cuneate leaf bases, and leaf glands in the axils of the secondary veins. The close relationship between F. semivestita and sect. Adenosperma is supported by the presence of lateral bracts on syconia, gynobasic styles, auriculiform seeds with a double ridge arising from the hilum, pistillodes in staminate florets, "Terminalia" branching, and entire leaves. Section Adenosperma itself is marked by the presence of sclereids in the receptacle, sessile pistillate florets, staminate perianth fused only at the base, and cuneate leaf bases.

The other major clade of dioecious figs, including sects. Ficus, Kalosyce, Rhizocladus, and Sycidium, has only two apomorphies: pubescent basal bracts and scalariform tertiary venation. Within this clade, there is morphological evidence for a lineage consisting of sects. Ficus, Kalosyce and Rhizocladus characterized by having bracts at the bottom of the fig stalk, two stamens per floret, mucronate anthers, and leaf glands in the axils of the basal and lateral veins. Section Ficus (subsect. Eriosycea) is characterized by axillary syconia, tuberculate seeds, a forked ridge arising from the hilum, filaments with epidermal hairs at the base, and the loss of cystoliths. Dioecious climbing figs (sects. Kalosyce and Rhizocladus) are marked by caducous basal bracts, flattened achenes, and distichous, entire leaves. In contrast to sect. Rhizocladus, which has a single apomorphy of syconia with sunken apical bracts, sect. Kalosyce is distinguished by several apomorphies. These include shifts to dispersed unistaminate florets with elongate pedicels, staminate perianth fused at base, the loss of mucronate connectives, leaf glands paired in the axils of the basal veins, reticulate tertiary venation, and glabrous, asymmetric leaves with sunken stomata.

Section Sycidium is heterogeneous in its morphology and is characterized by a single apomorphy (white ovaries) within the dioecious subclade. Scabrid twigs, petioles, leaves and syconia are common in this lineage. Subsection Paleomorphe (F. tinctoria and F. virgata) is distinctive, having syconia with sclereids and bracts at the bottom of the

stalk, lacking glandular hairs within syconia, and having functional gall ovaries, glabrous staminate tepals, mucronate anthers, hemi-epiphytic growth, glabrous stipules, and entire leaves with cuneate bases and reticulate tertiary venation.

Breeding system evolution

The phylogenetic analysis also provides insights on the evolution of breeding systems in Ficus. The phylogeny indicates one or two independent origins of dioecy from monoecy in Ficus depending on which island of most parsimonious trees is examined (Figure 9). The first island included seven equally parsimonious trees with monoecious F. albipila (sect. Oreosycea) as sister to a clade containing dioecious subg. Ficus plus monoecious subg. Sycomorus (Figure 9A). The second tree island showed sect. Oreosycea to be paraphyletic to subg. Ficus, Sycomorus and Urostigma. The monoecious stranglers (subg. Urostigma) were sister to a clade of mostly dioecious figs while F. albipila was sister to the other major dioecious clade (Figure 9B). Both separate and combined analyses unequivocally suggested two reversals from dioecy to monoecy within one of the dioecious lineages.

It has been argued in regard to the inference of character evolution that the characters of interest should be excluded from phylogenetic analysis in order to avoid circularity and bias. Indeed, morphological characters are sometimes excluded from phylogenetic analyses on the grounds that convergence in function can lead to inaccuracy and such arguments are often the basis for preferential use of independent molecular data (Herre et al. 1996; Van Noort and Compton 1996; Machado 1998). However, morphological and molecular data may show similar levels of homoplasy (Donoghue and Sanderson 1992) and molecular data also have convergent properties (Naylor and Brown

1998). Furthermore, it is possible that excluding the characters of interest can also yield biased or inaccurate results (de Queiroz 1996), while sensitivity analyses can examine the effect of excluding characters on inferences about character evolution (Donoghue and Ackerly 1996).

Exclusion of breeding system from the combined analysis resulted in one most parsimonious tree similar to the second island from the analysis based on all the characters (Figure 9B). The tree suggests that monoecy was ancestral, that dioecy has evolved twice, and that two reversals to monoecy occurred in one dioecious lineage. Exclusion of additional characters possibly linked to breeding system, such as the presence of staminodes in seed figs, setose long-styled florets, and funnelform stigmas in short-styled florets, resulted in the same topology. Exclusion of all morphological characters on the grounds that they are not independent of breeding system (e.g. Herre et al. 1996) also results in two gains of dioecy and two losses. Therefore, the inclusion or exclusion of morphological characters does not have a major impact on inferences about breeding system evolution, although whether dioecy evolved once or twice is unclear from the total evidence analysis. These issues are explored further in Chapter 3, using comparative methods (Harvey and Pagel 1991) to examine correlations between breeding systems and the evolution of pollinator traits.

An additional theoretical issue concerns the use of functional characters in taxonomy and phylogeny reconstruction. It has been suggested that coadaptation and convergence in functional traits might bias our conclusions about the extent of cospeciation in the interacting lineages (Wiebes 1994c, Herre et al. 1996). Examples of such traits include: (1) the shape of the ostiole in figs and pollinator head shape (van

Noort and Compton 1996); (2) the distribution of staminate florets in figs and behaviors or structures associated with pollen collection (Ramirez 1978); and (3) fig breeding system and pollinator ovipositor lengths (Ramirez 1980); Chapter 3). However, it can be argued that most characters, whether morphological or molecular, exhibit homoplasy and this is not sufficient for their exclusion from phylogenetic analysis (Donoghue and Sanderson 1992). Indeed, ITS sequences and morphology, for example, showed similar levels of homoplasy in Ficus (CI = 0.54 and CI = 0.46, respectively). Sensitivity analyses also showed that, at least in the case of breeding systems, excluding the character of interest did not substantially alter the overall results (de Queiroz 1996, Donoghue and Ackerly 1996). It can be argued that the same is true of functional traits in interacting lineages.

Although I tried to include all monoecious species in subg. Ficus (e.g. F. microdictya), it was not possible to obtain recent collections of monoecious F. pritchardii Seem., endemic to the Fiji Islands in the western Pacific. Corner (1970) transferred this species from sect. Oreosycea to subsect. Papuasyce on the grounds that, like F. microdictya from New Guinea, it is monoecious and shares additional characters including the presence of cauliflory, the fusion of the red pistillate perianth, and white ovaries. However, overall morphology and pollinator associations (Wiebes 1963) place the species closer to F. pungens and subsect. Sycocarpus. Ficus pritchardii possibly represents a third reversal to monoecy within a dioecious lineage.

Corner (1965) delimited subgenera on the basis of breeding system, but this character appears to be more homoplasious than morphology in general. The consistency index of breeding system ranged 0.25-0.33, depending on the tree island from the

combined analysis, compared to 0.46 for morphology overall. The weight that Corner placed on breeding system led to splitting monoecious Sycomorus from dioecious Neomorphe in spite of shared features including cauliflory, buttresses, and deciduous growth. Similarly, monoecious F. microdictya was at one time classified with sect. Oreosycea (Corner 1965). However, Corner (1962b, 1970) recognized the close relationship of monoecious F. microdictya to dioecious F. itoana in New Guinea and phylogenetic analysis shows the monoecy of F. microdictya to be a reversal within a dioecious lineage. Why, then, did Corner divide the figs primarily according to breeding system? Although shifts in breeding system are widespread in flowering plants, taxonomists have often recognized genera and subgenera on the basis of breeding systems (Renner and Ricklefs 1995). Corner (1985) viewed the constraints of the pollination mutualism in dioecious figs as irreversible. Contrary to expectations based on taxonomic evidence, shifts from dioecy to monoecy may be more common than the reverse (Weiblen et al. In press).

Ficus is unique in that functional dioecy results from the interaction of genetic factors controlling floral development and the impact of pollinator larvae on seed maturation (Valdeyron and Lloyd 1979). The genetics of sex determination in Ficus are known from crossing studies in the edible fig, F. carica (Storey 1975). A pair of linked loci, each affecting the abortion of staminate florets and the distribution of style lengths, are responsible for gynodioecious morphology. A stable 1:1 ratio of progeny results when heterozygous gall figs (GgAa) are crossed with homozygous seed figs (ggaa), where G is dominant for short-styled pistillate florets while g is recessive for long-styled

florets and A is dominant for the development of staminate florets while a is recessive for the abortion of staminate florets.

The evolutionary sequence that could lead to the origin and loss of this unique sex determining mechanism is unknown (Valdeyron and Lloyd 1979). Evidence from selection models suggest that a possible pathway to dioecy involves a gynodioecious intermediate step through the evolution of male sterility (Charlesworth and Charlesworth 1978). However, functional dioecy in Ficus results from the interaction of pollinator ovipositor lengths and heterostylous florets in seed and gall figs (Ganeshaiah et al. 1995; Chapter 3). It seems plausible that changes in pollinator behavior and morphology could have specific effects on the fig breeding systems and this hypothesis can be tested in a phylogenetic framework. The extent of coevolution in style length distributions and pollinator ovipositor lengths is examined in Chapter.

Congruence with pollinator classification

Congruence among fig and pollinator classifications has provided a basis for much speculation on the extent of coevolution between Ficus and the Agaonidae (Ramirez 1974, Corner 1985, Wiebes 1987, Compton 1996). The close correspondence of fig and pollinator taxonomy could be interpreted as direct evidence for cospeciation of the interacting lineages but congruence could also be a taxonomic artifact. Artificial agreement could arise if information from one group contributed to the classification of the other. Corner classified most of Ficus without knowledge of the pollinators (but see pp. 395-396 in Corner 1962c). On the other hand, Wiebes (1994a) admitted the influence of the botanical classification in his concepts of pollinator species and genera.

Phylogenetic relationships provide valuable information for identifying instances of conflict and congruence in the taxonomy of the associated lineages (Wiebes 1987). Phylogenetic information can evaluate whether particular instances reflect evolutionary events or taxonomic artifacts.

Agreement between fig and pollinator classifications is generally supported by phylogenetic analyses based on ITS sequences and fig morphology (Figure 10). Remarkably, there was no homoplasy in the associations of pollinator genera (CI = 1.00). Seven out of twelve pollinating genera were each associated with well-supported clades of host figs (>50% bootstrap; Figure 7). Five of these clades represent taxonomic groups including: (A) Blastophaga-pollinated sect. Ficus, (B) Tetrapus-pollinated sect. Pharmacosycea, (C) Lipporhopalum-pollinated subsect. Paleomorphe, (D) Pleistodontes-pollinated sect. Malvanthera, and (E) Platyscapa-pollinated sect. Urostigma. In addition, a clade including the distichous-leaved climbers of sects. Kalosyce and Rhizocladus is pollinated by Wiebesia. One of the major clades of dioecious figs is pollinated by Ceratosolen; corresponding subg. Sycomorus sensu Ramirez (1977). However, some genera were associated with paraphyletic groupings of Ficus. Paraphyletic sect. Oreosycea, for example, is pollinated by Dolichoris. Also, Kradibia-pollinated subsect. Sycidium is paraphyletic to Lipporhopalum-pollinated subsect. Paleomorphe.

Specific cases of conflict between taxonomy and phylogeny are also worthy of consideration. The Ceratosolen-pollinated clade, in particular, does not agree with the classification of Corner (1965). We find that in one case the conflict between fig and pollinator taxonomy is reconciled upon consideration of phylogenetic relationships. Corner (1960a) related F. pungens to Kradibia-pollinated subsect. Sycidium while

Wiebes (1963b) placed the pollinator of F. pungens under Ceratosolen. However, morphology and ITS sequences strongly support the placement of F. pungens in a Ceratosolen-pollinated clade. The source of Corner's error becomes apparent upon consideration of the early taxonomic history of F. pungens. Miquel (1967) first placed the species in subg. Covellia (i.e. in the Ceratosolen-pollinated clade). Later, Corner (1960a) argued that King (1888) had mistaken a new species of sect. Sycocarpus as F. pungens and he went on to describe this Mollucan endemic as F. calcarata (1960b). However, it is apparent from the earlier descriptions and illustrations that F. calcarata is none other than F. pungens from the Mollucas (King 1888).

Corner (1960) excluded F. pungens and a number of species from sect. Sycocarpus (Covellia), placing them in subsect. Sycidium on the sole basis of having free tepals and in spite of their cauliflorous habit. He designated ser. Pungentes to include F. pungens and F. minahassae; sister species that can be difficult to separate when sterile. The two differ in the position of the cauliflorous syconia; tightly fascicled in F. minahassae and more or less scattered along the leafless branchlets in F. pungens. They are geographically isolated in eastern and western Malesia, and together with their pollinators, C. pygmaeus and C. nanus, could represent an instance of allopatric cospeciation (Chapter 3). In light of the evidence from fig morphology, ITS sequences, and pollinator relationships, it is now indisputable that F. pungens belongs to a Ceratosolen-pollinated clade. These conclusions also draw attention to ser. Prostratae and ser. Phaeopilosae, which Corner (1960a) removed from Covellia and placed in sect. Sycidium based on few characters. At least in the case of F. pungens, it appears as though the insects are better practitioners of taxonomy than Corner thought.

Another interesting case concerns the placement of F. semivestita. Corner (1960b) described the species under sect. Neomorphe based on leaves, gall figs and growth form. In spite of similarity in buttresses, girth and height in the canopy, F. semivestita is the only member of sect. Neomorphe with axillary figs. Also, Corner's original description was incomplete because seed figs were unavailable at the time. Recent collections of seed figs (Appendix 1) provide additional characters that suggest the placement of F. semivestita in sect. Adenosperma, including the presence of a gynobasic style and auriculiform seeds with a double ridge arising from the hilum. "Terminalia" branching, axillary figs, and the presence of lateral bracts on figs also support a closer relationship to Adenosperma than to Neomorphe, and the distribution of F. semivestita in New Guinea is consistent with endemic sect. Adenosperma.

Wiebes (1963) suggested a close relationship between Ceratosolen grandii, the pollinator of F. semivestita, and C. appendiculatus, the pollinator of F. variegata (sect. Neomorphe). This affinity, however, is based on two homoplasious morphological characters (i.e. the absence of cerci in the male genitalia, and the fusion of three apical segments of the antennae). Phylogenetic relationships inferred from mitochondrial genes indicate that C. grandii is more closely related to pollinators of sect. Adenosperma than to C. appendiculatus (Chapter 2). The results once again suggest that traditional classification of dioecious figs could be improved based on by phylogenetic analyses and pollinator relationships.

Disagreement between pollinator relationships and the classification of Corner (1965) also involves the placement of F. pseudopalma and F. rivulares in Blastophaga-pollinated subsect. Ficus. Corner (1960a) recognized a monotypic ser. Pseudopalmae,

based on the common occurrence of bistaminate flowers in F. pseudopalma and in subsect. Ficus, although Ceratosolen bakeri was later shown to be the pollinator of F. pseudopalma (Wiebes 1963). Corner (1960a) also designated monotypic ser. Rivulares for F. rivularis; later noting that bistaminate flowers also occur in sect. Sycocarpus, along with other shared features (Corner 1967). Ramirez (1977) noted similarities between F. rivularis and sect. Sycocarpus, including the partial fusion of the perianth, the auriculiform seed, and the gynobasic style in seed figs. Confirming the incorrect placement of F. rivularis in subsect. Ficus, Ramirez (1977) correctly predicted that the pollinator would be a species of Ceratosolen (Wiebes 1991b).

Wiebes (1981) noted close similarities within a group of Ceratosolen species that included the pollinator of F. pseudopalma. He went on to recognize this group as subg. Strepitus (Wiebes 1994a). The results of phylogenetic analyses including F. theophrastoides, F. dammaropsis, F. itoana and F. microdictya, although not well resolved, are congruent with a Strepitus-pollinated clade (Figure 10). All but two species of Strepitus are associated with figs in sect. Sycocarpus (subsects. Auriculisperma, Theophrastoides, Dammaropsis and Papuasyce). The remaining pollinators are associated with F. pseudopalma and F. rivularis. Ramirez (1977) reconciled the conflict by grouping all Ceratosolen-pollinated figs including F. pseudopalma and F. rivularis under subg. Sycomorus. Morphological evidence suggests that F. pseudopalma and F. theophrastoides may be sister taxa (Corner 1967, Wiebes 1981). It is interesting to note that F. pseudopalma and F. rivulares are restricted to the Philippines while their nearest relatives occur in New Guinea and especially the Solomon Islands. The close similarities

and geographic isolation of these species and their pollinators are also suggestive of allopatric cospeciation (cf. F. pungens and F. minahassae).

The overall congruence between fig and pollinator classifications is striking (Figure 10), although fig phylogeny alone cannot distinguish between taxonomic artefacts and coevolutionary processes as explanations for congruent patterns. Reciprocal phylogenetic studies are needed to address this point, given that the classification of pollinators was not independent of fig taxonomy (Wiebes 1994a) and the revised classification of Ficus was based on pollinator taxonomy (Ramirez 1977). Chapter 2 presents a phylogenetic analysis of the pollinators, while phylogenetic patterns of fig and pollinator associations are examined in Chapter 3.

Table 1: The classification and distribution of Ficus L. according to Berg (1989). The arrangement of sections within subgenera is alphabetical.

subgenus	section	spp.	distribution
Ficus	Adenosperma Corner	23	Malesia
	Ficus	60	Malesia, Asia and Africa
	Kalosyce (Miq.) Corner	20	Malesia and Asia
	Neomorphe King	6	Malesia and Asia
	Rhizocladus Endl.	55	Malesia and Asia
	Sinosycidium Corner	1	Asia
	Sycocarpus Miq.	75	Malesia and Asia
	Sycidium Miq.	105	Malesia, Asia and Africa
Pharmacosycea Miq.	Oreosycea (Miq.) Corner	50	Malesia and Africa
	Pharmacosycea	20	America
Sycomorus (Gasp.) Miq.	Sycomorus	13	Africa and Malesia
Urostigma (Gasp.) Miq.	Americana	120	America
	Conosycea (Miq.) Corner	65	Malesia, Asia and Africa
	Galoglychia (Gasp.) Endl.	75	Africa
	Leucogyne Corner	2	Asia
	Malvanthera Corner	20	Malesia
	Stilpnophyllum Endl.	1	Asia
	Urostigma	20	Malesia, Asia and Africa

Table 2: Ficus species selected for phylogenetic analysis and their pollinating Agaoninae.

subg.	section	Ficus species	pollinating Agaoninae
Ficus	Adenosperma	adenosperma Miq.	Ceratosolen (C.) adenospermae
		ochrochlora Ridley	Ceratosolen (C.) sp. "riparianus"
	Ficus	grossularioides Burm. f.	Blastophaga (V.) malayana
		padana Burm. f.	Blastophaga (V.) intermedia
	Kalosyce	punctata Thunb.	Wiebesia punctatae
		ruginerva Corner	Wiebesia sp.
	Neomorphe	auriculata Lour.	Ceratosolen (C.) emarginatus
		nodosa Teysm. et Binn.	Ceratosolen (C.) nexilis
		robusta Corner	Ceratosolen (C.) cf. nexilis
		semivestita Corner	Ceratosolen (C.) grandii
		variegata Bl.	Ceratosolen (C.) appendiculatus
	Rhizocladus	baeuerlenii King	Wiebesia sp. "brusi"
		odoardi King	Wiebesia sp. "frustrata"
	Sycidium	conocephalifolia Ridley	Kradibia jacobsi
		copiosa Steud.	Kradibia copiosae
		phaeosyce Laut.	Kradibia sp. "salembensis"
		pungens Reinw. ex Bl.	Ceratosolen (C.) nanus
		tinctoria Forst. f.	Liporrhopalum cf. gibbosae
		trachypison K. Schum.	Kradibia sp. "ohuensis"
		wassa Roxb.	Kradibia wassae
		virgata Reinw. ex Bl.	Liporrhopalum virgatae
	Sycocarpus	bernaysii King	Ceratosolen (R.) hooglandi
		botryocarpa Miq.	Ceratosolen (R.) corneri
		dammaropsis Diels	Ceratosolen (S.) abnormis
		hispidioides S. Moore	Ceratosolen (R.) dentifer
		itoana Diels	Ceratosolen (S.) armipes
		microdictya Diels	Ceratosolen (S.) sp. "kaironkensis"
		septica Burm f.	Ceratosolen (C.) bisulcatus
		theophrastoides Seem.	Ceratosolen (S.) vissali
Pharmacosycea	Oreosycea	albipila (Miq.) King	Dolichoris sp.
		edelfeltii King	Dolichoris inornata
		hombroniana Corner	Dolichoris sp. "hombronianae"
	Pharmacosycea	insipida Willd.	Tetrapus costaricanus
		maxima P. Mill.	Tetrapus americanus
Sycomorus	Sycomorus	botryoides Baker	Ceratosolen (C.) blommersi
		racemosa L.	Ceratosolen (C.) fusciceps
		sur Forssk.	Ceratosolen (C.) capensis
Urostigma	Americana	pertusa L.	Pegoscapus silvestrii
	Conosycea	microcarpa L.	Eupristina (P.) verticillata
		pellucido-punctata Griff.	Waterstoniella brevigena
	Malvanthera	destruens C.T. White	Pleistodontes rigisamos
		hesperidiiformis King	Pleistodontes plebejus
		xylosycia Diels	Pleistodontes rieki
	Urostigma	prasinicarpa Elm.	Platyscapa ficheri
		superba Miq.	Platyscapa corneri
		virens Ait.	Platyscapa coronata

Table 3: Templeton test results for incongruence between morphological and ITS data sets. For each data set, the tree length (L) resulting from rival constraint searches was compared to the length of shortest trees resulting from unconstrained searches. The largest sum of the signed rank differences for (N) characters was used to compute the non-parametric test statistic (z).

ITS data and tree vs.	L	rank sum	N	z	p
morphology MP tree	567	115.5	96	-8.6348	<0.0001
morphology strict consensus	555	123.0	94	-8.6687	<0.0001
morphology 50% bootstrap	464	77.5	23	-2.0298	0.0424
morphology 70% bootstrap	453	1.5	2	0.0000	1.0000
morphology 90% bootstrap	453	0	2	-	-
combined consensus (Figure 7)	463	80.5	23	-1.9612	0.0499

Morphology and tree vs.	L	rank sum	N	z	p
ITS MP tree	385	77.0	38	-4.3727	<0.0001
ITS strict consensus	372	125.0	37	-3.5736	0.0004
ITS 50% bootstrap	362	107.0	31	-2.8950	0.0038
ITS 70% bootstrap	353	106.5	26	-1.8557	0.0635
ITS 90% bootstrap	349	120.0	26	-1.5410	0.1233
combined consensus (Figure 7)	349	110.0	25	-1.5230	0.1278

Figure 1: Illustrations of fig characters and states. (1) hollow twig in cross section; (2) twig with a waxy gland below each node; (3) leaf gland at the base of the midrib; (4) leaf glands paired in the axils of the basal veins; (5) solitary leaf gland in the axil of a basal vein; (6) leaf glands in the axils of secondary veins; (7) midrib; (8) basal vein; (9) secondary vein; (10) tertiary veins; (11) fine venation; (12) sessile syconium; (13) peduncle with bracts at the bottom; (14) peduncle with median bracts; (15) peduncle with bracts at the apex; (16) syconium basal bracts; (17) lateral bracts; (18) apical bracts; (19) ostiolar bracts; (20) monoecious fig with staminate florets dispersed; (21) seed fig with long-styled pistillate florets; (22) gall fig with short-styled pistillate florets and staminate florets around the ostiole; (23) pistillate floret with free tepals; (24) pistillate floret with tepals fused at the base; (25) pistillate floret with tepals fused and enclosing the ovary; (26) pistillate floret without tepals; (27) glabrous and entire perianth; (28) abaxial pubescence on perianth; (29) ciliate perianth margin; (30) dentate perianth margin; (31) glabrous and simple style; (32) setose style; (33) divided style; (34) funnelform style; (35) lenticular achene with a single ridge; (36) compressed achene; (37) tuberculate achene; (38) achene with two ridges arising from the hilum; (39) unistaminate floret; (40) bistaminate floret; (41) staminate floret with a pistillode; (42) staminate floret with a functional gall ovary; (43) filament with epidermal hairs at the base; (44) mucronate anther. Illustrations are redrawn from Corner (1967).

Figure 2: The geographical distribution of Ficus species richness. **(A)** Numbers of Ficus species (and the percentage of dioecious species) in the three tropical regions (Berg 1989). Species richness of dioecious figs is highest in Malesia when compared to Africa

and Americ B) Ficus species (and the percentage of dioecious species) in India, China, Peninsular Malaysia, the Philippine Islands, Borneo, Java, Sumatra, New Guinea and Australia according to Corner (1958). Overall species richness in Malesia is highest in the islands of New Guinea and Borneo; also the main centers of diversity and endemism for dioecious figs.

Figure 3: The strict consensus of 218 ITS trees with two additional clades from the 50% bootstrap consensus. Bootstrap percentages and decay values are listed above and below the branches, respectively. Closed circles indicate those nodes that are congruent with the morphology strict consensus (Figure 5). Open circles indicate conflicting nodes. Ficus sections and subgenera are shown in brackets. Open and closed bars mark monoecious and dioecious species, respectively.

Figure 4: ITS phylogram selected from one of 208 most parsimonious trees. Branch lengths are proportional to nucleotide substitutions supporting each node (also indicated above the branches). Gains and losses of indels reconstructed under parsimony (ACCTRAN) are mapped on the tree. Closed and open boxes indicate gains and losses, respectively. Ficus sections and subgenera are shown in brackets.

Figure 5: The strict consensus of 6 trees resulting from the morphological analysis modified to include one additional clade from the bootstrap consensus. Bootstrap percentages and decay values are listed above and below the branches, respectively. Closed circles indicate those nodes that are congruent with the ITS strict consensus

(Figure 3). Open circles indicate conflicting nodes. Ficus sections and subgenera are shown in brackets. Open and closed bars mark monoecious and dioecious species, respectively.

Figure 6: Null distributions for the incongruence length difference test (ILD; Farris et. al. 1994) for (**A**) ITS and morphological data sets, and (**B**) ITS nucleotide substitutions and indels. Arrows indicate the sum of the tree lengths for the data partitions. Null distributions represent the sum of tree lengths from random partitions of the combined data where the size of each partition is equal to the number of characters in the designated data partitions. ITS and morphological data significantly rejected the null hypothesis of congruence (P = 0.01), whereas ITS nucleotide substitutions and indels did not (P = 0.93).

Figure 7: The strict consensus of eight most parsimonious trees recovered in the combined ITS and morphological analysis with two additional clades recovered in the 50% bootstrap consensus (see Results). A third clade with 63% support (indicated parenthetically) was present in seven of the eight most parsimonious trees. Bootstrap percentages and decay values are listed above and below the branches, respectively. Ficus sections and subgenera are shown in brackets. Open and closed bars mark monoecious and dioecious species, respectively.

Figure 8: One of eight most parsimonious trees recovered in the combined analysis of ITS and morphology, selected at random for reconstruction of morphological

apomorphies. Ficus sections and subgenera are shown in brackets. Open and closed bars mark monoecious and dioecious species, respectively.

Figure 9: Alternative reconstructions of fig breeding system under parsimony. (**A**) One origin of dioecy from monoecy and two reversals to monoecy within the dioecious clade. (**B**) Two independent origins of dioecy and two reversals to monoecy within one of the major dioecious lineages. Only monoecious taxa are labeled for the sake of simplicity.

Figure 10: The associations of pollinating fig wasp genera mapped on one of the most parsimonious trees from the combined analysis of fig morphology and ITS sequences. Ficus sections and subgenera are shown in brackets.

Figure 1

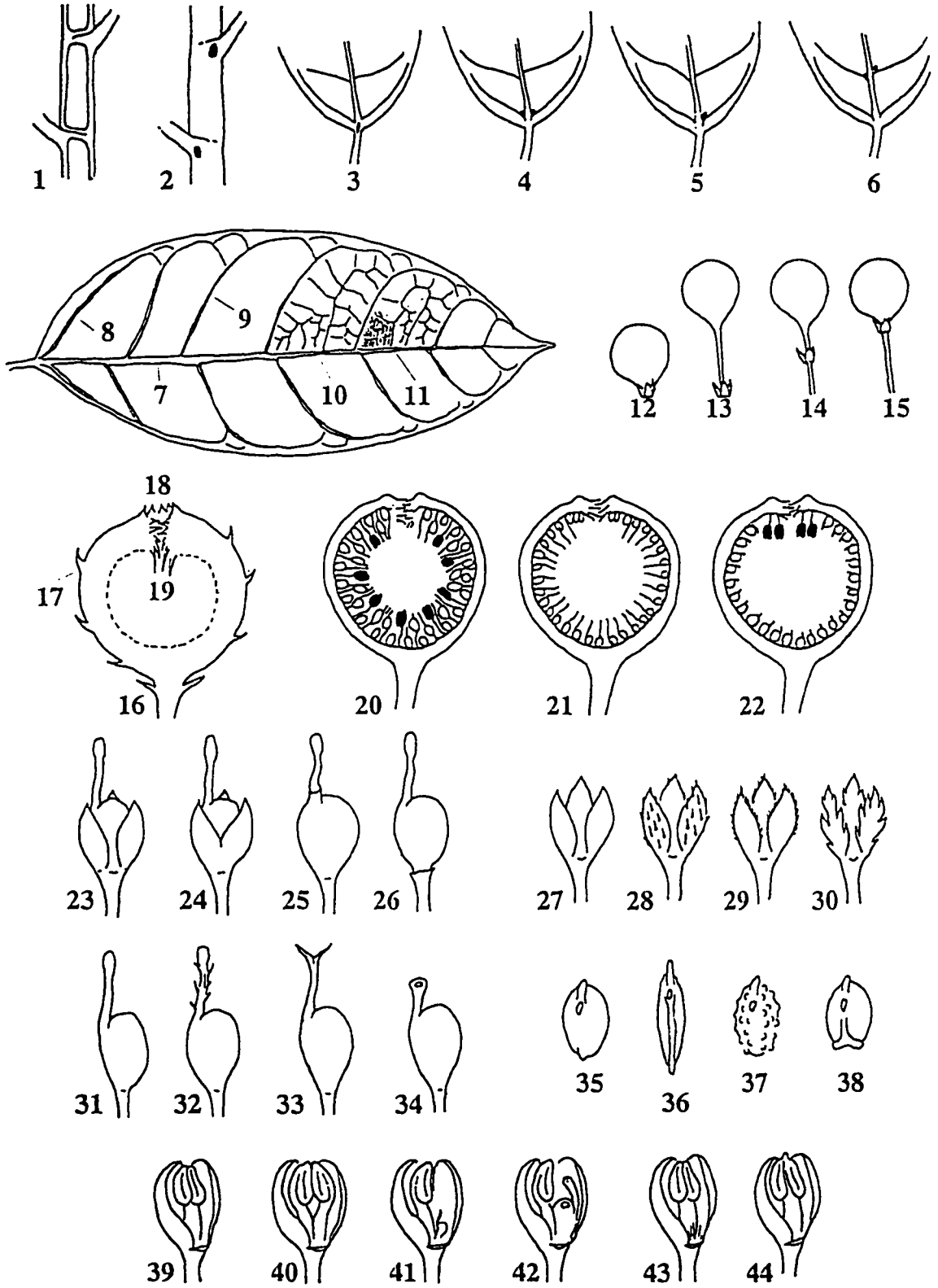
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Figure 2A

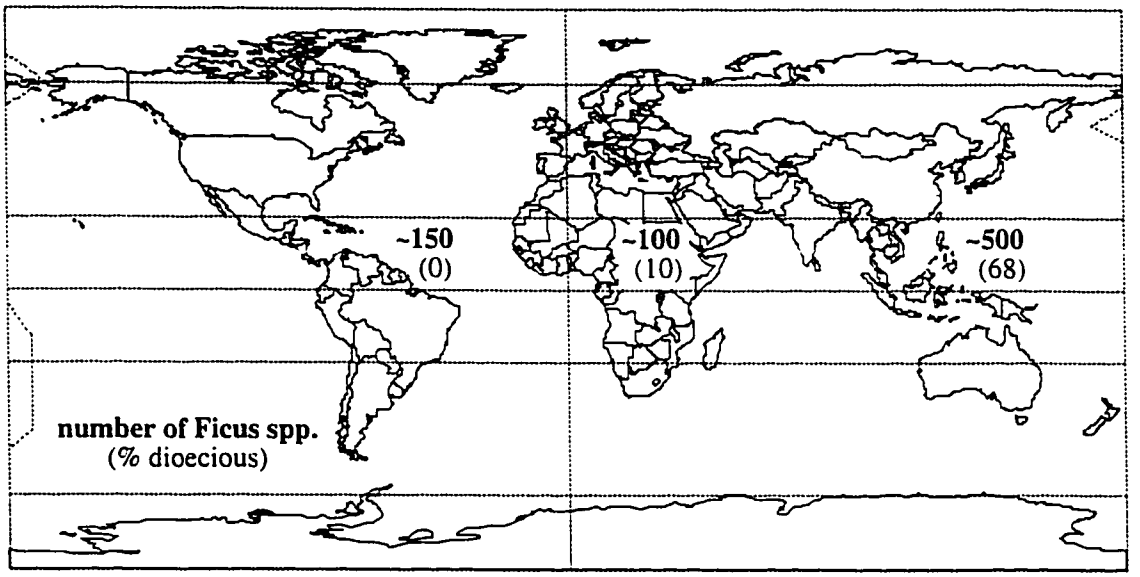


Figure 2B

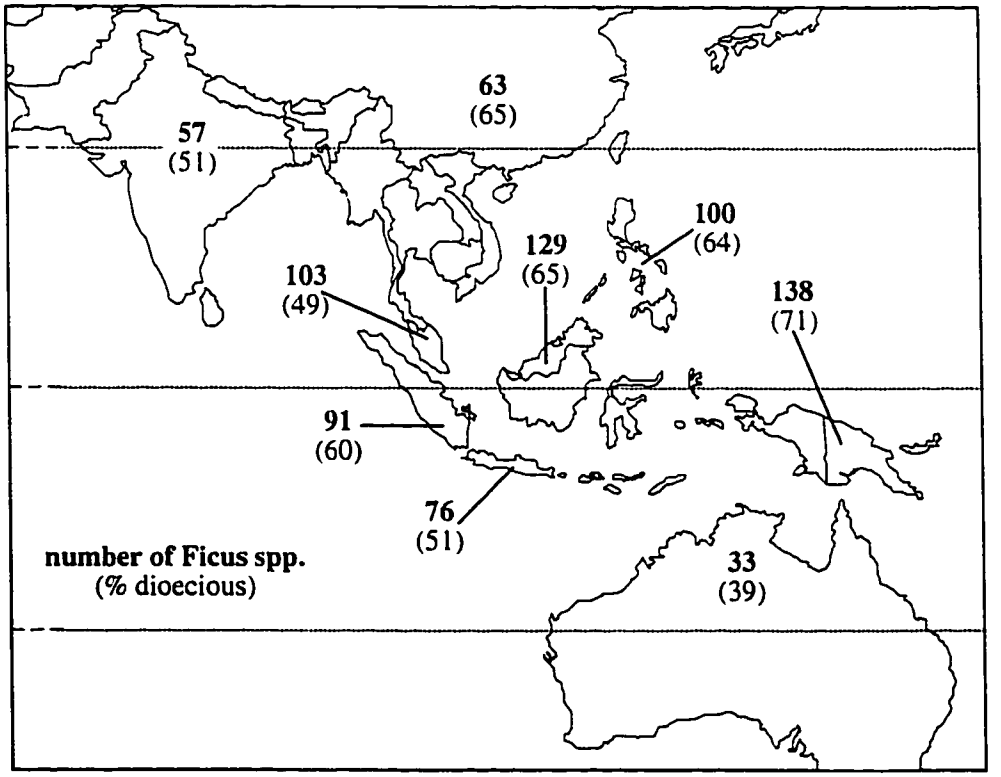
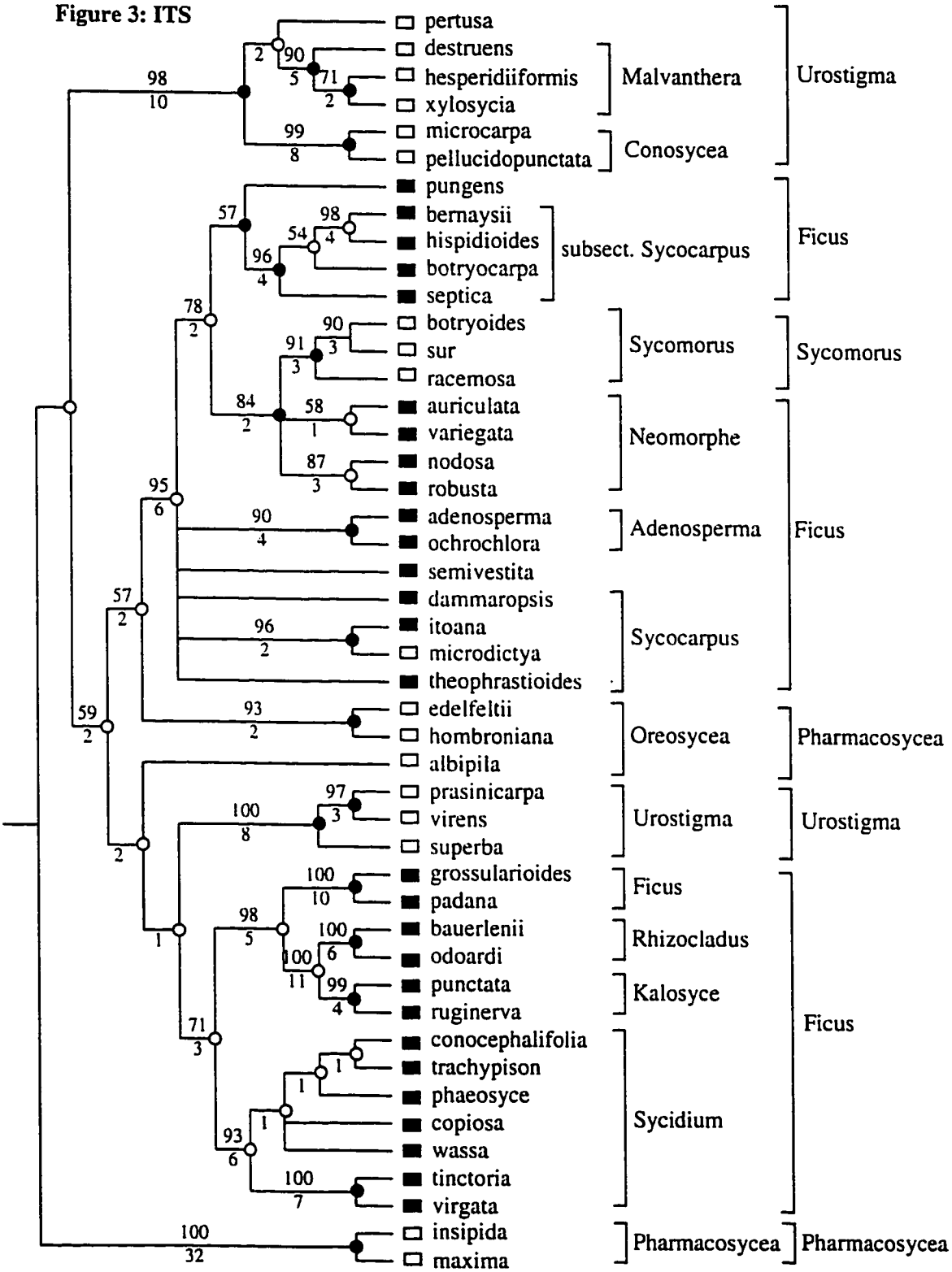


Figure 3: ITS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racemosa
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robusta
adenosperma
ochrochlora
semivestita
dammaropsis
itoana
microdictya
theophrastioides
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prasinicarpa
virens
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grossularioides
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odoardi
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ruginerva
conocephalifolia
trachypison
phaeosyce
copiosa
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tinctoria
virgata
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Conosycea
subsect. Sycocarpus
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Neomorphe
Adenosperma
Sycocarpus
Oreosycea
Urostigma
Ficus
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Figure 4: ITS phylogram

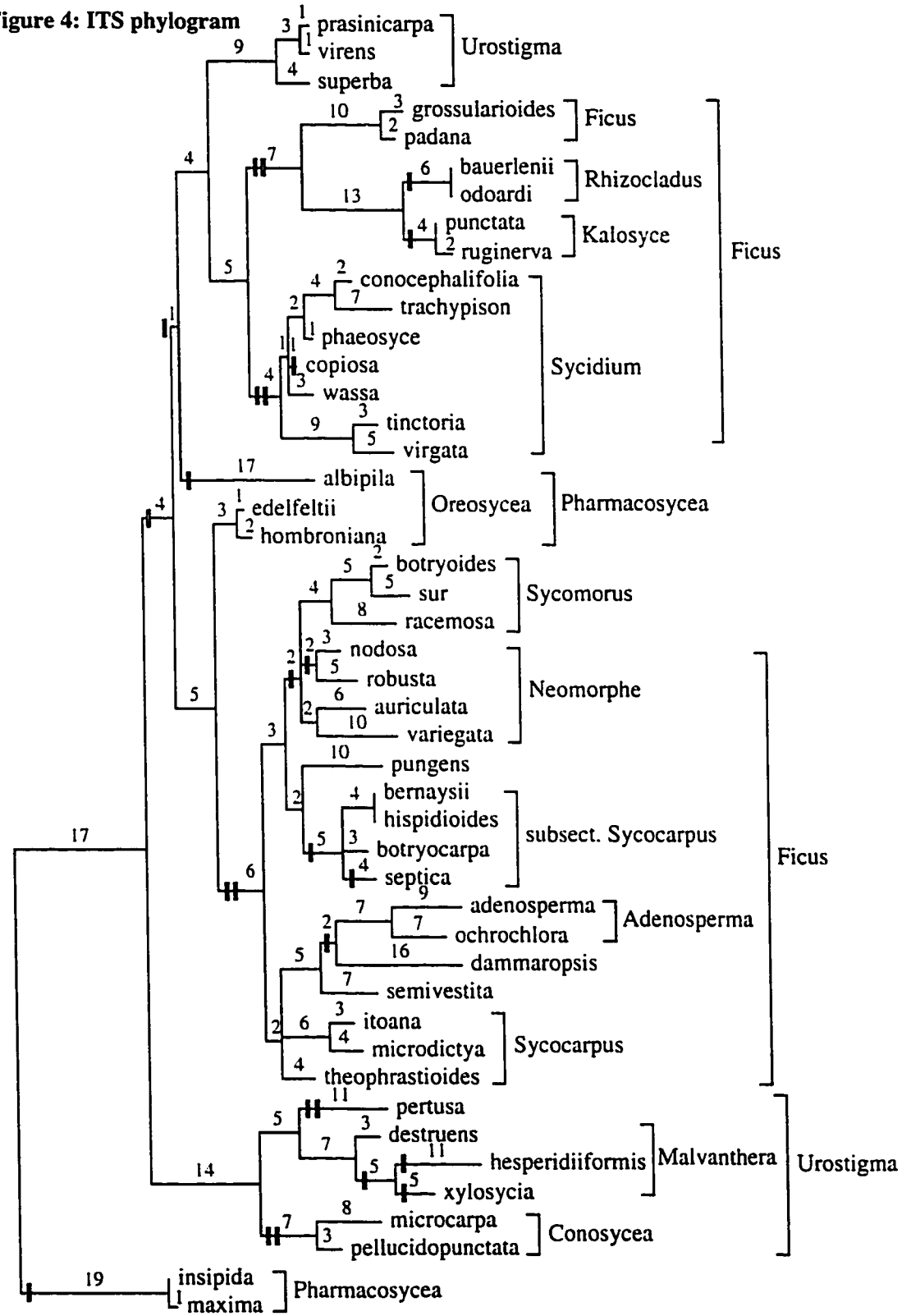


Figure 5: morphology

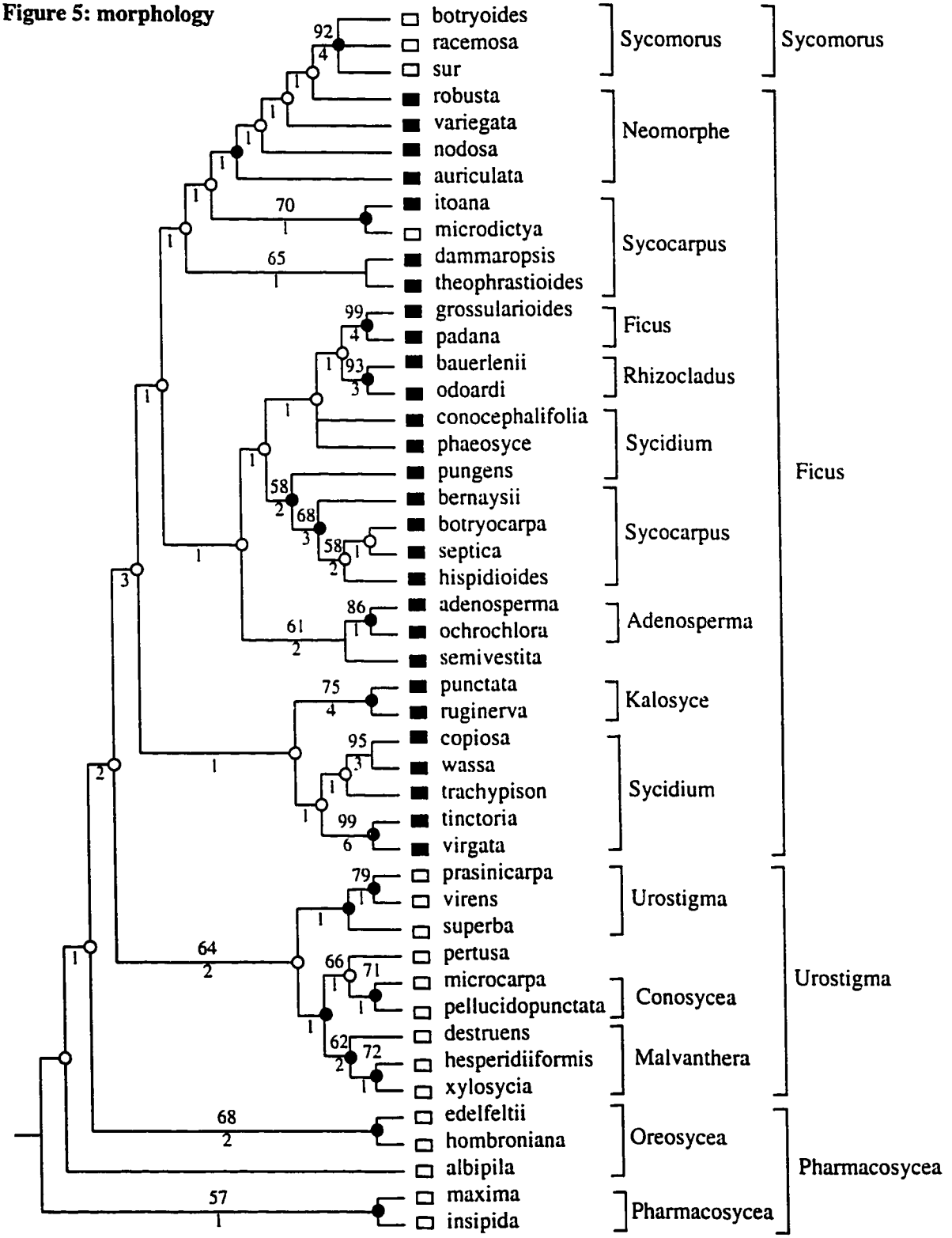


Figure 6A: ITS vs. morphology

P = 0.01

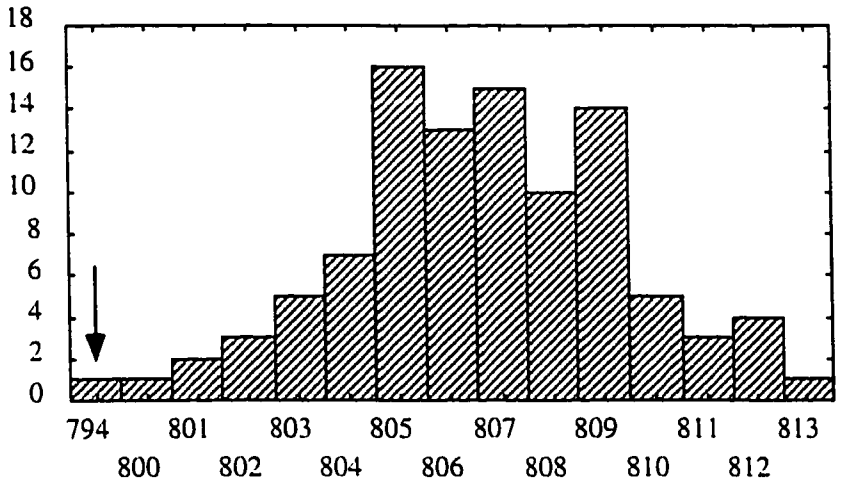


Figure 6B: ITS substitutions vs. indels

P = 0.93

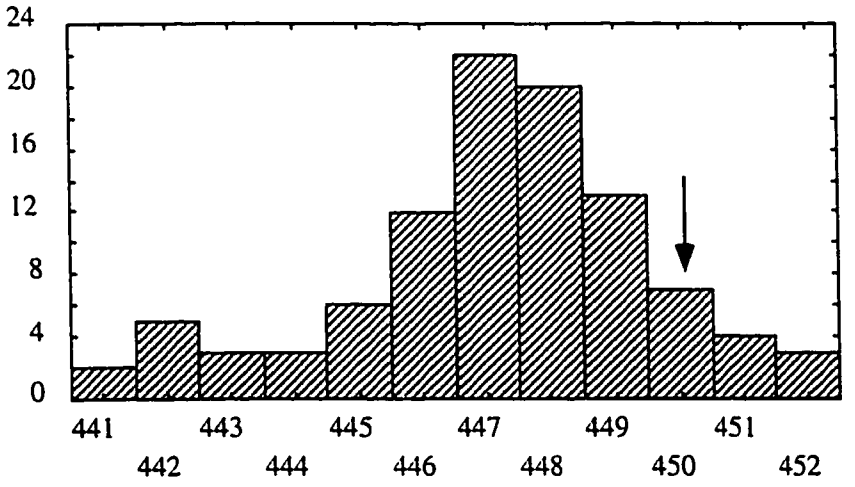


Figure 7: combined

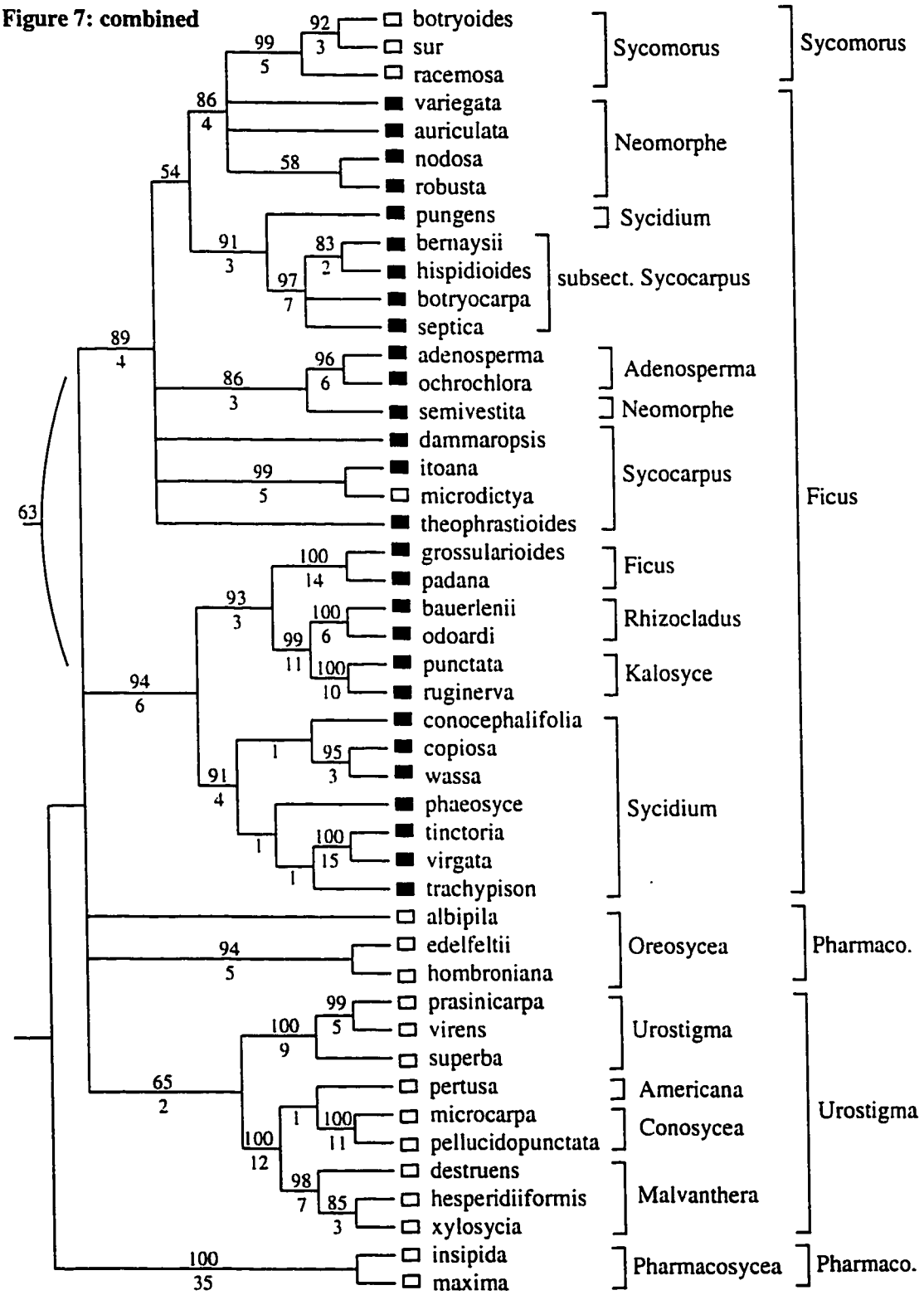
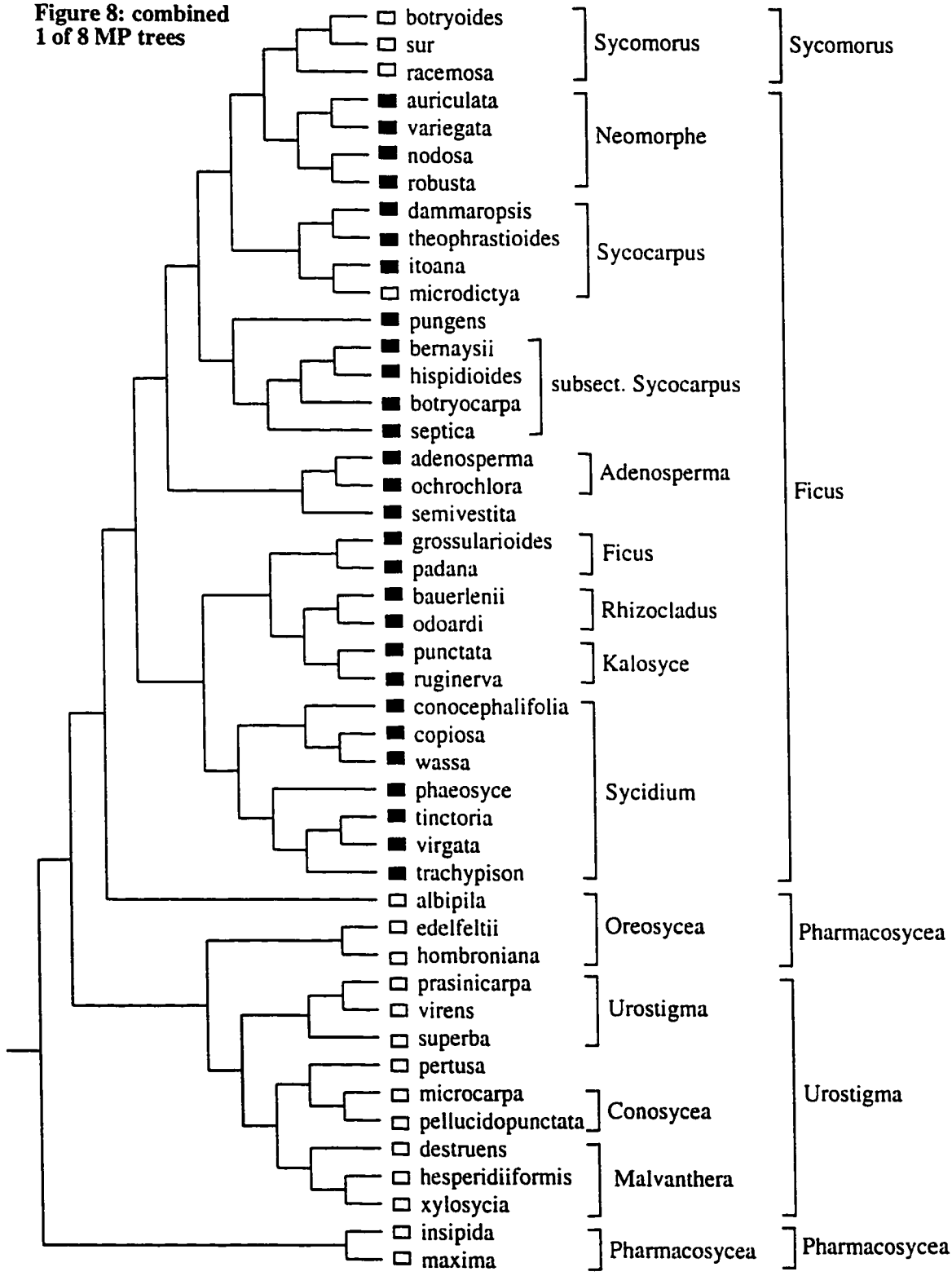


Figure 8: combined 1 of 8 MP trees



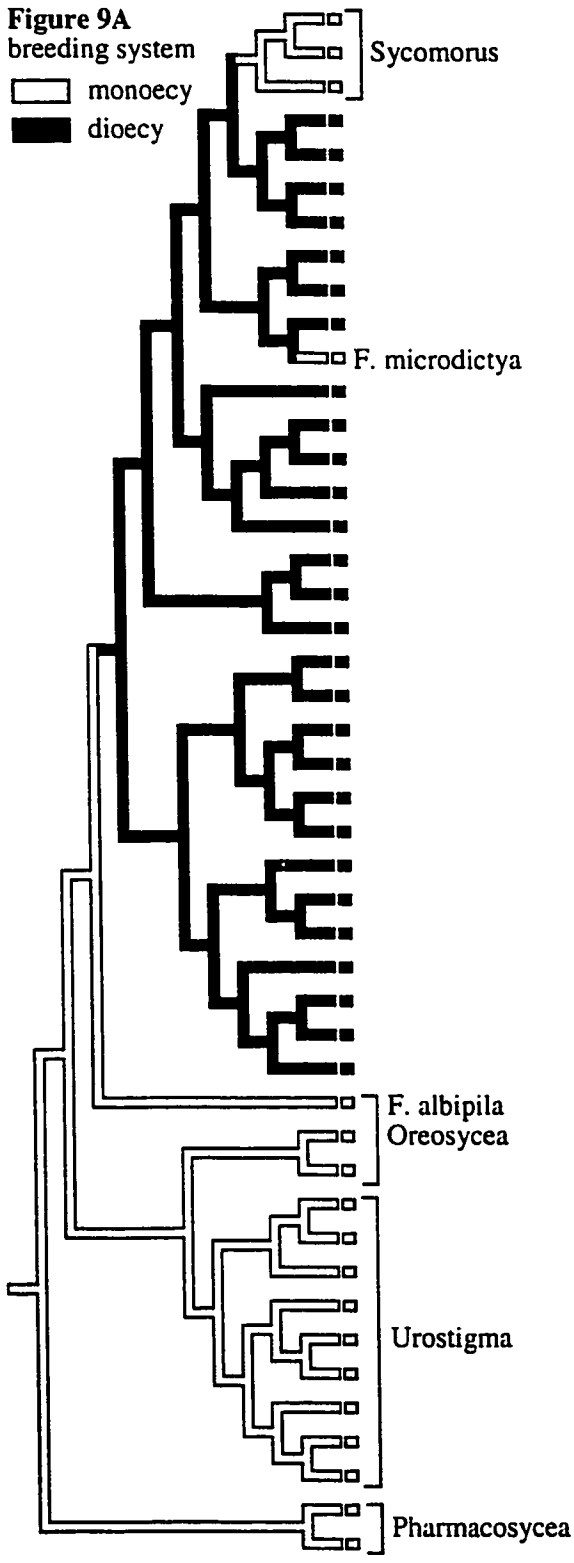


Figure 9A
breeding system
monoecy
dioecy
Sycomorus
F. microdictya
F. albipila
Oreosycea
Urostigma
Pharmacosycea

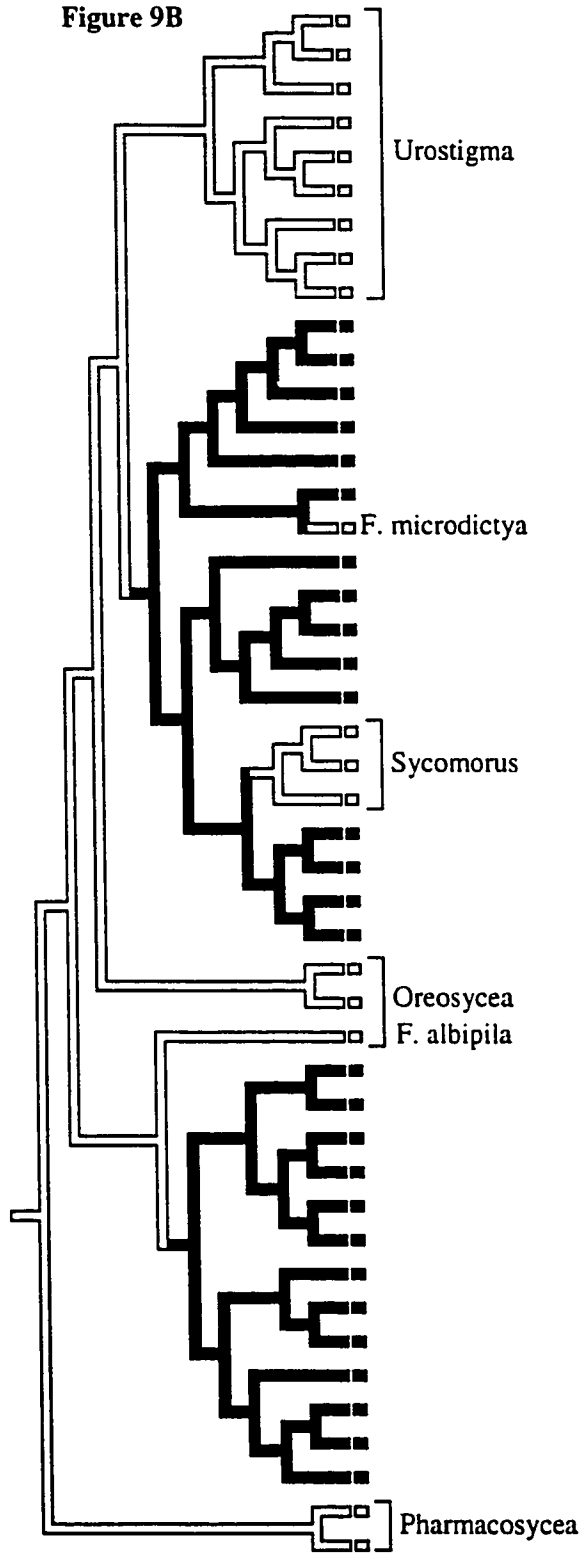
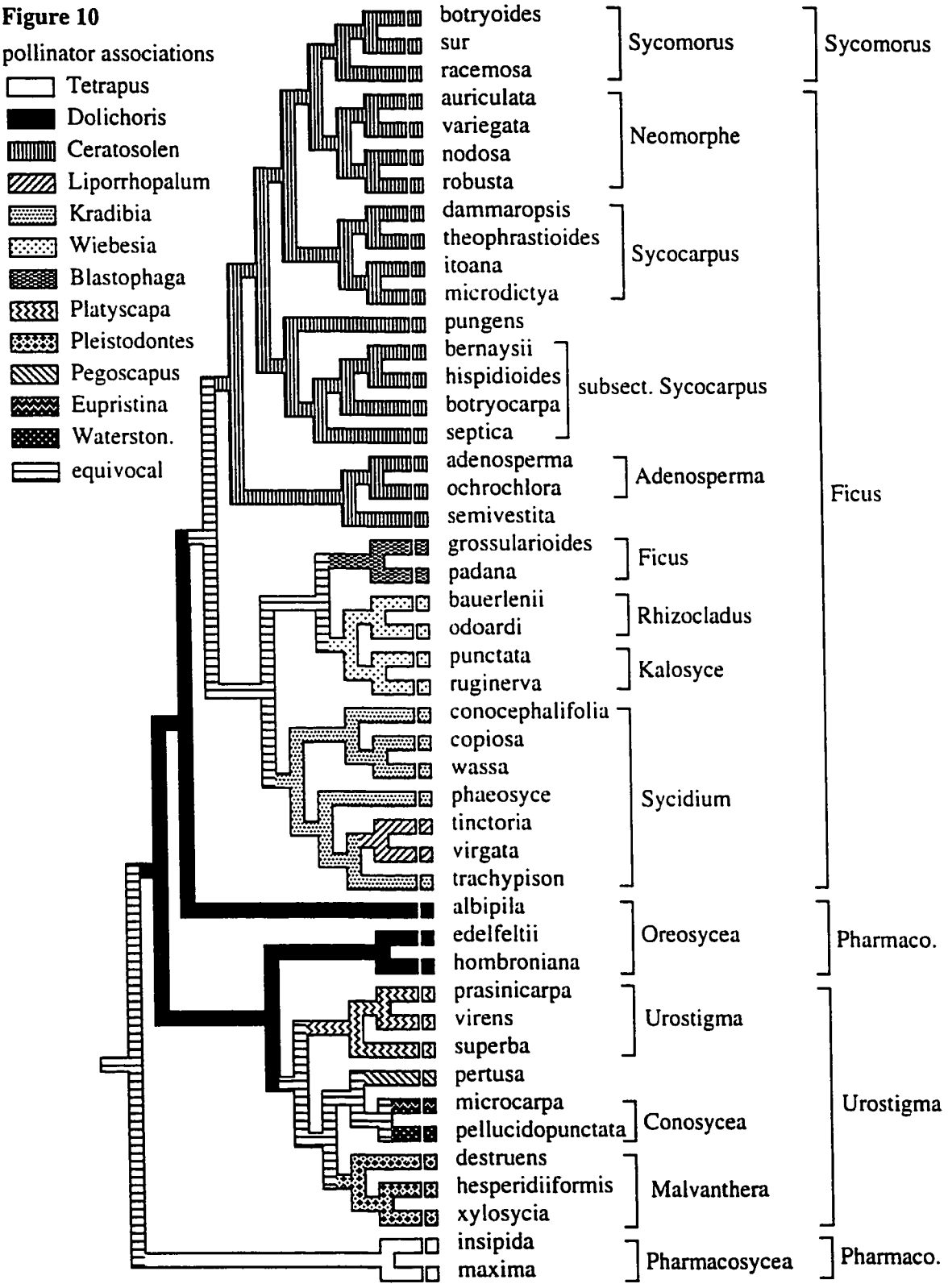


Figure 9B
Urostigma
F. microdictya
Sycomorus
Oreosycea
F. albipila
Pharmacosycea

Figure 10


pollinator associations
Tetrapus
Dolichoris
Ceratosolen
Liporrhopalum
Kradibia
Wiebesia
Blastophaga
Platyscapa
Pleistodontes
Pegoscapus
Eupristina
Waterston.
equivocal
botryoides
sur
racemosa
Sycomorus
auriculata
variegata
nodosa
robusta
Neomorphe
dammaropsis
theophrastioides
itoana
microdictya
Sycocarpus
pungens
bernaysii
hispidioides
botryocarpa
septica
subsect. Sycocarpus
adenosperma
ochrochlora
semivestita
Adenosperma
grossularioides
padana
Ficus
bauerlenii
odoardi
Rhizocladus
punctata
ruginerva
Kalosyce
conocephalifolia
copiosa
wassa
phaeosyce
tinctoria
virgata
trachypison
Sycidium
albipila
edelfeltii
hombroniana
Oreosycea
prasinicarpa
virens
superba
Urostigma
pertusa
microcarpa
pellucidopunctata
Conosycea
destruens
hesperidiiformis
xylosycia
Malvanthera
insipida
maxima
Pharmacosycea
Sycomorus
Ficus
Pharmaco.
Urostigma
Pharmaco.

CHAPTER 2

Phylogenetic relationships of dioecious fig pollinators based on mitochondrial DNA sequences and morphology

"...it is clear that the wasps have classified the figs better than the botanists."

E. J. H.(Corner 1955), p. 430

Introduction

The family Agaonidae (Hymenoptera: Chalcidoidea) includes several hundred parasitic wasp species that are closely associated with the inflorescence or syconium of Ficus (Boucek 1988). All fig wasps are confined to syconia as larvae and their specialized diets are restricted to fig embryos, galled ovaries or other fig wasp larvae (Boucek 1988). The life histories of fig wasps show substantial variation in modes of oviposition and in the outcomes of their interactions with hosts, whether mutualistic or antagonistic (Chapter 4). Recent molecular phylogenetic studies suggest that fig pollination evolved once and characterizes the subfamily Agaoninae (Machado 1998).

Pollinating fig wasps are obligate mutualists with peculiar morphological adaptations, extreme host specificity, and life cycles that are tightly synchronized with fig reproductive phenology (Wiebes 1979a). As outlined in the previous chapter, the fig/pollinator mutualism has provided a basis for much speculation on the nature and extent of coevolution involved. The diversity of fig wasp assemblages is indeed an asset in evolutionary studies but the application of comparative methods to the fig/pollinator mutualism has been hindered by the lack of robust estimates of phylogeny for fig and pollinator lineages (cf. Wiebes 1982b, Ramirez 1991). However, the development of molecular phylogenies for neotropical fig pollinators (Machado et al. 1996) and fig wasps in general (Machado 1998) have recently opened the door to comparative studies (e.g. Cook et al. 1997).

The pollinators of dioecious figs are of particular interest due to apparent evolutionary conflicts with their host plants (Kjellberg et al. 1987a, Grafen and Godfray 1991, Anstett et al. 1997). Questions regarding the evolutionary stability of pollination in dioecious figs arise from the observation that pollinators show no preference for gall syconia despite the fact that pollinators of seed syconia leave no offspring (Patel et al. 1995). Another question concerns the evolution of the length of the pollinator ovipositor in relation to the length of the styles in the heterostylous florets of dioecious figs

(Ganeshaiah et al. 1995). Ramirez (1980) noted that the pollinators of dioecious figs tend to have short ovipositors compared to monoecious fig pollinators but the proposition that ovipositor lengths have coevolved with fig breeding systems has not been tested in a phylogenetic framework. This chapter presents a phylogenetic analysis of the dioecious fig pollinators and provides the framework for studies of morphological adaptation and speciation (Chapter 3).

Pollinator life cycles and morphological specialization

The overall life cycles of dioecious figs and their pollinators are illustrated by the example of Papuasian F. nodosa and its obligate pollinator, Ceratosolen nexilis (Figures 1A-1C). As described in the previous chapter, functional dioecy in F. nodosa results from how C. nexilis affects the heterostylous florets in two types of syconia on separate plants. Ceratosolen nexilis pollinates and lays eggs in the pistillate florets of gall and seed syconia. Only eggs deposited between the integument and the nucellus of fig ovules will hatch where the larvae feed on endosperm. Pollinator larvae consume the short styled florets of gall syconia in this manner (Figure 1D). In seed syconia, the florets are unharmed by egg laying because the pollinators fail to fully penetrate the long styles with their ovipositors. The timing of arrival, oviposition and pollination are closely synchronized with the timing of receptivity in pistillate florets (Chapter 4). In addition, the eclosure of the pollinators from gall syconia coincides with the release of pollen from staminate florets. Asynchronous reproductive phenology at the population level also provides emerging pollinators with a source receptive figs (Kjellberg and Maurice 1989, Spencer et al. 1996) and within-plant synchrony is a general feature of the fig life cycles.

Morphological specialization in pollinators is further associated with the functioning of the life cycle. For example, dimorphism in C. nexilis (Figures 1A-1B) reflects the functional roles of each sex. Males are apterous and have vestigial or reduced eyes, antennae and tarsi. On the other hand, females have functional wings, eyes and

antennae. Male participation in the life cycle is restricted to the cavity of the host fig while females are responsible for locating and colonizing new hosts. Male Agaoninae emerge first and chew holes in other galled ovaries where mating occurs prior to female emergence. The abdominal segments of male pollinators are telescopic and curled beneath the body, enabling the genitalia to be inserted into galls containing females. Given that the number of foundresses is few, related male offspring are in local competition for mates and there is a strong possibility of mating between siblings (Hamilton 1967). The effects of local mate competition and inbreeding favor the evolution of highly female-biased sex ratios (Herre 1985), and so, fig wasps have served as an important model system for testing predictions from sex allocation theory (Charnov 1982). Fighting between males has not been observed in Agaoninae, in contrast to some non-pollinators that possess striking adaptations for intraspecific combat (e.g. Sycoryctinae; Hamilton 1979). However, armature on the fore tibia, enlarged fore and hind femora, and retractable antennae seem to be associated with the burrowing activities of male Agaoninae. Tunneling through the ostiolar bracts or the fig wall by males provides an exit for the mated and pollen-laden females. Male fig wasps also possess unique respiratory adaptations to life in some figs that are fluid-filled during development (Compton and McLaren 1989).

Female pollinators are characterized by modifications of the head and antennae in response to the shape of ostioles (van Noort and Compton 1996) and by the evolution of mechanisms for the transport of pollen (Ramirez 1978). For example, the female head is specially flattened for entering through the ostiole. The head also bears mandibular appendages with rows of ventral lamellae or teeth that push against the ostiolar bracts during passage through to the ostiole. In most pollinator species, the antennal scapes fold back into a deep groove on the dorsal surface of the head and the third segment bears a spine that serves both as a hook for prying at the outer bracts of the ostiole and as a point of breakage for the distal segments during passage through the ostiole. Pollinators also

have evolved mechanisms for the pollen transport, including corbiculae on the fore coxae, pockets on the mesothorax, or grooves between the abdominal segments (Ramirez 1978, Boucek 1988). In general, pollinator life cycles and morphology provide a rich source of adaptive hypotheses that can be tested with phylogenetic information (Chapter 3).

Host specificity

The associations between fig and pollinator species are generally host specific (Ramirez 1970, Wiebes 1979a, Rasplus 1994, Michaloud et al. 1996). In most cases, the geographic distribution of pollinator species closely matches that of the host. Although the one-on-one specificity is suggestive of cospeciation (Ramirez 1974, Wiebes 1987), the occasional breakdown of host specificity has provided a basis for speculation on alternate modes of speciation in the fig/pollinator interaction (Michaloud et al. 1996). Rasplus (1994) outlined different scenarios in which more than one species of pollinator is associated with a particular host. The co-occurrence of pollinating and cheating species of Agaoninae in the same fig is relatively rare (e.g. pollinating Ceratosolen arabicus and non-pollinating C. galili in African F. sycomorus; Compton et al. 1991). In addition, well-documented cases of two pollinator species in sympatry are few (e.g. C. flabellatus and C. silvestrianus in F. sur; Kerdelhue et al. 1997) and might be explained by differences in habitat preference (Michaloud et al. 1986).

The most common departure from one-to-one specificity is the situation in which two pollinator species are geographically isolated across the host species range. Fifteen cases are known from the Malesian region (Rasplus 1994) and these frequently involve allopatric host subspecies or varieties (e.g. Liporrhopalum gibbosae and L. rutherfordi from F. tinctoria ssp. gibbosa and ssp. parasitica, respectively). There are five additional cases in the region in which pollinator subspecies are allopatric across the range of a single host species (e.g. C. bisulcatus ssp. bisulcatus and ssp. jucundus in the southern

and northern range of F. septica; Wiebes 1982a). Rasplus (1994) suggested that different rates of speciation resulting from different rates of dispersal across islands could account for such patterns. This hypothesis and alternatives (Michaloud et al. 1996) are explored in Chapter 3.

Cases in which different host species are associated with the same pollinator are less common. When two host species occur in sympatry and share the same pollinator, differences in host habitat preference and limited pollinator dispersal have been proposed as a means of reproductive isolation (Michaloud et al. 1986). However, artifacts of botanical classification account for the apparent breakdown of host specificity in several cases. For example, C. appendiculatus is known to pollinate widespread F. variegata and endemic F. viridicarpa in peninsular Malaysia (Rasplus 1994). However, F. viridicarpa differs from F. variegata only in the color of the ripe syconium and the two are just one species (G. Weiblen, pers. obs.). A more difficult taxonomic problem involves the C. notus group associated with the host figs of ser. Congestae (Wiebes 1994a), in which the fig characters are overlapping (i.e. F. congesta and F. nota) and pollinators are recognized only on the basis of their associations.

The general pattern of one-to-one host specificity is also supported by natural and artificial experiments. In a natural example involving the recolonization of volcanic islands, population expansion by colonizing fig species has been observed following the introduction of specific pollinator species (Compton et al. 1994b). Furthermore, the naturalization of exotic Ficus in Florida has occurred only after the introduction of their specific pollinators from India (Nadel et al. 1992). A few reports of breakdown in specificity involve visits of local pollinators to exotic Ficus resulting in hybridization (Ware and Compton 1992, Ramirez 1994), but fertile F1 progeny have not been documented. In general, patterns of host specificity and pollinator life cycles suggest coevolutionary scenarios that still remain largely unexplored in a phylogenetic

framework. This chapter provides a phylogenetic analysis of fig wasps that can be combined with that of figs and used in tests of cospeciation (Chapter 3).

Classification, distrubution and phylogeny of dioecious fig pollinators

The obligate mutualistic pollinators of figs are assigned to the subfamily Agaoninae while the non-pollinator wasps are classified under Epichrysomallinae, Otitesellinae, Sycoecinae, Sycoryctinae and Sycophaginae (Boucek 1988). Boucek (1988) supposed that the pollinators and non-pollinators together constitute a monophyletic Agaonidae. However, molecular phylogenetic studies do not support this view (Machado 1998); rather, the non-pollinating subfamilies are more closely related to other chalcid families (Pteromalidae and Torymidae) than they are to the Agaoninae.

The taxonomy of pollinating fig wasps has received less attention than has that of Ficus, but more than 300 species in 16 genera are currently recognized (Berg and Wiebes 1992, Wiebes 1994c, Wiebes 1995b). Wiebes (1982) divided the pollinating genera into two groups, the Agaonini and Blastophagini, based on characters of the female head, although Boucek (1988) pointed out that neither male characters nor host associations support this division. Molecular phylogenetic analyses indicate that neotropical Tetrapus is a sister group to the rest of the pollinating Agaoninae (Machado 1998). The position of Tetrapus is consistent with fossil and morphological evidence (Brues 1910, Wiebes 1995a) and also agrees with the phylogenetic position of their host figs in neotropical sect. Pharmacosycea (Herre et al. 1996).

The overall congruence of fig and pollinator classification has been interpreted as evidence for parallel diversification (Wiebes 1987). However, the taxonomy of dioecious figs (Corner 1965) is incongruent with a proposed phylogeny of the pollinators (Wiebes 1982b). For example, the division of subg. Sycomorus and Ficus is incongruent with the generic limits of Ceratosolen pollinators as discussed in the first chapter. A more

detailed study of the relationships of dioecious figs and those of their pollinators is needed to examine patterns of conflict and congruence in their classification.

The pollinators of dioecious figs are classified in five genera of which two, Blastophaga and Ceratosolen, are further divided into subgenera (Table 1). As with the dioecious figs, their pollinators are restricted to the old world tropics and their center of diversity is in Malesia (Wiebes 1994). For example, 120 species of dioecious fig pollinators are known from Malesia compared to 23 species in Africa and Madagascar, where the fig wasp fauna is more completely described than anywhere else in the world (Berg and Wiebes 1992). Moreover, all genera and all subgenera of dioecious fig pollinators are present in Malesia, while only three such genera occur in Africa. For this reason, the Malesian region was the focus of sampling for a phylogenetic analysis of the dioecious fig pollinators in this study.

In contrast to dioecious figs (Chapter 1), their pollinating wasps have not been grouped taxonomically (cf. Corner 1965, Wiebes 1994). Ramirez (1978) suggested that the pollinators of dioecious figs might together constitute a monophyletic group, although Wiebes (1982b, 1994) argued against this on the basis of overall morphology. In particular, Wiebes (1994) suggested that Blastophaga might be more closely related to Platyscapa and Dolichoris than to other pollinators of dioecious figs. Wiebes (1994) also suggested that Kradibia, Liporrhopalum and Wiebesia belong to a clade that is distinct from the largest fig wasp genus, Ceratosolen. This genus includes pollinators of both monoecious and dioecious species (Chapter 1), but the associations of its three subgenera do not correlate with the distribution of fig breeding systems.

The most recent phylogenetic studies agree with the suggestion of Wiebes (1994) that the pollinators of dioecious figs are not monophyletic (Machado 1998). Ceratosolen, Kradibia and Liporrhopalum appear to belong to a clade and Ceratosolen may be monophyletic; the position of species of Wiebesia and Blastophaga is not resolved (Machado 1998). However, the pollinators of dioecious figs have not received a detailed

phylogenetic analysis. Sampling was minimal in the global study (Machado 1998), and regional studies in Panama (Machado et al. 1996) and Japan (Yokoyama 1994, Yokoyama 1995) have focused on monoecious figs. Machado (1998) emphasized the need for more intensive sampling of dioecious fig pollinators and the sampling scheme outlined in this chapter provides an essential compliment to previous studies (see *Taxon sampling*).. In the present study, representatives of each of the five genera associated with dioecious figs were included. The focus of sampling was Ceratosolen due to its taxonomic complexity and broad range of host associations.

Issues in phylogenetic analysis

As discussed in the previous chapter, issues concerning separate versus combined analyses of different data sets also apply to reconstructing the phylogenetic relationships of dioecious fig pollinators. Two adjacent genes (e.g. COI and COII) can be analyzed separately and the gene trees could be regarded as independent estimates of species phylogeny. However, mitochondrial genes do not provide independent estimates of species phylogeny because they are inherited as a single unit or haplotype (Moore 1995). Studies have repeatedly shown that different mitochondrial genes yield highly similar topologies that do not always reflect species phylogeny (Brown et al. 1994a, Machado 1998, Naylor and Brown 1998). Additional sources of characters are needed to provide independent estimates of species phylogeny, such as nuclear genes (Brower and DeSalle 1994, Hoelzer 1997) or morphology (e.g. Liljeblad and Ronquist 1998). Morphological characters, in particular, can provide a basis for interpreting cases of incongruence in the context of species phylogeny (Brown et al. 1994b, Normark and Lanteri 1998).

With regard to pollinating fig wasps, it has been argued that morphology may be more indicative of the functional constraints imposed by host associations than of phylogenetic relationships (Hill 1967; Herre et al. 1996; Machado 1998). Examples of traits and the features of figs with which they are associated include (1) pollinator head

shape and the arrangement of ostiolar bracts (van Noort and Compton 1994); (2) mechanisms for pollen transport and the distribution of staminate florets (Ramirez 1978); and (3) pollinator ovipositor lengths and fig breeding systems (Ramirez 1980). However, the notion that convergence in functional traits leads to inaccurate estimates of phylogeny has not been evaluated through comparable analyses based on morphological and molecular data.

Another important issue for any method of phylogenetic analysis concerns the validity of assumptions about the evolutionary process and the sensitivity of the results to violations of those assumptions (Swofford et al. 1996). Under the optimality criterion of maximum parsimony, for example, inaccurate inferences can result from the failure to take into account unequal rates of evolution in different lineages (i.e. long-branch attraction; Felsenstein 1978). In the case of molecular data, inconsistency can also result from the failure to take into account biases in base composition, rates of nucleotide substitution and rate heterogeneity across sites (Naylor and Brown 1998). It is possible to incorporate such information in explicit models of molecular evolution (Yang 1993, Yang 1994a, Yang 1994b) and to evaluate the fit of the data to different models under maximum likelihood (Goldman 1993a, Goldman 1993b). Statistical tests compared alternative evolutionary models and the best model, with the fewest additional parameters, was used to reconstruct phylogeny from mitochondrial DNA sequences.

Methods

Taxon sampling and sources of characters

The evolutionary relationships of dioecious fig pollinators were examined through phylogenetic analysis of 44 species (Table 2). Sampling focused on representatives of the major taxonomic divisions of pollinators (genera and subgenera), but was constrained by the availability of recent collections with adequate host records. All taxa included here had paired collections of hosts. At least two representatives of each genus, except for Eupristina

and Deilagaon, known to pollinate monoecious figs in Malesia, were sampled. Thirty-two species of dioecious fig pollinators including at least two species of each genus, but with particular emphasis on Ceratosolen, including 18 species representing all three subgenera. The pollinators of host species included in Chapters 4 and 5 were also sampled. Most collections were made by the author between 1995 and 1997 in Australia, Indonesia, Papua New Guinea and the Solomon Islands.

An attempt was made to include all of the pollinators associated with species sampled in the phylogenetic analysis of dioecious figs (Chapter 1) for comparative purposes (Chapter 3). Although 39 out of 46 host species were included, unpublished sequences for Tetrapus and Pegoscapus were not available at the time of this study (Machado 1998). Due to inadequate preservation, it was not possible to amplify mitochondrial DNA in three cases (Wiebesia sp. from F. ruginerva, Dolichoris sp. from F. albipila and Platyscapa coronata from F. virens). Three pollinator species not associated with hosts from Chapter 1 were also sampled, including Ceratosolen vechti from F. lepicarpa (sect. Sycocarpus), Dolichoris vasculosae from F. vasculosa (sect. Oreosycea) and Waterstoniella dubium from F. dubia (sect. Conosycea). In addition, Ceratosolen medlerianus, from F. mollior, was substituted for C. adenospermae, the closely related pollinator of F. adenosperma. A non-pollinating fig wasp, Apocryptophagus spinitarsus (Sycophaginae), was included for rooting purposes.

Mitochondrial DNA sequences are routinely used to reconstruct phylogenetic relationships in Hymenoptera (Cameron et al. 1992, Derr et al. 1992b, Derr et al. 1992a, Dowton and Austin 1994). Coding sequences from cytochrome oxidase genes have proven useful for resolving phylogenetic relationships at the species level in fig wasps (Machado 1998) and other insect groups (i.e. Brown et al. 1994b, Simon et al. 1994). The main source of characters for this analysis was mitochondrial cytochrome c oxidase subunit I (COI). As a supplement to COI, partial sequences from cytochrome oxidase II (COII), the leucine tRNA, and morphological characters were analyzed separately and in combination.

Mitochondrial DNA

Sources of DNA included adult males and females reared from figs and preserved in 70-95% ethanol and stored at room temperature. Although genomic DNA was extracted from collections up to 27 years old, the best results were obtained from specimens less than one year old. Voucher specimens for DNA sources are deposited in the Entomology Department at the Museum of Comparative Zoology of Harvard University (Appendix 6). Details on the rearing, sorting and identification of pollinators are provided in Chapter 4. Genomic DNA was extracted from 1-10 pollinators reared from the same fig; due to inbreeding and low foundress numbers per fig (Herre 1985), pollinators from the same fig are likely to be similar genetically and may even share the same mitochondrial DNA haplotype.

Genomic DNA was isolated using reagents from the QIAamp® Tissue Kit (QIAGEN® Inc.) and an extraction protocol modified for small insects. First, any traces of ethanol were removed from the specimens under vacuum for 5 min. Wholly dried specimens were ground in Eppendorf tube mortars containing 90 µL ATL buffer and 10 µL proteinase K solution. Specimens in ATL buffer were incubated at 50° C for 24 hr. After 12 hr, a 10 µL aliquot of proteinase K solution was added to each tube. After incubation, specimens were vortexed with 110 µL AL buffer and incubated at 70° C for 10 min. Extracts were centrifuged at 13000 rpm for 5 min. The supernatant was transferred to a clean tube and vortexed with 110 µL absolute ethanol. Cleaning of genomic DNA extracts followed the QIAamp@ protocol with one exception: in order to increase yield, genomic DNA was incubated at 70° C for 5 min in 90 µL water prior to elution from QIAamp® spin columns. 1:10 dilutions of the genomic extracts were used in PCR.

Primers designed from various insect groups were used to amplify ~1900 bp including mitochondrial cytochrome c oxidase subunit I (COI), the leucine tRNA (UUR), and part of the cytochrome c subunit II (COII). It was not possible to amplify reliably the

entire fragment. Rather, three overlapping fragments (~700-900 bp) were obtained using primer combinations Juan-Nancy, New Jerry-Pat, and sw2618-Maryln (Table 3). The thermal conditions for amplification included: (A) denaturation at 96° C for 3.5 min; (B) 35 cycles of denaturation at 94° C (30 sec), annealing at 45° C (60 sec), and extension at 72° C (30 sec); and (C) final extension at 72° C for 5 min. Protocols for DNA sequencing are described in Chapter 1; all species were sequenced in both directions.

The length of the entire aligned fragment was 2083 bp but only 1932 bp were considered for analysis after the exclusion of 151 ambiguous positions at the 3' and 5' ends. The COI portion comprised 1602 bp out of the 1932 bp. The leucine tRNA (73 bp) is located between COI and COII (257 bp). COI in fig wasps also includes an insertion of variable length at the 3' end of the molecule. The position of stop codons indicated that the insertion is located within COI rather than downstream of the coding region (cf. Machado 1998). Moreover, the 3' tail of COI is exposed to the cytosol and may not be subject to the same selective constraints as the membrane-bound portion of the molecule (B. Farrell, pers. comm.). Due to the presence of the insertion, 174 additional positions with ambiguous alignment were excluded, reducing the length of the analyzed sequence to 1724. The alignment is deposited in TreeBASE (http: www.herbaria.harvard.edu/treebase).

Smaller insertions and deletions of three, six and nine bp were also present in COI and the reading frame was preserved in each instance. In some cases, sister taxa shared the same indels. For example, K. copiosae and K. wassae shared six bp and three bp insertions at positions 410-415 and 1371-1372. Ceratosolen nexilis and C. cf. nexilis shared a three bp insertion at positions 1088-1090. Autapomorphic indels were also present in Waterstoniella dubium and Wiebesia punctatae. All indels, including a seven bp indel in the non-transcribed leucine tRNA, were not included in the analyses.

Morphology

Fifty seven skeletal features were coded as discrete characters for phylogenetic analysis (Appendix 5). Published descriptions provided an initial source of character state information (Grandi 1916a, Grandi 1916b, Grandi 1925b, Grandi 1925a, Grandi 1927, Grandi 1928, Grandi 1931, Grandi 1952b, Grandi 1952a, Wiebes 1963b, Wiebes 1963a, Wiebes 1964, Wiebes 1965, Hill 1967, Hill 1969, Wiebes 1974, Wiebes 1977b, Wiebes 1977a, Wiebes 1979b, Wiebes 1980, Wiebes and Abdurahim 1980, Wiebes 1981, Wiebes 1982b, Wiebes 1989, Wiebes 1991a, Wiebes 1992, Wiebes 1993b, Wiebes 1993a, Wiebes 1994a, Wiebes 1994c, Wiebes 1994b, Wiebes 1995a, Wiebes 1995b). Characters included external features of the head, antennae, mouthparts, thorax, legs, spiracles and genitalia. The position and number of hairs, teeth and lamellae were major sources of character states. Characters and states taken from the taxonomic literature were later confirmed by examination of specimens using light microscopy. Approximately 1.4% of the character matrix consisted of polymorphic character states.

Specimens were cleared for light microscopy following protocols in Noyes (1982) and mounted in 85% lactic acid. Best results were obtained upon clearing in 10% potassium hydroxide for 60 min. Specimens were rinsed twice in deionized water (10 min per rinse) and transferred to 50% ethanol for 10 minutes. They were dissected and then mounted in depression slides containing 85% lactic acid; observations were made using Nomarsky illumination at 100X and 400X magnification with a Nikon BX60 light microscope. For permanent storage, slides were sealed with a second cover slip and ringed with Permount® (Fisher Scientific Inc.). Specimens in alcohol were also prepared for scanning electron microscopy using the acetone drying method of van Noort (1995). The extensive type collection of J. T. Wiebes at the Rikjmuseum van Natuurlijke Historie, Leiden (RMNH) and specimens from the Bishop Museum, Honolulu (BISH) were also consulted. Specimens scored for morphological characters are listed in Appendix 6. Appendix 7 includes a character matrix for 43 species of pollinating

Agaoninae. Some characters were not applicable to the outgroup, Apocryptophagus spinitarsus (Sycophaginae), and these were scored as missing data.

Phylogenetic analyses

Phylogenetic analyses under the optimality criterion of parsimony were performed as in the previous chapter. In addition, distance and maximum likelihood methods were used to estimate phylogenies from the mtDNA data. Due to extreme A-T bias in fig wasp mtDNA sequences, genetic distances corrected for multiple substitutions were used to calculate a neighbor-joining tree (Tamura and Nei 1993, Machado 1998). Under maximum likelihood, the estimation of model parameters for different tree topologies is slow in PAUP* and model parameters were therefore estimated using the neighbor-joining tree (Swofford et al. 1996). Parameters were estimated for models of nucleotide substitution differing with regard to assumptions about base frequencies and types of substitutions. Models included JC (Jukes and Cantor 1969), F81 (Felsenstein 1981), K80 (Kimura 1980), HKY85 (Hasegawa et al. 1985), SYM (Zharkikh 1994), and GTR (Rodriguez et al. 1990). Parameters for the heterogeneity of substitutions across sites (Γ; Yang 1994b) and the proportion of invariant sites (I) were also estimated. The goodness-of-fit of models was compared using likelihood ratio tests (Goldman 1993a) as implemented by Posada and Crandall (1998). In the case of nested models, the likelihood ratio statistic is X^2 distributed with the degrees of freedom equal to the difference in the number of parameters between the models. The null hypothesis that a model with more parameters is no better than a simpler model was rejected at $p < 0.01$. The model with the best fit and the fewest additional parameters was used to infer phylogeny under maximum likelihood using parameter estimates from the neighbor-joining tree. A heuristic search was performed in PAUP* using the neighbor-joining topology as a starting tree for branch swapping (Swofford et al. 1996).

Results

Mitochondrial DNA

Complete sequences for the region including COI, the leucine tRNA and the 5' end of COII were obtained for 33 species. Partial sequences were obtained for another 11 species. In C. vechti, C. vissali and D. inornata, it was not possible to amplify 600 bp of the sequence at the 5' end of COI. Also, the 3' end of COI was not sequenced for W. brevigena. The leucine tRNA plus COII fragment posed the most difficulties in amplification and sequencing. This sequence was not obtained for nine species; C. emarginatus, C. medlerianus, C. riparianus, C. cf. nexilis, D. hombronianae, D. inornata, P. fischeri, W. dubium and W. brevigena. Prior to combined analyses of the COI and COII gene regions, an incongruence length difference test (Farris et al. 1994) was performed on a data set including only the 33 taxa with complete sequences. Incongruence between COI and COII genes was not statistically significant ($p = 0.15$) based on 100 partition homogeneity replicates with 10 random addition sequence replicates per partition replicate (Figure 2A). In the absence of significant conflict, complete and partial sequences for COI and COII were analyzed in combination.

Out of 1724 positions considered for the mtDNA analysis, 381 (22%) were invariant, 325 (19%) were autapomorphic and 1018 (59%) were parsimony informative. Only phylogenetically informative sites were included in parsimony analyses whereas all sites were included in ML analyses. Overall base composition was highly A+T biased (75%) and base frequencies differed significantly among species ($X^2 = 191.6$, df =129, $p < 0.005$) although this test did not take into account the potential for phylogenetic autocorrelation. Base composition was similar to the general patterns for insect mitochondrial DNA (Clary and Wolstenholme 1985, Brown 1989, Liu and Beckenbach 1992, Brown et al. 1994b). First and second codon positions were less A-T biased on average (66% and 65%) and did not differ significantly among species ($X^2 = 105.9$ and $X^2 = 47.8$). A-T bias was most extreme at third positions (80%) and significant

heterogeneity among species in base frequencies could be attributed to third positions (X^2 = 232.3, $p < 0.0001$).

A comparison of transitional and transversional nucleotide substitutions with respect to genetic distance and codon position is shown in Figure 3. As a result of A+T bias in base composition, transversions were more common than transitions. Furthermore, the absence of a correlation between genetic distance and numbers of substitutions is indicative of saturated change at some positions. Transitions were saturated at all three positions but less so at first and second positions than at third positions. Transversions did not exhibit patterns of saturation; being most abundant at third positions and least abundant at second positions. Due to base composition bias and saturation in transitions, Machado (1998) favored the calculation of Tamura-Nei genetic distances on transversions only and the same approach was implemented using the method of neighbor-joining (Figure 4).

Sequence divergences between species were quite large after correcting for multiple substitutions. Machado (1998) showed that genetic distances between pollinating fig wasp genera are comparable to distances between insect orders, perhaps related to their unsual life history (Liu and Beckenbach 1992). In particular, sequence divergence within Ceratosolen is larger than between insect orders and subg. Rothropus shows an apparent further increase in the rate of nucleotide substitution (Machado 1998). Genetic distances between 20 species of Ceratosolen are shown in Table 4. After correcting for multiple substitutions, between-species nucleotide divergence ranged from 5% to 28% and averaged 20% ($\pm$0.5%) within Ceratosolen.

Log likelihood ratio tests comparing models of nucleotide substitution indicated that the general time reversible model with a discrete approximation of the gamma distribution provided the best fit (Table 5). Under maximum likelihood, the data rejected the assumptions of equal base frequencies (JC versus F81), an equal ratio of transition and transversion rates (F81 versus HKY85), equal rates of transitions and transversions

(HKY85 versus GTR), and equal substitution rates across sites (GTR versus GTR+Γ). Taking into account the proportion of invariable sites (I), however, did not significantly improve the fit of the model. Parameters for the GRT+Γ model were estimated at 0.6441, 3.8993, 2.7537, 2.4547, and 2.4422. The shape parameter α was estimated to equal 0.5977. Although the GTR+Γ model was the most appropriate model available, it must be emphasized that violations of the model assumptions could lead to inconsistent phylogenetic inferences (Swofford et al. 1996). The potential for deviations from the model assumptions to affect phylogenetic inferences will be addressed in the discussion.

mtDNA parsimony analysis

Maximum parsimony searches resulted in a single shortest tree (Figure 5; L = 6806; CI = 0.29) based on all informative mtDNA characters. Twenty six of out 43 nodes in the MP tree were supported by >50% bootstrap support. After rooting the tree with non-pollinating Apocryptophagus, Dolichoris was paraphyletic to the rest of the Malesian pollinator genera with moderate bootstrap support (70%). The pollinators of dioecious figs fell into two clades although support for each clade was weak (<50% bootstrap values). One clade included Liporrhopalum, Kradibia and most of Ceratosolen; the other clade consisted of Blastophaga, Wiebesia, some Ceratosolen, and the pollinators of monoecious subg. Urostigma.

Liporrhopalum plus Kradibia was a well-supported group and there was strong support for the derivation of Liporrhopalum within paraphyletic Kradibia. In the largest Ceratosolen clade, there was strong support for the monophyly of subg. Rothropus plus C. bisulcatus. A single clade not present in the most parsimonious tree was recovered in the bootstrap consensus with 54% support, consisting of C. vechti as sister to New Guinean Rothropus plus C. bisulcatus. There was also strong support for a clade including subg. Strepitus (except C. vissali) and the pollinators of sect. Adenosperma, C. medlerianus and C. sp. "riparianus". Ceratosolen grandii, the pollinator of F. semivestita

from sect. Neomorphe, appeared to be more closely related to pollinators of sect. Adenosperma. This result agrees more closely with the phylogeny of the dioecious figs than with classification (Chapter 1) although instances of conflict with host classification and phylogeny are also apparent. For example, C. vissali, from F. theophrastoides and endemic to the Solomon Islands, appeared to be more closely related to C. blommersi from Madagascar than to C. abnormis, its putative New Guinea relative from F. dammaropsis in subsect. Papuasyce. Extreme sequence divergence in C. vissali and the possibility of phylogenetic inconsistency will be discussed in the context of maximum likelihood results.

There was strong support for a clade including Blastophaga, Wiebesia and the pollinators of subg. Urostigma. Wiebesia was monophyletic and appeared as the sister group to a clade including Blastophaga, Eupristina, Platyscapa, Pleistodontes and Waterstoniella. New Guinean Wiebesia brusi and W. frustrata, reared from sect. Rhizocladus were more closely related to each other than to Bornean W. punctatae from sect. Kalosyce. Pleistodontes was also monophyletic and Australian P. rigisamos appeared as the sister to the two New Guinean species. Eupristina, Platyscapa, and Waterstoniella belonged to a poorly supported clade that was sister to monophyletic Blastophaga.

mtDNA maximum likelihood analysis

According to the Kishino-Hasegawa test as implemented in PAUP*, the ML tree was significantly better than the neighbor-joining tree used to estimate model parameters and as a starting tree in heuristic searches under maximum likelihood (Table 6A). The ML tree, however, did not significantly reject the MP tree. Twenty eight nodes supported by >50% bootstrap values in the MP searches were congruent with the topology obtained under maximum likelihood (ML) and fourteen nodes were in conflict (Figure 6; L = -31520.43815), but only two of the conflicting nodes were supported by >50% bootstrap

values under parsimony. Platyscapa and Waterstoniella were monophyletic in the ML tree, in contrast to weakly supported paraphyly in the MP tree. The sister relationship of K. sp. "salembensis" and K. sp. "ohuensis" with 92% bootstrap support was contradicted by the placement of K. sp. "salembensis" with K. jacobsi on a very short branch in the ML tree. The Strepitus clade with C. abnormis as sister to a lineage including C. armipes and C. sp. "kaironkensis" had 53% support under parsimony but the ML tree placed C. abnormis along a short branch with C. grandii and the pollinators of sect. Adenosperma.

In further contrast to the MP results, ML analyses based on the neighbor-joining tree showed Dolichoris vasculosae to be nested within a Ceratosolen clade. It is noteworthy that D. vasculosae represented the most divergent mtDNA sequence in the analysis. In the ML tree, D. vasculosae attached near some of the most diverged Ceratosolen species. (e.g. C. vissali and Rothropus). However, in the MP tree, D. vasculosae was sister to the rest of the Malesian pollinators apart from Dolichoris associated with Ficus ser. Nervosae. Neither relationship was strongly supported but the widely different placement of divergent taxa suggests the potential for inconsistent estimates of phylogeny using either method. The longest internal branch in the tree was a Ceratosolen lineage showing a significant increase in mtDNA substitution rates (Machado 1998). The combination of heterogeneity in the rate of nucleotide substitution among lineages and codon-specific variation in base composition were not taken into account by the GTR+Γ model. In contrast, the parsimony analysis assumed equal weights of all changes across all sites. The sensitivity of parsimony and maximum likelihood to the violation of different assumptions and the potential for inconsistent phylogenetic inferences are addressed in the discussion.

Also in contrast to the MP tree, Ceratosolen plus Kradibia and Liporrhopalum were monophyletic after excluding D. vasculosae from the ML tree. Subgenus Strepitus and the pollinators of sect. Adenosperma appeared as the sister group to most of Ceratosolen plus Kradibia and Liporrhopalum. Pollinators of sect. Neomorphe and subg.

Sycomorus are paraphyletic with respect to the pollinators of subsect. Sycocarpus (including Ceratosolen subg. Rothropus). Also in agreement with the MP results, C. nanus is the most basal member of the Ceratosolen lineage showing an accelerated rate of mtDNA substitution (Machado 1998). Furthermore, the paraphyly of Kradibia with respect to Liporrhopalum is consistent with the MP results.

Morphology

Fifty-four characters were phylogenetically informative out of the 22 characters taken from adult males and 35 from females. The morphological data alone yielded 256 most parsimonious trees of 317 steps (CI = 0.28). Only nine nodes were supported by >50% bootstrap values and all were present in the strict consensus tree (Figure 7). Overall, morphological data supported the monophyly of Ceratosolen, Kradibia, Liporrhopalum, Pleistodontes, and Wiebesia. In sharp contrast to the mtDNA analyses, Pleistodontes appeared as the sister group to the rest of the Malesian pollinators with 75% bootstrap support. Other genera associated with subg. Urostigma including Eupristina, Platyscapa, and Waterstoniella, were paraphyletic with respect to Dolichoris and the pollinators of subg. Ficus and Sycomorus, but relationships among the genera lacked resolution and support.

A clade consisting of the pollinators of dioecious figs and secondarily monoecious figs had weak bootstrap support (51%) and the relationship of Dolichoris to this clade was not resolved. Wiebesia associated with Ficus sect. Rhizocladus (W. brusi and W. frustrata) were monophyletic and W. punctatae from sect. Kalosyce was its sister group. The relationships of Blastophaga, Liporrhopalum and Wiebesia to the rest of the dioecious figs were not well resolved, although the Kradibia-Liporrhopalum clade detected in mtDNA analyses was contradicted by morphology. Instead, morphology suggested a sister relationship of Kradibia to a monophyletic and moderately supported Ceratosolen. Few nodes within Ceratosolen were resolved in the strict consensus, except

for three clades with high bootstrap support. These were the sister relationships of: C. medlerianus and C. sp. "riparianus" (pollinators of sect. Adenosperma) with 75%, C. armipes and C. sp. "kaironkensis" (pollinators of subsect. Papuasyce) with 90%, and C. nexilis and C. cf. nexilis (pollinators of Papuasian endemics in sect. Neomorphe) with 86% support

Conflict and congruence

Only nine out of 23 resolved nodes in the morphological strict consensus (Figure 6) were in absolute agreement with the mtDNA MP tree (Figure 5). However, the overwhelming majority of conflicting nodes were weakly supported in one analysis or the other. For example, only four out of 26 clades with >50% bootstrap support in the mtDNA analysis were contradicted by clades in the morphological bootstrap consensus. The strict consensus of morphological trees was less resolved and few clades received strong bootstrap support. However, seven out of ten nodes supported by >50% bootstrap values are in absolute agreement with the mtDNA MP tree. The three conflicting nodes in the 50% morphological bootstrap consensus include: (A) the sister relationship of Pleistodontes to the rest of the Malesian pollinators with 75% support, (B) a weakly supported clade of mostly dioecious fig pollinators (51%) and (C) the monophyly of Ceratosolen with 66%. Of the three clades, only the monophyly of Ceratosolen does not conflict with the mtDNA 50% bootstrap consensus.

An ILD test found that the sum of the tree lengths from separate analyses of mtDNA and morphological data was significantly less than the sum of tree lengths taken from random partitions of the combined data (Figure 2B). As with a similar result for Ficus in Chapter 1, the ILD points to significant conflict between pollinator mtDNA and morphology. However, the ILD alone does not indicate whether conflict results from different phylogenetic histories, different rates of change or systematic error in either data set. In the case of pollinator mtDNA and morphology, it is especially difficult to

interpret the results of the ILD test given the tendency for the larger data set to have a greater effect on tree lengths in random data partitions compared to the smaller data set. With 1018 characters in the mtDNA data set compared to 54 in the morphology data set, there is a high probability that random data partitions will include mostly mtDNA characters and the size of partitions alone could have a strong influence on the tree length distribution (Figure 2B).

In addition to the ILD test, a maximum likelihood approach to testing for incongruence between alternative tree topologies was applied to the mtDNA data (Kishino and Hasegawa 1989). The ML tree topology for mtDNA was compared to topologies from morphology and combined analyses using the Kishino-Hasegawa test as implemented in PAUP*. Likelihood scores for each tree were calculated under the GTR+Γ model using earlier estimates of model parameters. The ML tree was significantly more likely than a morphological MP tree, although an MP tree based on combined data was not rejected (Table 6A).

Results of Templeton tests for incongruence are reported in Table 6B, where P values indicate the probability of obtaining a higher test statistic by chance given the null hypothesis that rival trees are not different. Mitochondrial DNA rejected the shortest morphological trees and a reciprocal test based on morphological data also indicated that incongruence was significant. Tests of strict consensus trees as rival constraints were also significant in both directions. However, taking into account the relative bootstrap support for clades in morphological analyses had a measurable impact on the results of the test. At the $\alpha = 0.05$ significance level, mtDNA sequences did not reject the morphological 70% or 90% bootstrap consensus trees. These findings agree with the observation that those few clades with support from morphology are generally congruent with clades supported by mtDNA. By contrast, the shortest morphological tree rejected all rival topologies derived from mtDNA, including the 90% bootstrap consensus.

In addition, morphology strongly rejected a combined MP tree while the mtDNA tree and the combined tree did not show significant conflict. Ferguson (1998) argued that the results of reciprocal tests are inconclusive when the null hypothesis is rejected by one data set but not by the other. However, it seems reasonable to interpret unilateral rejection in terms of differences between data sets in total numbers of characters and levels of homoplasy. For example, the rejection of rival mtDNA and combined trees by morphological data is based on a small set of relatively homoplasious characters in the latter that do not provide a robust estimate of phylogeny when considered alone. On the other hand, what little phylogenetic signal is present in morphological data (i.e. clades with >70% bootstrap support) is not rejected by the larger and more robust mtDNA data set. Although some global tests of conflict are significant, differences between the separate analyses could result from systematic error, especially in the morphological data set. In any event, only a single highly supported case of incongruence was detected - the placement of Pleistodontes.

Combined analyses

The combined analysis recovered three most parsimonious trees of 7174 steps (CI = 0.29) differing from each other only in the placement of Ceratosolen associated with sects. Neomorphe and Sycomorus. The >50% bootstrap consensus was congruent with the strict consensus (Figure 8). Thirty-one nodes in the combined MP tree were supported by >50% bootstrap values compared to 26 and 10 nodes in the mtDNA and morphology MP trees, respectively. In general, clades with >50% bootstrap support in the separate and combined analyses were in agreement. The addition of morphological data to the mtDNA data did not have a dramatic impact on the relative support for clades. For example, out of 26 clades also present in the separate mtDNA tree (Figure 5), bootstrap support for eight clades increased, ten decreased and eight remained unchanged relative to the combined analysis. In large part, changes in bootstrap values between the mtDNA

and combined analyses were between 1% and 4%, although some exceptions are noted below.

Comparing the combined strict consensus and mtDNA trees (Figures 5 and 7), it is evident that 31 nodes were in agreement and six nodes were in conflict. Three of the conflicting nodes supported by bootstrap values in the combined analysis could reflect positive contributions from morphology (i.e. monophyly of pollinators from subg. Urostigma, Ceratosolen, and the sister relationship of C. dentifer and C. vechti). On the other hand, two clades in the morphological bootstrap consensus were not supported in the combined analysis (i.e. Pleistodontes as sister to the rest of the Malesian pollinators and monophyly of dioecious fig pollinators). Support for the monophyly of dioecious fig pollinators in the morphological analysis was weak (51%) but the position of Pleistodontes (75%) will be discussed at greater length.

The combined analysis strongly supported Dolichoris in a paraphyletic relationship to the rest of the Malesian pollinators (Figure 8). The pollinators of sect. Oreosycea ser. Nervosae (D. sp. "hombronianae" and D. inornata) were monophyletic and, although the placement of D. vasculosae was well supported in the combined analysis, the position of this species in ML analyses raised questions (see Discussion). In the combined tree, a Kradibia-Liporrhopalum clade was sister to the rest of the pollinators excluding Dolichoris but this relationship was weak (d = 1; <50% bootstrap). Relationships within the Kradibia-Liporrhopalum clade were in complete agreement with the separate mtDNA MP tree. Kradibia was paraphyletic with respect to Liporrhopalum, due to the position of pollinators associated with sect. Sycidium ser. Copiosae (K. copiosae and K. wassae). Pollinators of ser. Phaeopilosae (K. jacobsi and K. sp. "salembensis") plus K. sp. "ohuensis" were the sister group to a monophyletic Lipporhopalum.

A clade including Blastophaga, Eupristina, Platyscapa, Pleistodontes, Waterstoniella and Wiebesia was well-supported and sister to Ceratosolen. In this clade,

a monophyletic and well-supported Wiebesia appeared as the sister to the other genera. In Wiebesia, the pollinators of sect. Rhizocladus in New Guinea were more closely related to each other than to W. punctatae from sect. Kalosyce. In contrast to the mtDNA results, Blastophaga was sister to the clade of Urostigma pollinators (i.e. Eupristina, Platyscapa, Pleistodontes and Waterstoniella) in the combined analysis. Pleistodontes and Platyscapa were each monophyletic within Urostigma-pollinating lineage. Pleistodontes plebejus and Pleistodontes rieki from New Guinea were more closely related to each other than to Australian Pleistodontes rigisamos. Also, Waterstoniella appeared to be paraphyletic with respect to Eupristina but support for this relationship was weak (d = 1; <50% bootstrap).

The monophyly of Ceratosolen had marginal support in the combined analysis (56%). Within Ceratosolen, there was strong support for a clade including the pollinators of sect. Adenosperma plus Ceratosolen subg. Strepitus (but excluding C. vissali). The poorly supported relationship between Solomon Islands and Madagascar endemics, C. vissali, and C. blommersi, was unexpected and is discussed in more detail (see *Issues in phylogenetic analysis*). The Strepitus clade included pollinators associated with subsects. Papuasyce and Dammaropsis and was sister to C. grandii plus pollinators of sect. Adenosperma. Pollinators from subsect. Papuasyce (i.e. C. armipes and C. sp. "kaironkensis") and from sect. Adenosperma (i.e. C. medlerianus and C. sp. "riparianus") were each monophyletic and highly supported. In contrast to the Strepitus plus Adenosperma-pollinating clade, support for relationships in the rest of Ceratosolen was weak. Relationships among pollinators from sects. Neomorphe and Sycomorus were mostly either unresolved or poorly supported. Two highly supported clades included Papuasian endemics pollinating sect. Neomorphe (C. nexilis and C. cf. nexilis) and widespread Malesian and African species pollinating sect. Sycomorus (C. fusciceps and C. capensis). There was also strong support for C. nanus as sister to a clade including

mostly subg. Rothropus. Rothropus was paraphyletic due to the highly supported relationship of C. bisulcatus (subg. Ceratosolen) to C. corneri.

Discussion

Issues in phylogenetic analysis

Mitochondrial DNA sequences in this study provide new insights on the phylogenetic relationships of dioecious fig pollinators. An advantage of using mitochondrial DNA in phylogenetic analysis is that high interspecific sequence divergence provides a rich source of potentially informative characters (Simon et al. 1994). However, the inference of species phylogenies from mitochondrial gene trees can be problematical given that genes and species do not necessarily share the same history (Doyle 1992, Maddison 1995, Maddison 1997). For example, gene trees may differ from species trees when ancestral polymorphisms retained through speciation events subsequently become fixed in different lineages (i.e. lineage sorting; Hoelzer 1997). The potential for conflicts due to lineage sorting could be great if speciation predates the coalescence of alleles. In this regard, Moore (1995) argued that mtDNA haplotypes are less prone to lineage sorting due to smaller effective population sizes and shorter coalescence times than the alleles of nuclear genes.

Hybridization and introgression may also lead to incongruence between gene trees and species trees (McDade 1995) but hybridization among pollinators of figs does not seem likely for two reasons. First, there is no evidence of natural hybridization as a major force in the evolutionary history of host Ficus (see *Discussion* in Chapter 1). Second, reproductive isolation of sympatric pollinator species is a consequence of extreme specificity in host preferences. Furthermore, even in rare cases where two pollinator species inhabit the same host, intermediates have not been observed. For example, Kerdelhue et al. (1997) found no evidence of hybridization between sympatric Ceratosolen arabicus and C. flabellatus in West African F. sur, even when the two species

develop in the same fig. Therefore, it seems reasonable to assume that hybridization does not pose a major problem for the inference of pollinator species trees from mtDNA. However, additional evidence from nuclear genes for pollinators of figs would aid in evaluating these assumptions (Brower and DeSalle 1994, Page and Charleston 1997).

Other possible explanations for discrepancies between species trees and gene trees involve cases in which estimates of phylogeny are positively misleading due to systematic error or unequal rates of change (Cunningham 1997b, Huelsenbeck 1997). Inconsistent estimates of phylogeny under parsimony can result from the failure to take into account unequal rates of nucleotide substitution in different lineages (i.e. "long-branch attraction"; Felsenstein 1978). For example, unequal base composition and differing rates of nucleotide substitution across sites in fig wasp mtDNA are potential sources of systematic error that should be considered (Simon et al. 1994). In this regard, maximum likelihood has an advantage over parsimony in evaluating the fit of data to explicit models of molecular evolution (Swofford et al. 1996). Comparing the fit of pollinator mtDNA to different models indicated that a model assuming unequal base frequencies, unequal rates of transitions and transversions plus unequal rates of substitution rates across sites (Rodriguez et al. 1990, Yang 1994b) was significantly better than simpler models (Table 5). However, it is important to bear in mind that even the best model of molecular evolution under maximum likelihood may not be robust to violations of its assumptions (Swofford et al. 1996).

It is encouraging, however, that the overall results from parsimony and likelihood analyses of pollinator mtDNA are highly similar (cf. Figures 5 and 6). That equally weighted parsimony is not especially sensitive to A-T bias or transition bias at third positions (Figure 3) could be due to the influence of a large number of potentially informative characters at first and second codon positions (516 out of 988 potentially informative coding sites). It seems plausible that, in general, the phylogenetic signal expressed by transversions at all codon positions is greater than systematic errors

resulting from the saturation of transitions at third positions. Huelsenbeck and Hillis (1996) performed power analyses to demonstrate that unweighted parsimony analysis of more than 1000 bp can be as accurate as weighted parsimony and maximum likelihood under simple conditions. However, unweighted parsimony may converge on the wrong tree given unequal rates of change in different lineages.

Particular conflicts between the MP and ML results, especially in those lineages with unequal rates of substitution, require further consideration with respect to phylogenetic accuracy. An interesting example concerns Dolichoris vasculosae. This species was derived within Ceratosolen in the most likely topology assuming GTR+Γ while under parsimony the species was sister to all Malesian pollinators besides Papuasian Dolichoris. However, the derivation of D. vasculosae within Ceratosolen seems doubtful based on morphology and its association with F. vasculosa in sect. Oreosycea. Could it be that maximum likelihood is less consistent than unweighted parsimony in this case? Among the currently available models of molecular evolution, even the best fit can be over simplified (Chang and Campbell; In prep). For example, assumptions of the GTR+Γ model that base composition bias is equal across codon positions and across taxa are not met by pollinator mtDNA. In particular, A-T bias is 14-15% higher at third positions compared to first and second positions and there is significant heterogeneity among species in base composition at third positions. To examine the sensitivity of likelihood estimates to the effect of codon position bias, GTR+Γ model parameters were estimated separately for each codon position and then used to compare the likelihood scores of the ML and MP trees (Figures 5 and 6).

Likelihood estimates for ML and MP topologies under the different models are shown in Table 7. Models based on first and second positions agreed with the overall model in favoring the ML topology over the MP topology. Unexpectedly, however, the MP topology was more likely than the ML topology under the third codon position model. This result is surprising given that the ML topology was used to estimate the

parameters of the model. That some codon positions favor the exclusion of D. vasculosae from Ceratosolen but other codon positions do not suggests that GTR+Γ is too simple a model for nucleotide substitutions in pollinator mtDNA. The total likelihood of a GTR+Γ model that also takes into account codon position bias is obtained by summing the individual likelihoods for the three separate codon models (Table 7; -ln L = 30231.8935). In spite of tripling the number of parameters, this new model of mtDNA evolution significantly rejects GTR+Γ ($X^2 = 2577.09$, df = 18, $p < 0.0001$).

It is especially noteworthy that in the case of D. vasculosae parsimony appears to be more accurate than maximum likelihood. It is possible that unweighted parsimony could be more robust than maximum likelihood to deviations from the assumption of equal base composition across codon positions. Future maximum likelihood analyses of fig wasp mtDNA should consider new models allowing base frequencies to vary among codon positions. The effect of differential weighting of transversions and transitions under parsimony should also be examined (Simon et al. 1994). Additional sampling of taxa and nuclear genes (Brower and DeSalle 1994) may also aid in corroborating this unexpected result. A-T bias is often most extreme at third positions in insect mtDNA (Brower 1994, Brown et al. 1994a) and the potential for codon position bias to influence the results of phylogenetic analyses ought to be explored for mtDNA in general.

Conflict and congruence in mtDNA and morphology

It has been argued that molecular data provide an independent source of evidence for evaluating the classification and evolution of fig wasps when compared to morphology (Herre et al. 1996, Machado et al. 1996). In particular, convergence in the functional traits of pollinators in relation to their host plants (as discussed in Chapter 1) might lead to inaccurate phylogenetic hypotheses based on morphology alone. Although this may be true, it is also the case that mtDNA can fail to provide an accurate estimate of species phylogeny for a variety of reasons. In the case of the fig pollinators, there appeared to be

less phylogenetic signal in the morphological data set compared to mtDNA, as indicated by levels of homoplasy, clade support, and resolution in the separate analyses. This result is consistent with the notion that morphological convergence could obscure species relationships but, on the other hand, some phylogenetic signal was present in the morphological data set (Figure 7). The possibility of correlated evolution of pollinator traits in relation to host fig morphology is considered in Chapter 3.

The question for the present chapter is whether a combination of mtDNA and morphology provides a more accurate phylogenetic hypothesis for fig pollinators than mtDNA alone. Recent reviews advocate a conditional approach to the combination of morphological and molecular data sets based on statistical tests of congruence (Huelsenbeck et al. 1996). In the case of fig pollinators, morphological and mtDNA data sets were significantly incongruent according to an ILD test (Figure 2A; Farris et al. 1994), Kishino-Hasegawa tests (Table 6A; Kishino and Hasegawa 1989), and several Templeton tests (Table 6B; Templeton 1993, Larson 1994). However, as discussed in the preceding chapter, the source of statistically significant conflict is unclear. Global tests of incongruence, for instance, do not distinguish data sets with different histories from sources of systematic bias or random error (de Querioz et al. 1995; Mason-Gamer and Kellogg 1996, Cunningham 1997b, Cunningham 1997a). With respect to the latter possibility, Templeton tests have the advantage of considering levels of support for rival clades in separate analyses.

Templeton results indicated that significant conflict between mtDNA and morphology was attributed to weakly supported clades (i.e. <70% bootstrap support; Table 6B). In particular, unilateral rejection of mtDNA by morphology is consistent with the notion that incongruence resulted from noise (i.e. homoplasy) in the much smaller morphological data set. The only highly supported instance of incongruence between mtDNA and morphology (i.e. Pleistodontes), could have resulted from correlated homoplasy in features of the female head (see *Classification and Phylogeny*). The

overall similarity of the mtDNA and combined results suggest that most of the phylogenetic signal in the combined analysis was contributed by the molecular data set. However, it is also possible that phylogenetic signal hidden in the separate analyses was recovered in the combined analysis (Barrett et al. 1991). For example, Platyscapa was monophyletic in the combined analysis but this clade did not appear in either separate analysis. In any event, some unique clades in the morphological analysis also appeared in the combined analysis (i.e. monophyletic Ceratosolen). Given that more nodes were supported by bootstrap values in the combined analysis than in either separate analysis, it seems that the combined data provide the best-supported estimate of pollinator phylogeny (see also Chapter 1).

Other arguments against the inclusion of morphological characters in phylogenetic analysis involve concerns about circularity (see Chapter 1) and subjectivity in the delimitation of discrete states for continuous characters (Gift and Stevens 1997). Without question, future analyses of pollinator morphology would benefit from improved homology assessment of characters and states (Appendix 5) just as molecular data benefit from improved sequence alignment. Another improvement in morphological analysis would involve the ordering of states for characters showing transformation series (cf. Liljeblad and Ronquist 1998). For example, future analyses could consider reduction series in female wing venation, male eyes, male tarsi and mouthparts in both sexes (Ramirez 1978, Wiebes 1982b, Ramirez 1991). In general, further study of conflicts between mtDNA and morphological data sets would benefit from models correcting for biases in morphological and mtDNA evolution, i.e. new weighting schemes and models of nucleotide substitution. In the absence of strong incongruence between mtDNA and morphology the combined results will be used to discuss classification, morphological evolution, and the evolution of host associations (Figure 8).

Classification and phylogeny of dioecious fig pollinators

The combination of mtDNA and morphology provides new insights on the classification (Boucek 1988, Wiebes 1994c) and proposed phylogenetic relationships of the dioecious fig pollinators (Wiebes 1982b, Ramirez 1991, Machado et al. 1996). Machado (1998) demonstrated that the insertion at the end of COI occurs in all pollinating Agaoninae except Tetrapus and supports the position of this genus as the sister group to the rest of the pollinating fig wasps. The lack of an insertion in the outgroup, Apocryptophagus spinitarsus, is consistent with Machado (1998) but several ingroup taxa also lack the insert (i.e. C. emarginatus, K. copiosae and K. wassae). The division of the fig pollinators into two subfamilies, Agaoninae and Blastophaginae based on characters of the female head (Wiebes 1982b), is not supported by results from either the separate or combined evidence. This conclusion agrees with Boucek (1988), who regarded the two subfamilies as artificial and uncorroborated by male morphology. Interestingly, Wiebes (1982a) was concerned with the similarities between Pleistodontes and the pollinators of subg. Pharmacosycea (Dolichoris and Tetrapus). He regarded the similarities as pleisiomorphic but then argued, on the basis of several female head characters, that Tetrapus was more closely related to Pleistodontes than to Dolichoris. Morphology alone suggests that Pleistodontes is the sister group to the Malesian pollinators of figs, a similar position to that of Tetrapus in previous phylogenetic studies (Machado et al. 1996, Machado 1998). These studies showed that Pleistodontes belongs to a clade of Urostigma-pollinators and is not most closely related to Tetrapus. Mitochondrial data strongly suggest that the remarkable similarities between Tetrapus and Pleistodontes in the elongation of the female head and modifications of the mandibular appendage would reflect convergence. Convergent head shapes in African pollinating and non-pollinating fig wasps have been related to ostiole morphology (van Noort and Compton 1996). In a separate example, Machado (1998) suggested that morphological similarities between Pleistodontes and the pollinators of African sect. Galoglychia noted by Wiebes (1982b) are also due to convergence.

Although morphology weakly supports a clade including all five genera known to pollinate dioecious figs (e.g. Ramirez 1991), mtDNA strongly rejects the monophyly of the dioecious fig pollinators. Instead, there was 73% support for a close relationship between dioecious-pollinating Blastophaga and the pollinators of monoecious subg. Urostigma (Wiebes 1994). The overall results of separate and combined analyses (Figure 8) indicate that pollinators of dioecious figs (subg. Ficus) are paraphyletic with respect to the pollinators of subg. Urostigma and subg. Sycomorus (Figure 9; see *Evolution of host associations*). Four distinct clades of dioecious fig pollinators correspond to Ceratosolen, Blastophaga, Wiebesia, and Kradibia-Liporrhopalum. As discussed in Chapter 1, the Ceratosolen clade includes pollinators of both dioecious and secondarily monoecious species, thereby providing the opportunity to study pollinator traits that are correlated with shifts in fig breeding system (Chapter 3). The Kradibia-Liporrhopalum clade was not detected in earlier morphological cladistic analyses (Ramirez 1978, Wiebes 1982b, Ramirez 1991). More recently, this clade was detected using COI (Machado 1998) and additional sampling in the present study indicated that Kradibia is paraphyletic with respect to Liporrhopalum.

The combined results generally agree with the global phylogenetic analysis of fig pollinators (Machado 1998). For example, in both studies, the pollinators of subg. Urostigma and most genera were monophyletic. However, the position of two taxa in particular deserve further discussion. In the global study, Dolichoris sp. appeared to be closely related to the pollinators of subg. Urostigma but the sample was collected in a light trap and its host association is unknown (Machado 1998). Inclusion of pollinators reared from documented host plants reduces the uncertainty associated with material from light traps and incorrectly identified hosts (cf. Wiebes 1994 and Machado 1998). In the present study, none of the three Dolichoris spp. with known host associations (sect. Oreosycea) show a close relationship to the pollinators of subg. Urostigma. Such

different results underscore the importance of fully documented host associations in comparing phylogenetic studies.

The derivation of Wiebesia pumilae within Ceratosolen in the parsimony analyses of Machado (1998) is analogous to the problem exhibited by the mtDNA sequence from Dolichoris vasculosae. Much like D. vasculosae in the present study, W. pumilae represents the most diverged sequence in the global study and occupies different positions in parsimony and maximum likelihood topologies. In spite of these problems, the level of agreement between studies based on different samples of taxa (e.g. monophyly of Pleistodontes in both studies) and on different sources of evidence (i.e. mtDNA and morphology) is encouraging for future phylogenetic studies. In general, the combination of morphological and molecular data in phylogenetic analysis supports the generic limits of dioecious fig pollinators (cf. Kradibia). Morphological apomorphies for clades recovered in the combined analysis are discussed in terms of a single MP tree (Figure 9).

Morphological apomorphies

Morphological evolution inferred from the combined phylogeny supports the view that pollinators of figs show trends toward the reduction and loss of multiple features including mouthparts, tarsi, male eyes and female wing venation (Wiebes 1982). For example, the Malesian pollinators are distinguished from paraphyletic Dolichoris by eight unambiguous morphological changes. In females, these include the loss of the maxillary palpus and reduction of ventral lamellae on the mandibular appendage, loss of front coxal combs and a reduction in ovipositor length. In males, there is a reduction of mouthparts to a maxillolabial complex, and the separation of the mesonotum from the metanotum. It has been supposed that the elaboration of other features including the female antennae, mandibular appendages, and mesosternal pockets may reflect adaptation to host figs (e.g. Ramirez 1991; 1978). Similar adaptations to functional constraints imposed by different

hosts could have occurred independently in different lineages (e.g. vanNoort and Compton 1996). Most morphological features showed patterns of homoplasy, providing a basis for specific tests of adaptation hypotheses in a phylogenetic framework. Chapter 3 explores these issues in depth while the remainder of this discussion is devoted to morphological apomorphies for clades of special interest.

The clade including Blastophaga, Wiebesia and the pollinators of subg. Urostigma is marked by the loss of sternal corbiculae and the insertion of male antennae in a common groove. Pollinators of subg. Urostigma also share many features. These include eleven segments in the female antennae, a pointed apical process on the third segment, a single gland in the female mandible and the tooth-like modification of ventral lamellae on the mandibular appendage. In females, the fore tibia has two dorso-apical teeth and the hind tibia has two teeth; a bicuspidate axial and a tricuspidate antaxial. In males, the fore leg has five tarsi and the hind tibia has two bicuspidate teeth. Apomorphies for the pollinators of sect. Conosycea (Eupristina and Waterstoniella) are only found in males (i.e. two mandibular glands and the presence of three ventro-apical teeth in the male fore tibia). Platyscapa is characterized by nine ventral lamellae on the mandibular appendage, front coxal combs and sternal corbiculae, male hind tibia with a tricuspidate antaxial tooth, and four segments in the male antennae.

The female head in the Pleistodontes clade is apomorphic in six different features of the female head that were previously cited as pleisiomorphic characters shared with Neotropical Tetrapus (Wiebes 1982a). For example, the facial groove fitting the antennae in most Agaonines is closed in Pleistodontes, the scape and pedicel are elongate, the pedicel has few axial spines and the mandibular appendage is oriented subvertically with a row of many ventral lamellae (more than twelve). In males, there are seven segments in the antennae. Apomorphies for Blastophaga include the loss of setae on the female labium and the separation of the metanotum from the propodeum. Apomorphic features of the Wiebesia clade are a single subapical seta on the labium,

closed mesosternal pollen pockets, a longitudinal groove along the median of the mesoscutum in females and atrophied tarsi of the mid leg in males.

The Ceratosolen clade is morphologically distinct from its relatives in at least eight features. In females, the spiracular peritremata of the eighth urotergite are enlarged and ovoid. The male head is elongate, with few dorsal spines and a prominent trilobate margin to the epistoma. The male antennae are slender and the eyes are reduced. In the male thorax, the pronotum is elongate and the propodeal peritremata are enlarged. It is noteworthy that some highly supported clades (i.e. C. nanus plus Rothropus) with no previous taxonomic designation were not marked by changes in morphology. The elongate male hind tibia in males distinguishes Rothropus, a character that was erroneously omitted from Appendix 5 and the phylogenetic analysis. A reduced maxillolabial complex and clawed claspers in male genitalia are additional apomorphies for the Rothropus clade. There are no unambiguous changes in the Strepitus clade but an unnamed clade including the pollinators of sect. Adenosperma plus F. semivestita is characterized by three features. In this clade there are seven ridges in the female mandible, two glands in the male mandible, and a propodeum separated from the metanotum.

The second largest clade of dioecious fig pollinators (i.e. Kradibia plus Liporrhopalum) was first proposed by Wiebes (1994) in an effort to reconcile pollinator and host classification. However, Kradibia and Liporrhopalum were not most closely related in earlier morphological cladograms (Wiebes 1982a, Ramirez 1991). It is therefore unsurprising that morphological apomorphies for this clade are lacking. In contrast, the monophyly of Liporrhopalum with repsect to paraphyletic Kradibia is marked by eight apomorphies. Female features include elongate funicular segments in the antennae, a tricuspidate antaxial tooth in the hind tibia and obsolete forewing venation. Male apomorphies include slender antennae, the separation of the propodeum and metanotum, the reduction of tarsi in mid and hind legs plus genitalia with clawed

claspers. Overall trends in morphological evolution are of great relevance to the study of fig/pollinator coevolution and conflict in the mutualism. The next chapter examines correlated morphological evolution in the interacting lineages and the correlates of dioecious pollination in particular.

Evolution of host associations

It is possible to reconstruct the evolution of host associations in light of pollinator phylogeny, keeping in mind the caveats discussed in Chapter 1. Futuyma et al. (1993), for example, used such a reconstruction to demonstrate that genetic constraints could affect the evolution of host associations in Ophraella (Coleoptera: Chrysomelidae). This approach was also used by Brown et al. (1994a) to argue that speciation in the yucca moth family, Prodoxidae (Lepidoptera: Incurvarioidea) has resulted from multiple host shifts. In the case of fig pollination, it has often been suggested that patterns of host association are indicative of cospeciation (Ramirez 1974, Corner 1985, Wiebes 1987, Compton 1996). Reconstructing the evolution of host associations bears directly on this question. The cospeciation hypothesis predicts that host associations are conserved through evolutionary time but, on the other hand, host shifts result in homoplasy. Since Wiebes (1994a) relied on the botanical classification of Ficus as a guide in the placement of wasp species within genera, it is possible that congruence between pollinator classification and patterns of host association could be little more than a taxonomic artifact. However, the case for cospeciation in pollinator and host lineages is strengthened by evidence of monophyletic groups with conserved host associations. The evidence is even more compelling when clades without previous taxonomic designation show conservative associations.

Agreement between pollinator and fig classifications is generally supported by phylogenetic inferences from pollinator mtDNA and morphology (Figures 8-9). The host associations of pollinators showed less homoplasy (CI = 0.75 for host subgenera; CI =

0.80 for host sections) than either mtDNA or morphological characters (overall CI = 0.29 and 0.28, respectively). However, a number of exceptional cases are worthy of discussion, beginning with the association between paraphyletic Dolichoris and sect. Oreosycea in subg. Pharmacosycea. Fossil evidence and molecular phylogenetic studies (Chapter 1; Herre et al. 1996) suggest that Pantropical subg. Pharmacosycea is not monophyletic and, furthermore, that Paleotropical sect. Oreosycea is paraphyletic. The pollinators of sect. Oreosycea are also paraphyletic. From Figure 10, we infer that the ancestral host association of Paleotropical pollinators was with an Oreosycea fig. Although the associations with Ficus sections are equivocal at many internal nodes, pollinator phylogeny indicates that a single host shift from monoecious sect. Oreosycea to dioecious subg. Ficus sensu Corner was followed by multiple shifts to monoecious subg. Urostigma and Sycomorus (Figure 9). In this scenario, pollinators of dioecious figs (subg. Ficus) gave rise to the pollinators of monoecious strangler figs (subg. Urostigma). The conflict between this scenario and fig phylogeny is noteworthy, although the clades of interest are not well supported in either case. Conflicting scenarios for the evolution of dioecious fig pollination are discussed in Chapter 3.

Several monophyletic genera of pollinators are uniquely associated with host sections such as Blastophaga with sect. Ficus, Platyscapa with sect. Urostigma, and Pleistodontes with sect. Malvanthera. However, the apparent conservatism of host associations in each of the three clades could be spurious if the taxonomic limits of pollinator genera were set by host classification. Pollination of sect. Conosycea by a Eupristina-Waterstoniella clade provides additional evidence of conservatism in this regard. Also, a monophyletic Wiebesia is associated with a clade of dioecious root climbers (sects. Rhizocladus and Kalosyce; Chapter 1) and in particular, the W. brusi plus W. frustrata clade is associated with sect. Rhizocladus. Pollination of sect. Sycidium by the Kradibia-Liporrhopalum clade also supports the notion of conservatism. Paraphyletic

Kradibia is associated with paraphyletic subsect. Sycidium due to the derived position of Liporrhopalum.

Every instance of homoplasy in pollinator associations with Ficus sections could be attributed to the Ceratosolen clade. The genus was more intensively sampled in this study due to the complexity of host associations in subg. Ceratosolen, Rothropus, and Strepitus. One of three most parsimonious trees from the combined analysis indicated that the ancestral host for Ceratosolen was sect. Neomorphe (Figure 10) but the other two trees were equivocal in this respect (not shown). In the absence of information about host phylogeny, it is tempting to conclude from Figure 10 that two host shifts from sect. Neomorphe to sect. Sycocarpus have occurred independently. However, sect. Sycocarpus is not monophyletic (Chapter 1) and the inference of multiple host switching events from fig classification alone is misleading in this regard. Two additional examples illustrate how botanical classification can confuse the inference of shifts in pollinator associations.

The reconstruction of sect. Neomorphe as an ancestral host for Ceratosolen results from the position of C. grandii in relation to the pollinators of sect. Adenosperma. As discussed in the first chapter, C. grandii is associated with F. semivestita, which Corner (1960b) assigned to sect. Neomorphe based on incomplete collections from New Guinea. A complete description of morphology together with ITS sequence data suggest that F. semivestita is more closely related to sect. Adenosperma than to sect. Neomorphe (Chapter 1). Wiebes (1963b) recognized similarities between C. grandii and C. appendiculatus (also associated with sect. Neomorphe) in two features; the absence of cerci in male genitalia and the fusion of three apical segments of the female antennae into a club. It is noteworthy that separate and combined phylogenetic analyses of pollinator morphology and mtDNA indicate that C. grandii is more closely related to the pollinators of sect. Adenosperma than to C. appendiculatus. The morphological similarities between C. grandii and C. appendiculatus appear to reflect convergence as both characters are

homoplasious (CI = 0.09 and 0.18, respectively). A revised classification placing F. semivestita in sect. Adenosperma would alter the inference of an ancestral host for Ceratosolen from sect. Neomorphe to an equivocal situation involving four different sections.

A similar case of mistaken identity having a strong effect on inferences of host switching involves C. nanus, the pollinator of F. pungens in sect. Sycidium. From the reconstruction in Figure 10, we infer that the C. nanus lineage switched from sect. Sycocarpus to sect. Sycidium. Wiebes (1963b) asserted that pollinator undoubtedly belonged to Ceratosolen in spite of its association with Kradibia-pollinated subsect. Sycidium. However, phylogenetic analyses based on either molecular or morphological data indicate that F. pungens is more closely related to sect. Sycocarpus than to sect. Sycidium (Chapter 1). If the spurious placement of F. pungens in sect. Sycidium was corrected in a revised classification of Ficus, we would no longer infer an ancestral host shift in the case of C. nanus. These examples illustrate how important it is to consider the potential for host phylogeny to affect inferences on the conservatism or lability of pollinator associations. In addition to laying the groundwork for an improved classification, the combined phylogenetic results for pollinators and hosts will provide more substantive insights into coevolution (Chapter 3).

The pollinators of subg. Sycomorus present an additional problem with regard to the evolution of host associations. Ancestral state reconstruction in Ceratosolen suggests two shifts from subg. Ficus to Sycomorus (Figure 9); once from sect. Neomorphe and again from sect. Sycocarpus (Figure 10). Given that Sycomorus is monophyletic (Chapter 1), it would appear that the C. blommersi lineage represents a secondary switch to subg. Sycomorus. However, the sister relationship of C. blommersi from Madagascar and C. vissali from the Solomon Islands is poorly supported and seems questionable based on their geographic separation. A close relationship between such widely separated island endemics does not seem as plausible as the better-supported relationship

between the other pollinators of subg. Sycomorus; namely, widespread Indo-Australian C. fusciceps and African C. capensis. Implausible relationships, such as C. blommersi plus C. vissali could result from the failure of phylogenetic methods or mtDNA to accurately reveal species phylogeny. In addition to having the most diverged mtDNA sequences in the genus (Table 4), C. blommersi plus C. vissali are sister to a Rothropus clade showing an accelerated rate of nucleotide substitution (Machado 1998). The possibility of inconsistency (i.e. long-branch attraction; Felsenstein 1978) in the placement of C. blommersi and C. vissali could be tested in future studies using parametric bootstrapping methods (Huelsenbeck and Hillis 1996).

Lastly, clades including Rothropus and Strepitus further support the overall pattern of conservatism in the evolution of host associations. Strepitus is monophyletic with the exception of C. vissali, and is associated with a possible clade of Sycocarpus spp. (Figure 9 in Chapter 1). A clade including Rothropus plus C. bisulcatus is also associated with monophyletic subsect. Sycocarpus. In general, the inference of ancestral host associations based on pollinator phylogeny provides little evidence of host switching as a major factor in the evolution of the fig/pollinator mutualism. Pollinator phylogeny supports instances of taxonomic congruence at several levels and provides additional evidence in support of the cospeciation hypothesis. The following chapter will test models of cospeciation and scenarios for host switching in a broader phylogenetic framework.

Table 1: Classification of Malesian Agaoninae and their host figs from Wiebes (1994). Host associations below the level of Ficus sections are listed only for the dioecious figs. Conflicts between the botanical and entomological classifications are also noted.

genus (subgenus)	Ficus subgenus	section	subsect. or series
Dolichoris	Pharmacosycea	Oreosycea	-
Pleistodontes	Urostigma	Malvanthera	-
	"	Stilpnophyllum	-
Platyscapa	"	Urostigma	-
Deilagaon	"	Conosycea	-
Waterstoniella	"	"	-
Eupristina (Eupristina)	"	"	-
Eupristina (Parapristina)	"	Leucogyne	-
Blastophaga (Blastophaga)	Ficus	Ficus[1]	Ficus
Blastophaga (Valisia)	"	"	Eriosycea
Wiebesia[2]	"	Kalosyce	
	"	Rhizocladus	
Liporrhopalum	"	Sycidium	Paleomorphe
Kradibia	"	"	Sycidium[3]
	"	"	Varinga[4]
Ceratosolen (Ceratosolen)	"	Neomorphe	
	"	Sycocarpus	Sycocarpus[5]
	"	Sycidium	Prostratae
	"	"	Pungentes
	"	"	Phaeopilosae[6]
	"	"	Cyrtophyllae[4]
	"	Sycomorus[7]	
Ceratosolen (Rothropus)	"	Sycocarpus	Sycocarpus[5]
Ceratosolen (Strepitus)	"	"	Auriculisperma
	"	"	Dammaropsis
	"	"	Papuasyce[8]
	"	Ficus	Rivulares[1]
	"	"	Pseudopalmae[1]

[1] All Blastophaga are associated with sect. Ficus except for ser. Rivulares and ser. Pseudopalmae, which are pollinated by Ceratosolen (Strepitus) (see *Discussion* in Chapter 1).

[2] All Wiebesia are associated with sects. Kalosyce and Rhizocladus except for the type species, W. partita Boucek, which pollinates the New Guinea endemic F. primaria (sect. Sycidium ser. Copiosae). By assigning all of the pollinators of sects. Kalosyce and Rhizocladus to a single genus Wiebes (1993a) improved the match between the pollinator and host classifications.

[3] Most species in subsect. Sycidium are pollinated by Kradibia except for Liporrhopalum-pollinated F. montana (ser. Copiosae) and two other cases[2,6].

[4] Subsect. Varinga is pollinated by Kradibia with the exception of Ceratosolen-pollinated F. asperiuscula (ser. Cyrtophylleae).

[5] All species in subsect. Sycocarpus are pollinated by subg. Rothropus with two exceptions: F. hispida and F. treubii are pollinated by subg. Ceratosolen.

[6] In series Phaeopilosae, F. complexa is pollinated by Ceratosolen and Kradibia pollinates F. conocephalifolia.

[7] Sect. Sycomorus sensu Berg and Wiebes (1992). Ramirez (1977) proposed an expanded subg. Sycomorus including all species pollinated by Ceratosolen (see *Discussion* in Chapter 1).

[8] In subsect. Papuasyce, all species are pollinated by subg. Strepitus except for F. pritchardii. This monoecious species was placed with F. microdictya in subsect. Papuasyce on the basis of breeding system alone (Corner 1970). However, other botanical characters and pollinator associations (Wiebes 1963) suggest that F. pritchardii may be more closely related to F. pungens.

Table 2: Fig wasps (Agaoninae) selected for phylogenetic analysis. Subgenera are abbreviated (C)eratosolen, (P)arapristina, (R)othropus, (S)trepitus, and (V)alisia. Informal names of seven new species are introduced; descriptions are in prep.

Pollinator	Ficus species	section
Blastophaga (V.) intermedia Grandi	padana	Ficus
Blastophaga (V.) malayana Wiebes	grossularioides	Ficus
Ceratosolen (C.) appendiculatus Mayr	variegata	Neomorphe
Ceratosolen (C.) bisulcatus (Mayr)	septica	Sycocarpus
Ceratosolen (C.) blommersi Wiebes	botryoides	Sycomorus
Ceratosolen (C.) capensis Grandi	sur	Sycomorus
Ceratosolen (C.) emarginatus Mayr	auriculata	Neomorphe
Ceratosolen (C.) fusciceps (Mayr)	racemosa	Sycomorus
Ceratosolen (C.) grandii Wiebes	semivestita	Neomorphe
Ceratosolen (C.) medlerianus Wiebes	mollior	Adenosperma
Ceratosolen (C.) nanus Wiebes	pungens	Sycidium
Ceratosolen (C.) nexilis Wiebes	nodosa	Neomorphe
Ceratosolen (C.) sp. "riparianus"	ochrochlora	Adenosperma
Ceratosolen (C.) cf. nexilis Wiebes	robusta	Neomorphe
Ceratosolen (R.) corneri Wiebes	botryocarpa	Sycocarpus
Ceratosolen (R.) dentifer Wiebes	hispidioides	Sycocarpus
Ceratosolen (R.) hooglandi Wiebes	bernaysii	Sycocarpus
Ceratosolen (R.) vechti Wiebes	lepicarpa	Sycocarpus
Ceratosolen (S.) abnormis (Wiebes)	dammaropsis	Sycocarpus
Ceratosolen (S.) armipes Wiebes	itoana	Sycocarpus
Ceratosolen (S.) sp. "kaironkensis"	microdictya	Sycocarpus
Ceratosolen (S.) vissali Wiebes	theophrastoides	Sycocarpus
Dolichoris inornata Wiebes	edelfeltii	Oreosycea
Dolichoris sp. "hombronianae"	hombroniana	Oreosycea
Dolichoris vasculosae Hill	vasculosa	Oreosycea
Eupristina (P.) verticillata Waterson	microcarpa	Conosycea
Kradibia copiosae (Wiebes)	copiosa	Sycidium
Kradibia jacobsi (Wiebes)	conocephalifolia	Sycidium
Kradibia sp. "ohuensis"	trachypison	Sycidium
Kradibia sp. "salembensis"	phaeosyce	Sycidium
Kradibia wassae (Wiebes)	wassa	Sycidium
Liporrhopalum cf. gibbosae Hill	tinctoria	Sycidium
Liporrhopalum virgatae Hill	virgata	Sycidium
Platyscapa corneri Wiebes	superba	Urostigma
Platyscapa fischeri Wiebes	prasinicarpa	Urostigma
Pleistodontes plebejus Wiebes	hesperidiiformis	Malvanthera
Pleistodontes rieki Wiebes	xylosycia	Malvanthera
Pleistodontes rigisamos Wiebes	destruens	Malvanthera
Waterstoniella brevigena Wiebes	pellucidopunctata	Conosycea
Waterstoniella sp. "dubium"	dubia	Conosycea
Wiebesia sp. "brusi"	baeuerlenii	Rhizocladus
Wiebesia sp. "frustrata"	odoardi	Rhizocladus
Wiebesia punctatae Wiebes	punctata	Kalosyce

Table 3: List of primers for amplification and sequencing of cytochrome oxidase I and II. Locations correspond to the mitochondrial genome of Drosophila yakuba (Clary and Wolstenholme 1985). Except for Marcus and Brus, primers were compiled from published sources (Roehrdanz 1993, Simon et al. 1994, Machado 1998).

alias	location	sequence	source
Juan	S1514	5'-ACCAATCATAAAAATATTGG-3'	(Farrell)
Marcus	S1540	5'-ATATTTAATTTTTGGAAGATGAGC-3'	(Weiblen)
Brus	A1891	5'-GAAGMTAAAGGAGGGTAWACAG-3'	(Weiblen)
New Jerry	S2195	5'-TTGATTTTTTGGTCATCCGAAT-3'	(Roehrdanz)
Nancy	A2216	5'-CCCGGTAAAATTAAAATATAAACTTC-3'	(Harrison)
Rev. Dick	A2410	5'-GCTAATCATCTAAAAATTTTAATTCCTGTTGG-3'	(Crespi)
sw2618	S2918	5-'GCTCATTTTCATTATGTTYTATCTATRGG-3'	(Machado)
sw2642	S2642	5'-GGAGCWGTTTTTGCAATTTTTGGWAG-3'	(Machado)
Pat	A3014	5'-TCCAATGCACTAATCTGCCATATTA-3'	(Harrison)
Maryln	A3389	5'-TCATAAGTTCAATATCATTG-3'	(Harrison)

Table 4: Genetic distances among 20 Ceratosolen species. Species are numbered according to the sequence in Table 2. Above the diagonal are uncorrected (p) distances and below the diagonal are Tamura-Nei distances for transversions only.

3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
-	0.25	0.17	0.27	0.22	0.20	0.29	0.26	0.21	0.21	0.17	0.26	0.17	0.20	0.25	0.25	0.22	0.23	0.28	0.26
0.21	-	0.24	0.26	0.20	0.20	0.28	0.25	0.19	0.20	0.24	0.26	0.23	0.22	0.26	0.24	0.25	0.22	0.30	0.24
0.14	0.20	-	0.27	0.22	0.20	0.28	0.25	0.20	0.20	0.16	0.25	0.12	0.19	0.25	0.24	0.23	0.23	0.29	0.27
0.25	0.24	0.26	-	0.23	0.25	0.13	0.12	0.22	0.25	0.25	0.13	0.26	0.23	0.19	0.26	0.25	0.24	0.14	0.26
0.19	0.16	0.17	0.22	-	0.18	0.25	0.23	0.21	0.21	0.22	0.23	0.21	0.22	0.23	0.22	0.25	0.23	0.26	0.24
0.17	0.15	0.17	0.24	0.14	-	0.26	0.23	0.17	0.15	0.18	0.24	0.19	0.19	0.24	0.21	0.23	0.21	0.26	0.24
0.27	0.26	0.25	0.09	0.22	0.24	-	0.14	0.24	0.27	0.26	0.14	0.28	0.25	0.19	0.27	0.27	0.25	0.14	0.28
0.25	0.24	0.23	0.10	0.22	0.22	0.10	-	0.20	0.24	0.23	0.08	0.24	0.23	0.16	0.24	0.25	0.23	0.09	0.27
0.18	0.17	0.17	0.20	0.17	0.15	0.21	0.18	-	0.18	0.20	0.21	0.20	0.22	0.23	0.19	0.24	0.21	0.22	0.25
0.18	0.15	0.17	0.24	0.17	0.11	0.25	0.22	0.16	-	0.18	0.23	0.20	0.20	0.24	0.22	0.23	0.20	0.26	0.24
0.14	0.19	0.13	0.24	0.18	0.16	0.25	0.21	0.17	0.15	-	0.24	0.16	0.17	0.24	0.23	0.20	0.22	0.26	0.25
0.24	0.22	0.22	0.10	0.21	0.21	0.10	0.05	0.19	0.21	0.22	-	0.25	0.23	0.16	0.26	0.26	0.23	0.08	0.27
0.13	0.18	0.07	0.24	0.16	0.16	0.25	0.22	0.17	0.16	0.13	0.22	-	0.18	0.24	0.23	0.22	0.22	0.28	0.25
0.15	0.19	0.15	0.21	0.19	0.16	0.23	0.21	0.20	0.18	0.14	0.20	0.14	-	0.23	0.22	0.17	0.23	0.25	0.27
0.23	0.25	0.23	0.16	0.21	0.22	0.15	0.13	0.22	0.22	0.22	0.12	0.22	0.20	-	0.25	0.25	0.23	0.17	.026
0.24	0.21	0.22	0.24	0.18	0.19	0.25	0.23	0.16	0.19	0.20	0.24	0.20	0.20	0.23	-	0.24	0.10	0.28	0.24
0.18	0.24	0.20	0.23	0.22	0.21	0.25	0.23	0.24	0.22	0.16	0.23	0.18	0.14	0.23	0.23	-	0.26	0.28	0.26
0.21	0.19	0.20	0.23	0.20	0.19	0.23	0.23	0.18	0.19	0.20	0.22	0.20	0.21	0.22	0.08	0.25	-	0.25	0.25
0.28	0.28	0.28	0.12	0.26	0.26	0.10	0.06	0.20	0.25	0.27	0.06	0.27	0.25	0.13	0.27	0.28	0.25	-	0.27
0.21	0.18	0.24	0.25	0.19	0.20	0.28	0.27	0.22	0.21	0.22	0.26	0.22	0.23	0.25	0.23	0.23	0.22	0.28	-

Table 5: Log likelihood ratio tests comparing models of molecular evolution for mtDNA from Malesian pollinators of figs. Results are listed for models including JC (Jukes and Cantor 1969), F81 (Felsenstein 1981), HKY85 (Hasegawa et al. 1985), and GTR (Rodriguez et al. 1990). The addition of parameters for heterogeneity of substitutions across sites (Γ; Yang 1994b) and for the proportion of invariant sites (I) were also tested. Other models assuming equal base frequencies were rejected and are not listed (i.e. Kimura 1980) and Zharkikh 1994). Significance was evaluated at $\alpha = 0.01$ following a Bonferroni correction for multiple tests.

H_0	H_1	-ln L_0	-ln L_1	df	X^2	p
JC	F81	40578.06	39009.28	1	3137.56	<0.0001
F81	HKY	39009.28	38994.12	1	30.32	<0.01
HKY	GTR	38994.12	38104.28	4	1779.68	<0.0001
GTR	GTR+Γ	38104.28	35227.26	1	5754.01	<0.0001
GTR+Γ	GTR+Γ+I	35227.26	35223.35	1	7.81	n.s.

Table 6: **(A)** Kishino-Hasegawa test results for incongruence between trees obtained by maximum likelihood (ML), neighbor joining (NJ) and parsimony (MP) analyses of mtDNA from Malesian pollinators of figs. The log-likelihood scores of trees obtained from morphological and combined analyses were also compared to the mtDNA ML tree. **(B)** Templeton test results for incongruence between mtDNA, morphology, and combined data sets for Malesian pollinators of figs.

(A) mtDNA (ML tree) vs.	-ln L	-ln L diff	sd diff	T	p
mtDNA NJ tree	31591.28	70.85	24.99	2.8346	0.0046
mtDNA MP tree	31533.66	13.22	21.54	0.6138	n.s.
morphology MP tree	33007.14	1486.70	86.38	17.2102	<0.0001
combined MP tree	31536.72	16.28	23.09	0.7049	n.s.
(B) mtDNA (MP tree) vs.	L	rank sum	N	z	p
mtDNA NJ tree	6885	-25732.0	356	-3.3686	0.0008
mtDNA ML tree	6865	-19101.0	304	-2.9374	0.0033
morphology MP tree	7616	-20463.0	612	-17.1837	<0.0001
morphology strict consensus	7229	-24013.0	493	-12.1606	<0.0001
morphology 50% bootstrap	6858	-11354.5	238	-2.9969	0.0027
morphology 70% bootstrap	6839	-23174.0	321	-1.7671	n.s.
morphology 90% bootstrap	6806	0	0	-	-
combined strict consensus	6818	-3683.0	127	-1.0525	n.s.
morphology vs. rival	L	rank sum	N	z	p
mtDNA NJ tree	393	-25.5	39	-5.1452	<0.0001
mtDNA ML tree	382	-47.5	41	-5.0460	<0.0001
mtDNA MP tree	378	-57.5	41	-4.9379	<0.0001
mtDNA 50% bootstrap	351	-76.0	31	-3.4483	0.0006
mtDNA 70% bootstrap	341	-154.5	35	-2.7213	0.0065
mtDNA 90% bootstrap	337	-253.0	41	-2.4335	0.0150
combined strict consensus	356	-88.5	35	-3.8079	<0.0001

Table 7: Log-likelihood scores of mtDNA data from alternative tree topologies for the Malesian pollinators of figs. GTR+Γ model parameters were estimated separately for each codon position and then used to calculate the likelihood of the MP and ML topologies under each model (Figures 5 and 6).

	-ln L ML topology	-ln L MP topology	-ln L diff
overall	31520.43815	31533.66028	13.2221
first positions only	9559.80628	9601.19740	41.3911
second positions only	6384.54866	6414.69526	30.1466
third positions only	14287.53856	14282.85396	-4.6846

Figure 1: Ceratosolen nexilis Wiebes, the pollinator of Ficus nodosa Teysm. et Binn. (**A**) winged female (**B**) wingless male. Scale is 1 mm. (**C**) Cauliflorous figs in dioecious F. nodosa. A fig (syconium) in cross-section shows the bract-filled opening (ostiole) and hundreds of minute florets on the interior of the receptacle. Scale is 1 cm. (**D**) Life cycle diagram illustrating the interdependence of C. nexilis and F. nodosa. Due to differences in the style lengths of fig flowers, pollinators and seeds mature separately in two types of figs.

Figure 2: Null distributions for the incongruence length difference test (ILD; Farris et al. 1994) for (**A**) COI and COII genes, and (**B**) mtDNA and morphology from Malesian pollinators of figs. Arrows indicate the sum of tree lengths for the separate data partitions. Null distributions represent the sum of tree lengths from random partitions of the combined data where the size of each partition is equal to the number of characters in the observed partitions. COI and COII genes were not significantly heterogeneous ($P = 0.15$) but mtDNA and morphology rejected the null hypothesis of congruence ($P = 0.01$).

Figure 3: Pairwise genetic distance (uncorrected p') versus the absolute number of transitions and transversions for different codon positions in mitochondrial DNA sequences (COI and COII) from Malesian pollinators of figs. Open circles indicate transitions and points indicate transversions.

Figure 4: mtDNA phylogeny for Malesian pollinators of figs based on the neighbor joining (NJ) method using Tamura-Nei genetic distances and transversions only. The NJ tree was used to estimate maximum likelihood parameters for the GTR+Γ model of nucleotide substitution and as a starting tree for heuristic searches under maximum likelihood.

Figure 5: The single most parsimonious tree for mitochondrial DNA sequences (COI and COII) from Malesian pollinators of figs. The tree was rooted with non-pollinating Apocryptophagus spinitarsus (Sycophaginae). Bootstrap percentages and decay values excluding uninformative characters are listed above and below the branches, respectively.

Figure 6: Maximum likelihood phylogram for mitochondrial DNA sequences (COI and COII from Malesian pollinators of figs. The tree resulted from a heuristic search assuming a GTR+Γ model of nucleotide substitution with parameters estimated under maximum likelihood using the NJ tree (Figure 4). The ML tree was rooted with non-pollinating Apocryptophagus spinitarsus (Sycophaginae).

Figure 7: The strict consensus of 256 equally parsimonious trees for morphological data from Malesian pollinators of figs. Bootstrap percentages and decay values excluding uninformative characters are listed above and below the branches, respectively. Closed circles indicate those nodes that are congruent with the mtDNA MP tree (Figure 5). Open circles indicate conflicting nodes.

Figure 8: The strict consensus of three equally parsimonious trees for mtDNA sequences and morphological data from Malesian pollinators of figs. Bootstrap percentages and decay values excluding uninformative characters are listed above and below the branches, respectively.

Figure 9: The evolution of host associations in Malesian pollinators of figs reconstructed on one of the most parsimonious trees from the combined analysis of mtDNA and morphology. The classification of Ficus subgenera follows Corner (1965).

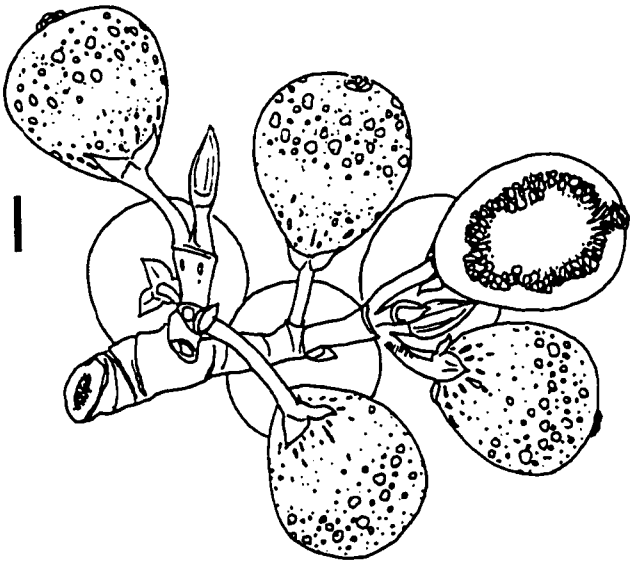
Figure 10: The evolution of host associations in Malesian pollinators of figs reconstructed on one of the most parsimonious trees from the combined analysis of pollinator mtDNA and morphology. Ficus sections sensu Corner (1965) are color coded.

Figure 1

A. Ceratosolen nexilis Wiebes (female)

B. Ceratosolen nexilis Wiebes (male)

C. Ficus nodosa Teysm. et Binn.



D. Life cycles of Ceratosolen nexilis and Ficus nodosa

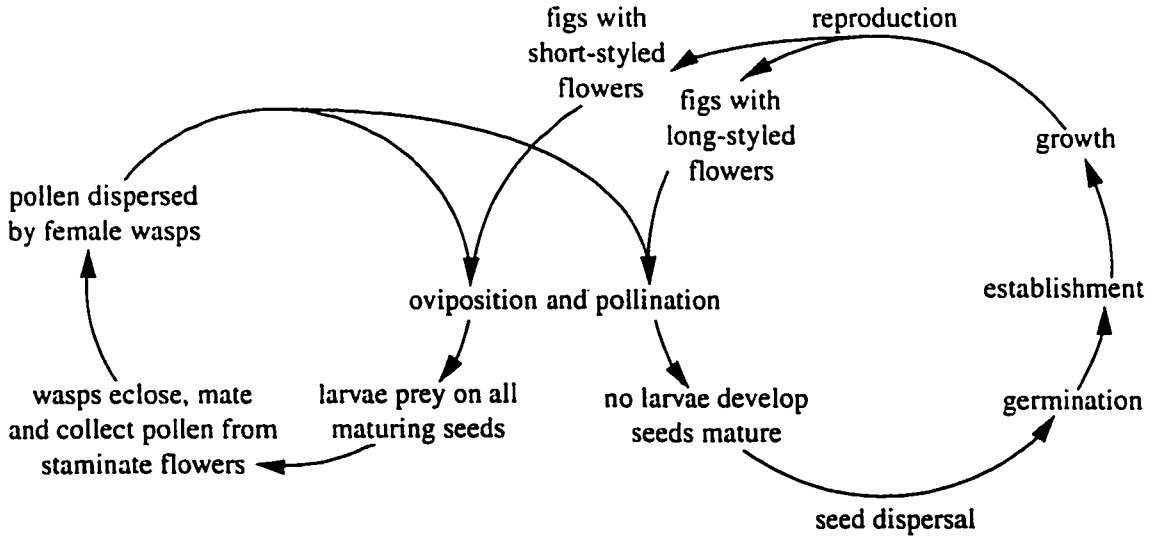


Figure 2A: COI vs. COII P = 0.15

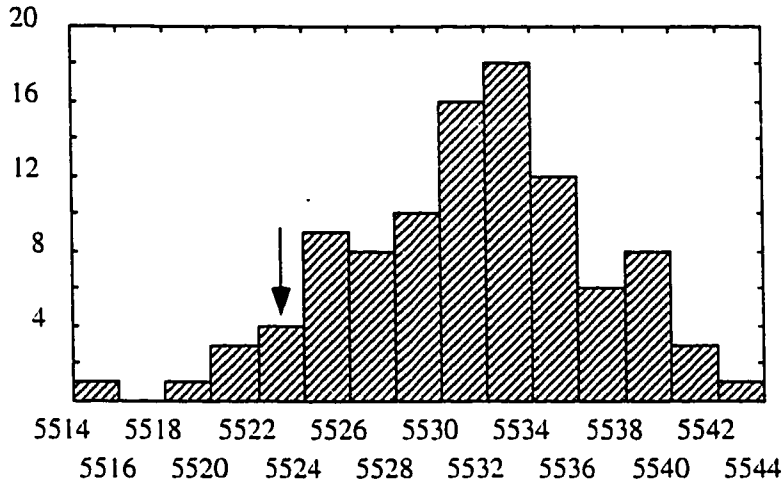


Figure 2B: mtDNA vs. morphology P = 0.01

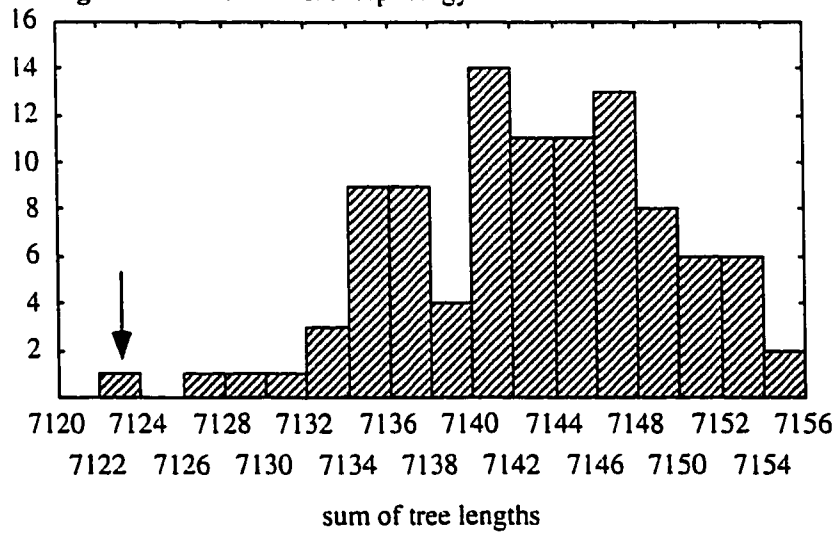


Figure 3

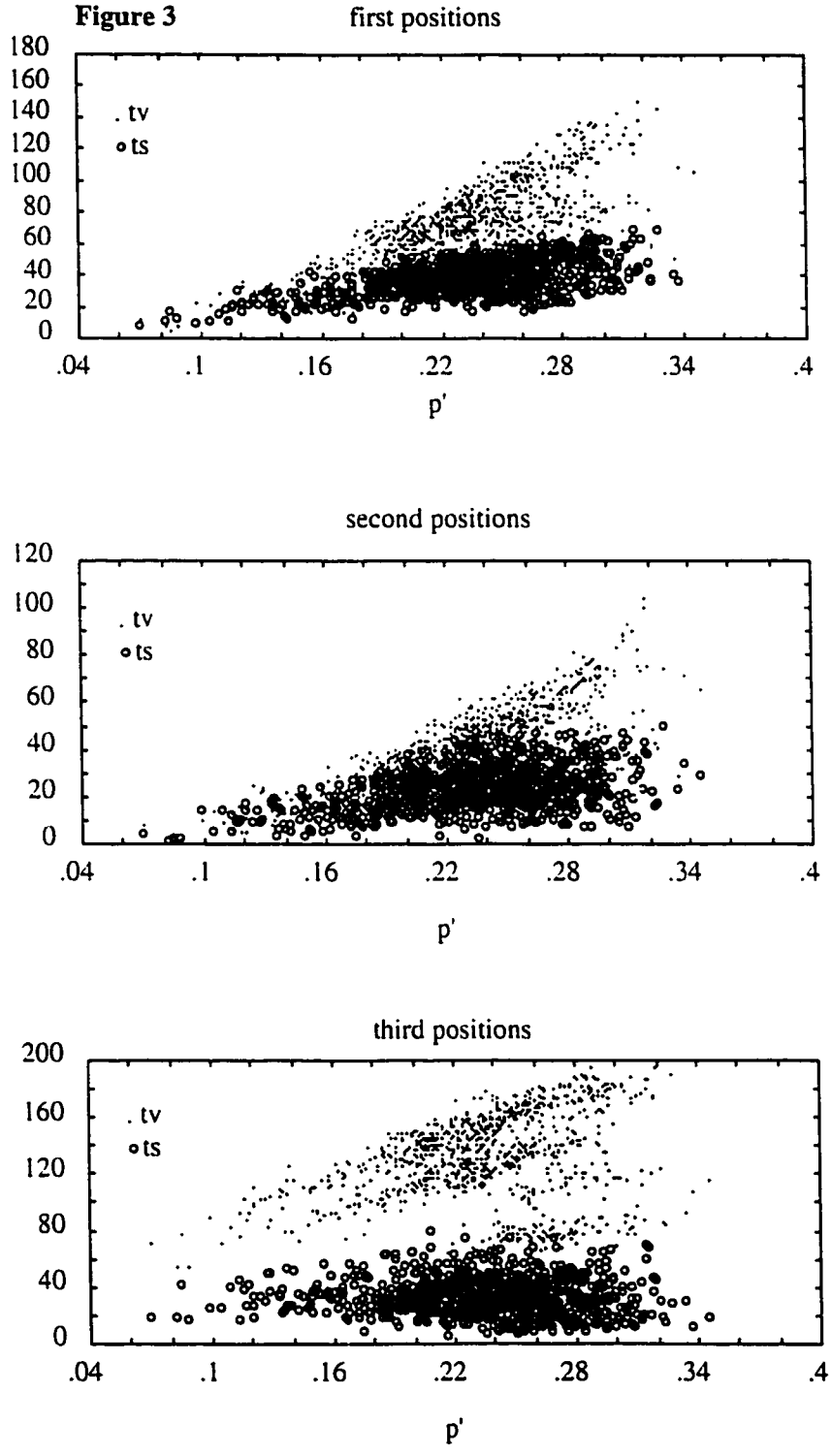

first positions
180
160
140
120
100
80
60
40
20
0
. tv
o ts
.04
.1
.16
.22
.28
.34
.4
p'
second positions
120
100
80
60
40
20
0
. tv
o ts
.04
.1
.16
.22
.28
.34
.4
p'
third positions
200
160
120
80
40
0
. tv
o ts
.04
.1
.16
.22
.28
.34
.4
p'

Figure 4: mtDNA NJ phylogram

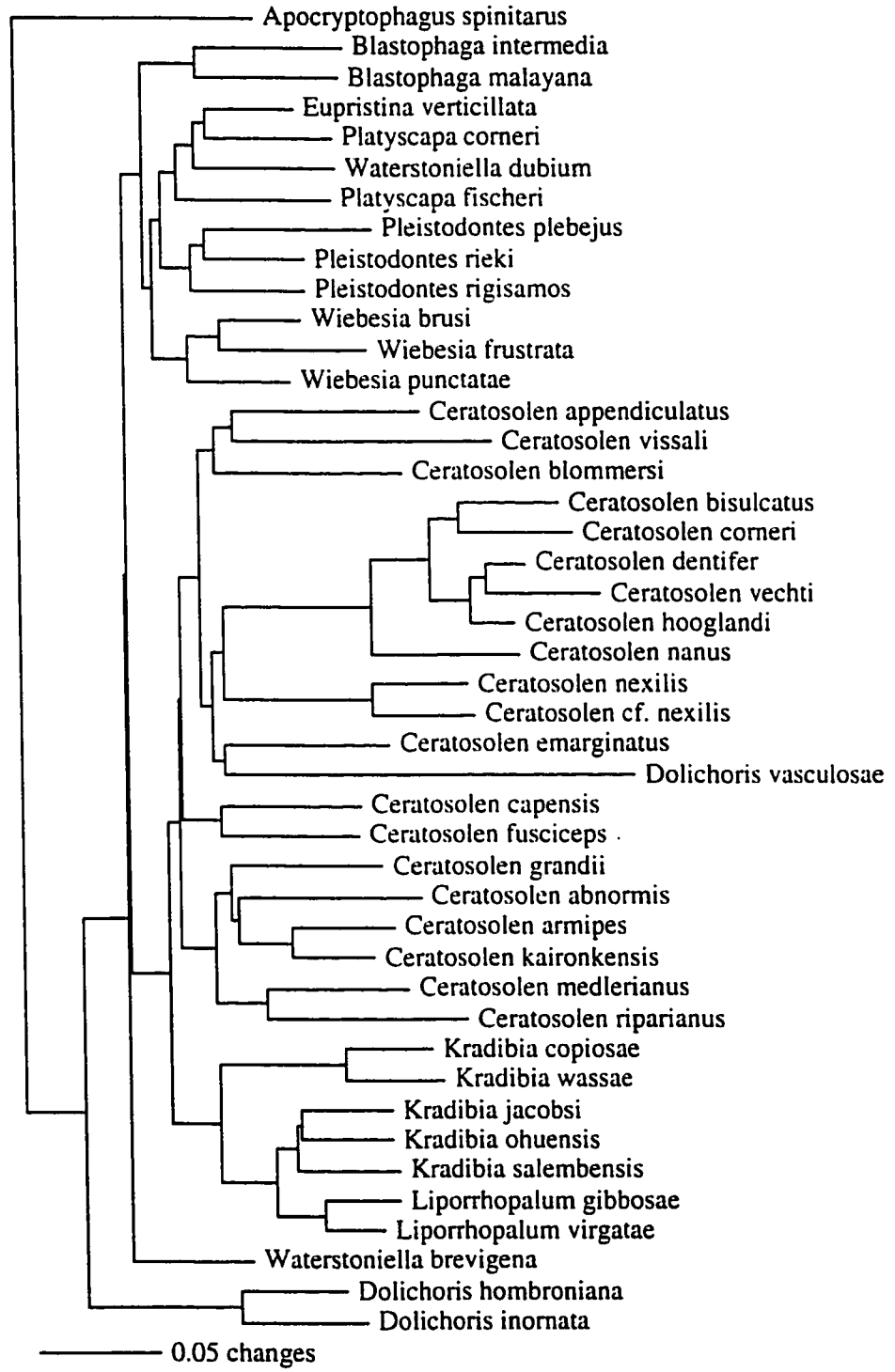


Figure 5: mtDNA MP tree

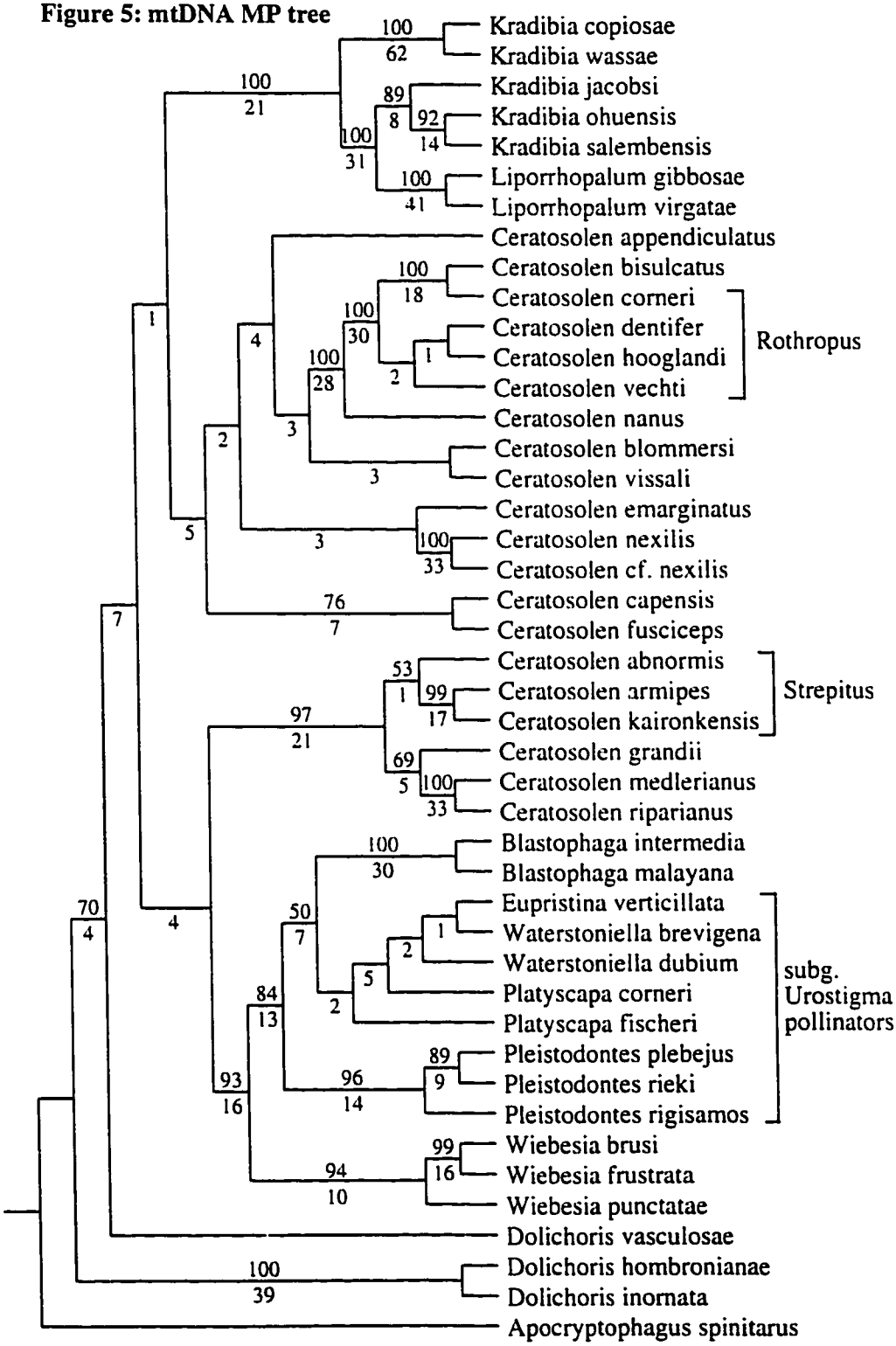


Figure 6: mtDNA ML phylogram

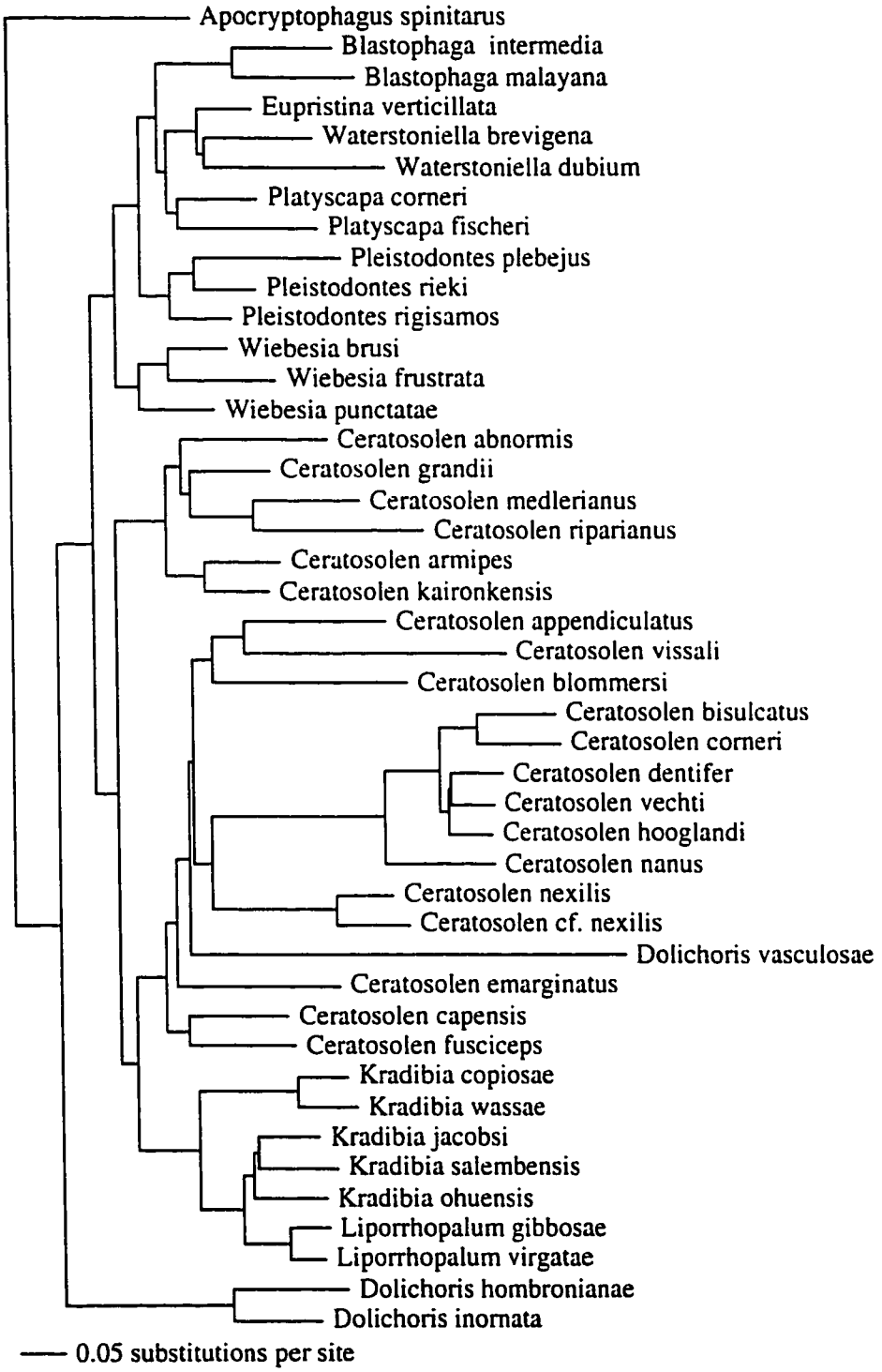


Figure 7: morphology

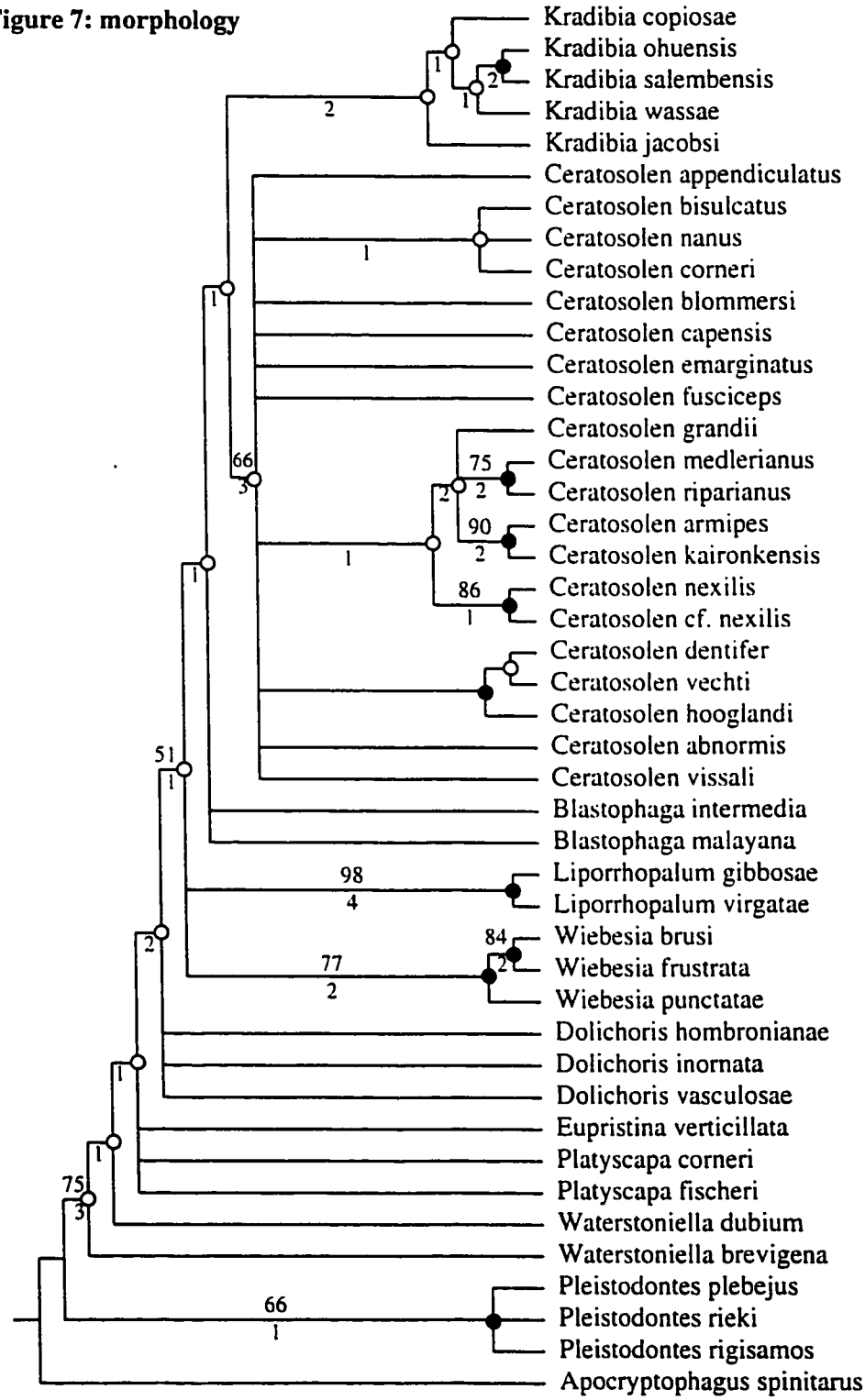


Figure 8: combined

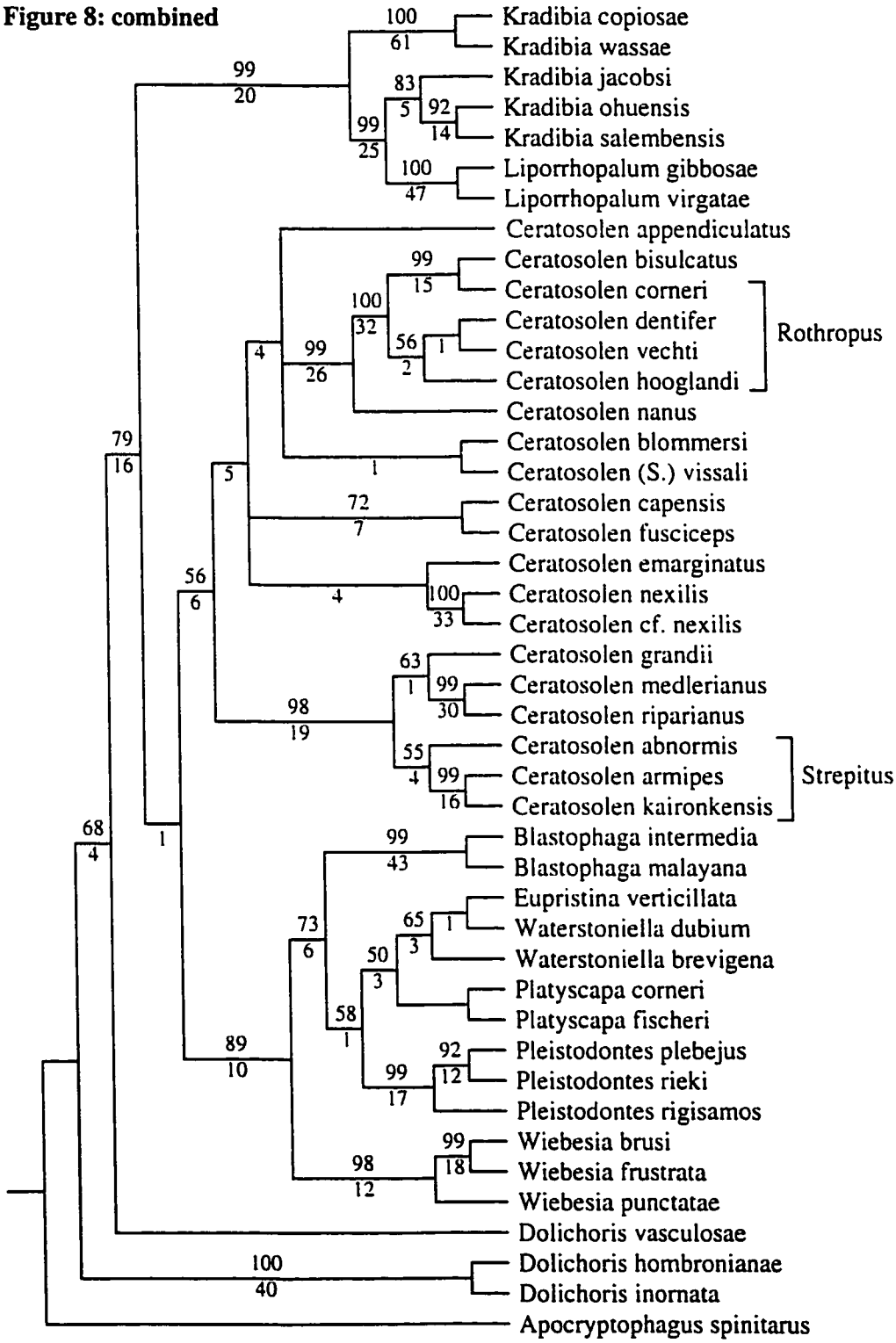


Figure 9

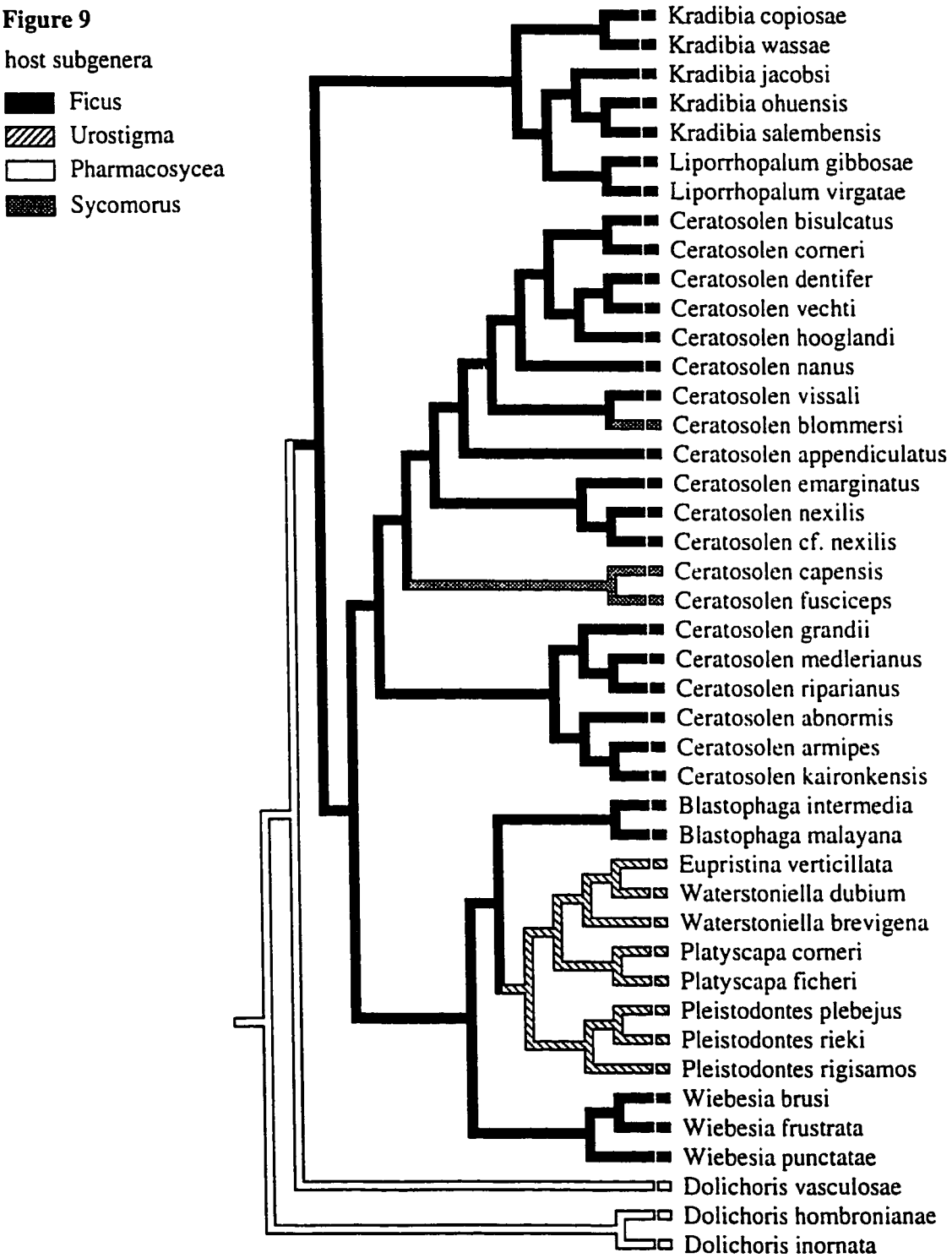
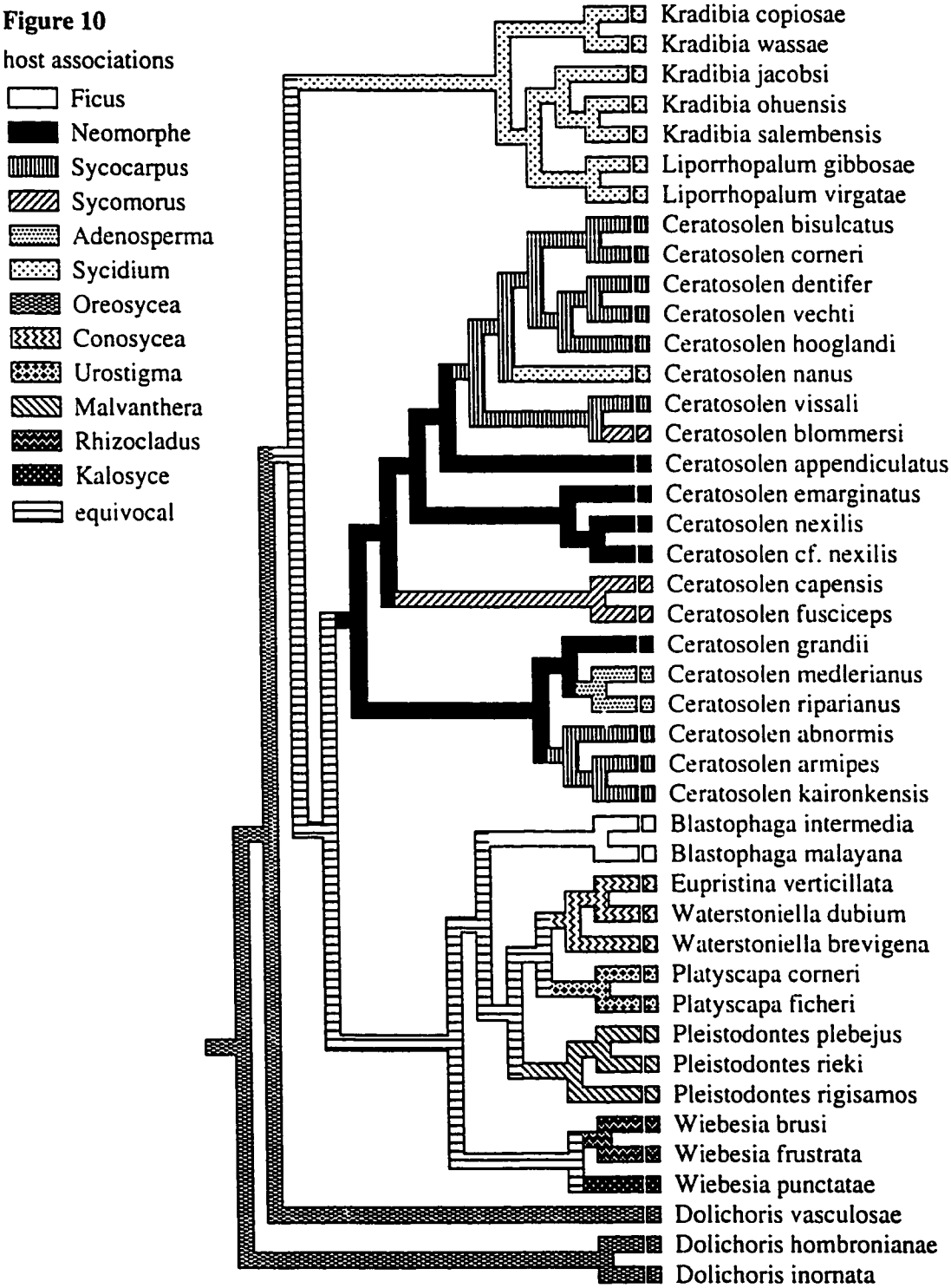

host subgenera
Ficus
Urostigma
Pharmacosycea
Sycomorus
Kradibia copiosae
Kradibia wassae
Kradibia jacobsi
Kradibia ohuensis
Kradibia salembensis
Liporrhopalum gibbosae
Liporrhopalum virgatae
Ceratosolen bisulcatus
Ceratosolen corneri
Ceratosolen dentifer
Ceratosolen vechti
Ceratosolen hooglandi
Ceratosolen nanus
Ceratosolen vissali
Ceratosolen blommersi
Ceratosolen appendiculatus
Ceratosolen emarginatus
Ceratosolen nexilis
Ceratosolen cf. nexilis
Ceratosolen capensis
Ceratosolen fusciceps
Ceratosolen grandii
Ceratosolen medlerianus
Ceratosolen riparianus
Ceratosolen abnormis
Ceratosolen armipes
Ceratosolen kaironkensis
Blastophaga intermedia
Blastophaga malayana
Eupristina verticillata
Waterstoniella dubium
Waterstoniella brevigena
Platyscapa corneri
Platyscapa ficheri
Pleistodontes plebejus
Pleistodontes rieki
Pleistodontes rigisamos
Wiebesia brusi
Wiebesia frustrata
Wiebesia punctatae
Dolichoris vasculosae
Dolichoris hombronianae
Dolichoris inornata

Figure 10


host associations
Ficus
Neomorphe
Sycocarpus
Sycomorus
Adenosperma
Sycidium
Oreosycea
Conosycea
Urostigma
Malvanthera
Rhizocladus
Kalosyce
equivocal
Kradibia copiosae
Kradibia wassae
Kradibia jacobsi
Kradibia ohuensis
Kradibia salembensis
Liporrhopalum gibbosae
Liporrhopalum virgatae
Ceratosolen bisulcatus
Ceratosolen corneri
Ceratosolen dentifer
Ceratosolen vechti
Ceratosolen hooglandi
Ceratosolen nanus
Ceratosolen vissali
Ceratosolen blommersi
Ceratosolen appendiculatus
Ceratosolen emarginatus
Ceratosolen nexilis
Ceratosolen cf. nexilis
Ceratosolen capensis
Ceratosolen fusciceps
Ceratosolen grandii
Ceratosolen medlerianus
Ceratosolen riparianus
Ceratosolen abnormis
Ceratosolen armipes
Ceratosolen kaironkensis
Blastophaga intermedia
Blastophaga malayana
Eupristina verticillata
Waterstoniella dubium
Waterstoniella brevigena
Platyscapa corneri
Platyscapa ficheri
Pleistodontes plebejus
Pleistodontes rieki
Pleistodontes rigisamos
Wiebesia brusi
Wiebesia frustrata
Wiebesia punctatae
Dolichoris vasculosae
Dolichoris hombronianae
Dolichoris inornata

CHAPTER 3

Coevolution in dioecious fig pollination:

Insights from phylogeny

"So each investigator discovers, according to his predilection, a solution to the Tree of Life... All may be right, for the idea of the tree expresses what the plant world has done for the animal."

E. J. H. Corner (1985), p.164

Introduction

Coevolution is broadly defined as "reciprocal change in interacting species" (Thompson 1994, p. 8) and coevolution in plant-insect interactions is of long-standing biological interest (Ehrlich and Raven 1964, Janzen 1980, Futuyma and Slatkin 1983, Futuyma and Keese 1992). Especially striking cases of coevolution involve mutualisms between pollinating seed predators and their host plants (Thompson 1994a). Classic examples include the fig wasps, the yucca moths, and their respective hosts (Corner 1985, Bronstein 1992, Pellmyr et al. 1996b). Recent advances in phylogenetic analysis (Maddison and Maddison 1992, Swofford et al. 1996, Swofford 1998) and comparative methods (Brooks and McLennan 1991, Harvey and Pagel 1991, Eggleton and Vane-Wright. 1994) have opened new approaches to understanding coevolution in ecological interactions (Huelsenbeck and Rannala 1997). The use of comparative methods in studying the fig-pollinator mutualism, however, has been limited by the availability of phylogeny estimates for fig and pollinator lineages (Yokoyama 1995, Herre et al. 1996, Machado et al. 1996, Machado 1998). Molecular phylogenetic analyses invite a closer examination of coevolutionary hypotheses drawn from the species-specific associations and interdependent life histories of figs and their pollinators (Ramirez 1974, Wiebes 1979a, Herre 1989). This chapter examines two major components of coevolution. First, phylogenetic evidence for cospeciation in the history of fig/pollinator associations is presented. Second, hypotheses of reciprocal adaptation in the dioecious fig/pollinator mutualism are tested through comparisons of interacting traits.

Coevolution in plant/pollinator mutualisms

Mutualisms involving pollinating seed predators and their host plants are characterized by resource conflicts and mutualisms also serve as model systems for studying the evolution of interactions (Pellmyr 1997a, Herre et al. 1999). In the exchange of pollination services for larval food, seeds are the common currency in which the success of subsequent generations is measured (Janzen 1979a). In theory, the consumption of too many or too few seeds by

pollinators could drive a mutualism toward parasitism or extinction (Pellmyr and Huth 1994). However, reciprocal selection on mutualists could maintain an equilibrium between resource allocation by the host plant and resource consumption by the pollinator (Kiester et al. 1984). Other instances of resource trade-offs between pollinating seed predators and host plants involve weevils and cycads (Norstog and Nicholls 1997), beetles and palms (Henderson 1986), flies and globeflowers (Pellmyr 1992) as well as gall midges and Monimiaceae (Feil 1992). However, the role of coevolution in maintaining the stability of mutualism can prove elusive (Janzen 1980, Herre and West 1997). In this regard, obligate mutualisms, such as fig pollination, are ideal for testing hypotheses of coadaptation and cospeciation (Bronstein and McKey 1989).

Cospeciation and parallel diversification

Cospeciation occurs when an ancestral association between species splits into descendant associations (Ronquist and Nylin 1990). Cospeciation can be an appropriate model for the diversification of endosymbiotic interactions (Cavanaugh 1994) or for interacting species with tightly linked reproductive cycles (Kiester et al. 1984). Phylogenetic evidence has supported cospeciation between aphids and their endosymbiotic bacteria (Moran and Baumann 1994) as well as pocket gophers and their parasitic lice (Page 1996). Although patterns of phylogenetic congruence are suggestive of cospeciation, host switching over evolutionary time can result in patterns of phylogenetic incongruence even in highly host-specific interactions (Kiester et al. 1984, Rasplus 1994). Previous comparisons of fig and pollinator phylogenies based on limited sampling were supportive of cospeciation (Yokoyama 1995, Herre et al. 1996). Phylogenies for the dioecious figs and their pollinators (Chapters 1 and 2) provide a broader sampling for testing the cospeciation hypothesis (Farrell and Mitter 1990).

The overall patterns of one-on-one specificity and lifecycle interdependence suggest a history of cospeciation in the fig/pollinator mutualism (Ramirez 1974, Wiebes

1987). However, the breakdown of host specificity suggests an alternative mode of speciation in the fig/pollinator interaction (Michaloud et al. 1996). The most common departure from specificity involves the geographic isolation of two pollinator species across the range of a single host species. In a survey of the Malesian region, Rasplus (1994) counted 20 cases of multiple allopatric pollinator species or subspecies per host species. For example, Platyscapa fischeri and P. hsui were recently shown to pollinate F. caulocarpa in different parts of its geographic range (Wiebes 1977a, Chen and Chou 1997). Different rates of speciation that are suggested by such patterns could result from different rates of dispersal across islands (Rasplus 1994), or alternatively, from different rates of local adaptation in pollinators and hosts. In either case, the overall pattern is consistent with a geographical model of diversifying coevolution (Thompson 1994a). The substantial radiation of dioecious fig and pollinator lineages in the Malesian region is especially appropriate for phylogenetic tests of cospeciation.

Tests of parallel diversification in fig and pollinator lineages could be based on methods for comparing morphological and molecular datasets in Chapters 1 and 2. For example, statistical tests under parsimony (Larson 1994) and likelihood (Kishino and Hasegawa 1989) could evaluate the significance of conflicts between fig and pollinator topologies. However, the combination of datasets not sharing a common phylogenetic history is problematical (de Queiroz 1993, Huelsenbeck et al. 1996). Therefore, more explicit methods for comparing host and associate tree topologies were used to infer the history of fig/pollinator associations (Brooks 1979, Brooks and McLennan 1991, Page 1994a). The reconciled tree approach (Page 1994a), for example, generates a combined host and associate tree that maximizes parallel divergence and minimizes the duplication and loss of associations under parsimony. A randomization test for cospeciation involves calculating the probability of obtaining the observed number of cospeciation events solely by chance (Page 1996).

Current knowledge of fig/pollinator interactions, however, runs contrary to several assumptions of the reconciled tree method. Due to linked lifecycles, the loss of a pollinator association implies the extinction of the host lineage unless accompanied by a contemporaneous host switch. The rarity of multiple pollinator species on the same host in sympatry (cf Michaloud et al. 1986, Kerdelhue et al. 1997) argues against the duplication of host associations (i.e. pollinator speciation in the absence of host speciation) except under allopatric conditions. The potential for host switching should also be included in tests of cospeciation (Ronquist 1995, Ronquist 1997, Ronquist 1998). Other statistical approaches to cospeciation involve the application of maximum likelihood methods in molecular phylogenetics; for example, Huelsenbeck et al. (1997) outlined tests of topological congruence, speciation time and rate of nucleotide substitution in host and associate lineages. Here I use likelihood and explicit models of molecular evolution to assess whether the observed incongruence between fig and pollinator phylogenies could arise from sampling error (i.e. chance mutation and drift in different genes with the same underlying phylogenetic history). Some limitations of this approach will also be discussed.

Coevolution in dioecious fig pollination

The reproductive biology of dioecious figs has attracted interest due to apparent resource tradeoffs (Herre and West 1997) and evolutionary conflicts with pollinators (Kjellberg et al. 1987a, Grafen and Godfray 1991, Patel et al. 1995, Anstett et al. 1997). Morphological specialization in dioecious figs and their pollinators has been interpreted as adaptation involved in stabilizing mutualisms (Ramirez 1978, Wiebes 1979a, Ramirez 1980, Corner 1985, Murray 1985, Berg 1990b, Ganeshaiah et al. 1995). For example, style lengths of fig flowers may select for optimal ovipositor lengths in pollinators (Ganeshaiah et al. 1995). On the other hand, the fitness costs of seed predation by pollinator larvae may drive the evolution of style lengths (Bronstein 1988a). However,

correlated changes in style and ovipositor lengths (e.g. Murray 1985) have not been examined in a phylogenetic context.

Monoecious fig species show a unimodal distribution of style lengths while dioecious species show a bimodal distribution (Bronstein 1988b, Kathuria et al. 1995, Nefdt and Compton 1996). Ecological studies have indicated that pollinator ovipositors can penetrate even the longest styles in monoecious figs but that most oviposition occurs in florets with shorter styles, possibly due to time-efficiency constraints on egg laying behavior (Nefdt and Compton 1996). Monoecious fig and pollinator species also show positively correlated style and ovipositor lengths which has been interpreted as evidence of interspecific adaptation (Nefdt and Compton 1996). This chapter examines this conclusion through a comparative approach taking into account the non-independence of species as data points (Harvey and Pagel 1991). However, a prediction of the Red Queen hypothesis, that coadaptation leads to runaway evolution of style and ovipositor length, contradicts the partitioning of seed resources according to differences in these traits (Ramirez 1980).

Recent evidence from monoecious figs indicates that the length of the style alone does not account for the stability of the fig/pollinator mutualism (Kathuria et al. 1995). In place of functional morphology, West and Herre (1994) suggested that a developmental barrier might protect a pool of fig ovaries (the "unbeatable seeds" hypothesis) while Murray (1985) suggested that the selective abortion of entire figs might stabilize levels of seed and pollinator production. Constraints on pollinator body size and egg loads have also been supposed (Herre 1989). For example, exploitation of seed resources by pollinators could be reduced by changes in the configuration of the ostiolar bracts that limit the size and number of foundresses (Ware and Compton 1994b, Nefdt and Compton 1996). Fig ovule size could also constrain pollinator body size and egg load. In addition, stabilizing effects have been proposed for population dynamic factors including local mate competition (Herre 1985, Herre 1987) and parasitoid load

(Kerdelhue and Rasplus 1996b). Herre and West (1997) suggested that, in general, the mitigation of ecological and evolutionary conflicts in mutualisms may involve the interaction of multiple factors (see also Pellmyr and Huth 1994). Disentangling the effects of many factors on mutualism stability requires detailed studies of particular species interactions (e.g. Herre and West 1997) but, on the other hand, finding general patterns requires a comparative approach.

For example, resource conflicts in dioecious fig pollination are quite different from the monoecious fig/pollinator mutualism (Nefdt and Compton 1996). The monoecious and dioecious life cycles as summarized in Figure 1 illustrate an overall difference in the seed/pollinator tradeoff. In contrast to monoecious species, the maturation of seed and pollinator larvae in dioecious species is segregated in two types of syconia on separate plants and each requires pollination by adult female fig wasps (Weiblen et al. 1995). Seeds of long-styled pistillate florets are unharmed by the ovipositing females while the galls of short-styled florets are destroyed by pollinator larvae (see also Chapter 1). The bimodal distribution of style lengths in dioecious figs neatly divides the maturation of pollinator larvae and seeds into gall figs and seed figs.

Questions regarding the stability of dioecious fig pollination arise from the observation that pollinators show no preference for gall figs despite the fact that pollinators of seed figs leave no offspring (Patel et al. 1995). However, that there are nearly 350 dioecious species indicates that a substantial radiation in dioecious fig lineages has occurred. Possible explanations for the origin and maintenance of dioecious fig pollination include mimicry, seasonality, vicarious selection and escape from parasitoids (Kjellberg et al. 1987a, Kjellberg et al. 1987b, Grafen and Godfray 1991, Kerdelhue and Rasplus 1996a, Anstett et al. 1997). The details of these hypotheses are described in Chapter 4. The present study focuses on the general observation that pollinators of dioecious figs tend to have shorter ovipositors than pollinators of monoecious figs (Ramirez 1980). Identifying the evolutionary correlates of shifts in fig

breeding systems could be the key to understanding the stability of fig pollination in general.

Relatively little attention has been focused on the bimodal style length distribution in dioecious figs compared to the unimodal distribution in monoecious species (Figure 1). Models of coevolution predict that ovipositor and style lengths will be highly correlated due to the increased fitness associated with access to fig ovaries (Kiester et al. 1984). In theory, longer ovipositors will be selected so that the entire style length distribution in monoecious species is accessible to pollinators (Murray 1985). However, if an efficiency cost is imposed on oviposition in longer styled florets then, effectively, the optimum style length for oviposition in a pollinator population is less than the mean style length of the host fig population (Ganeshaiah et al. 1995, Nefdt and Compton 1996). On the other hand, ovipositor lengths of dioecious fig pollinators closely match the first mode of the style length distribution (i.e. gall figs in Figure 1). Failure to distinguish gall and seed figs prior to entering the "tomb blossom" (Berg 1990b) combined with low heritable variation in ovipositor length could effectively prevent pollinator populations from reaching the second mode of the style length distribution. Could increased variability in ovipositor length arising through mutation result in the breakdown of dioecious pollination, leading to extinction or a shift from dioecy to monoecy in the host fig population? Phylogenetic methods provide a first step toward evaluating adaptive hypotheses (Baum and Larson 1991, Losos and Miles 1994) including sequences of character change (Donoghue 1989) and correlations among characters (Maddison 1990).

Coadaptation and comparative methods

Studies of character evolution can evaluate hypotheses of adaptation using available estimates of phylogeny (Coddington 1988, Donoghue 1989, Baum and Larson 1991, Brooks and McLennan 1991, Frumhoff and Reeve 1994 but see Leroi et al. 1994). In

particular, the availability of fig and pollinator phylogenies provides the opportunity to address whether transitions between monoecy and dioecy are correlated with shifts in the ovipositor lengths of the associated pollinators. Comparative methods for discrete characters test whether gains and losses of a particular trait are significantly correlated with the origin or loss of another trait. Such tests can be applied under parsimony (Maddison 1990, Maddison 1994) or likelihood criteria (Pagel 1994, Pagel 1997).

Homoplasy in fig and pollinator morphological traits is not necessarily an impediment to phylogenetic inference and it is essential to inferences of coadaptation (Chapter 1; see also de Queiroz 1996). Convergent evolution of pollinator morphology has been attributed to the functional constraints and morphological similarity of host figs (Herre et al. 1996) but this scenario has not been examined in an explicit phylogenetic framework. For example, evidence for the modification of the female head, antennae and mouthparts in relation to ostiole morphology deserves more attention using a comparative approach (van Noort and Compton 1996). In addition, the evolution of specialized pollen-carrying pockets in fig wasps could be examined in relation to the abundance and position of staminate florets in host figs (Ramirez 1978).

This chapter examines several instances of morphological convergence using comparative methods. For example, Frank (1984) argued that the stereotypical behavior of pollinators invites comparative study. An intriguing case of convergence involves the emergence of pollinators from syconia in relation to the positioning of staminate florets. Staminate florets may be positioned around the ostiole or dispersed among the pistillate florets. At anthesis, the male wasps chew an opening through the ostiolar bracts or the fig wall and the females collect pollen on exiting the syconium. Another adaptive hypothesis involves the evolution of fluid-filled syconia and the breathing apparatus of the associated pollinators. In particular, Ceratosolen-pollinated figs have syconia that fill with fluid during the interfloral phase between pollination and maturity (Chapter 1; Baker 1913, Berg and Wiebes 1992). Compton and McLaren (1989) showed that fig wasp

respiratory openings (spiracular peritremata) are capable of repelling fluid and suggested that their enlargement is adapted to respiration in an aquatic environment.

Correlated evolution of continuous characters can also be tested using comparative methods based on independent contrasts (Martins and Garland 1991, Garland et al. 1992). As noted by Felsenstein (1985), comparing the ancestral traits of sister groups based on explicit models of character evolution and phylogeny is preferable to treating species as independent data points (i.e. Nefdt and Compton 1996, van Noort and Compton 1996). The method of independent contrasts provides a powerful test of the hypothesis that style and ovipositor lengths are finely tuned (Murray 1985). Additional adaptive hypotheses for the evolutionary stability of the fig/pollinator mutualism can be tested in this manner. This chapter outlines two examples. First, the correlation between gall size and pollinator body size was used to test the hypothesis that wasp body size is regulated by fig resource allocation. Second, the hypothesis that ovipositor lengths in non-pollinating fig wasps are coevolved with fig size was also tested. Comparative methods were originally developed for the correlation between two traits in a lineage (i.e. pollinator body size and ovipositor length). These methods have not been applied previously to interacting lineages (i.e. pollinators and figs) and this chapter highlights the potential for a new approach to the study of coadaptation.

Methods

Sampling strategy

Coevolutionary studies were based on pairs of fig and pollinator species included in separate phylogenetic analyses in the preceding chapters (Table 1). Sampling was limited to representatives of major taxonomic groups: subgenera and sections of Ficus (Chapter 1) and genera and subgenera of Agaoninae (Chapter 2). Sampling was most intensive for the dioecious figs and their pollinators, especially members of the genus

Ceratosolen and their dioecious host species. An effort was made to include all species from Chapters 1 and 2, but DNA sequences for several taxa were not available at the time of this study. Ficus nuclear ribosomal DNA sequences (nrDNA) and pollinator mitochondrial DNA sequences (mtDNA) were available for 39 pairs of species. Inclusion of three incomplete pairs, as described below, raised the total to 42.

An unpublished mtDNA sequence for Tetrapus costaricanus (Machado 1998) was not available to match the outgroup nrDNA sequence for F. insipida (sect. Pharmacosycea). However, Neotropical sect. Pharmacosycea and Tetrapus are respectively sister groups to the rest of the figs and pollinators (Herre et al. 1996). Ficus insipida was used to root the fig phylogeny while non-pollinating Apocryptophagus spinitarsus was used to root the pollinator phylogeny. Additionally, mtDNA was not available for Ceratosolen adenospermae, the pollinator of F. adenosperma, but since the morphology, host associations and geography of C. adenospermae and C. medlerianus are indicative of a close relationship, C. medlerianus was paired with F. adenosperma to represent the pollinators of Papuasian sect. Adenosperma. Finally, mtDNA from the pollinator of F. albipila was not available, but Dolichoris vasculosae, the pollinator of closely related F. vasculosa (sect. Oreosycea ser. Vasculosae), was paired with F. albipila. The three additions served to approximate the diversity of fig and pollinator clades for purposes of comparison above the species level.

Tests of cospeciation

Comparisons of fig and pollinator phylogenies could be based on "independent" molecular data in order to avoid problems associated with morphological convergence (Herre et al. 1996, van Noort and Compton 1996). However, homoplasy in molecular data can also be misleading (Donoghue and Sanderson 1992) and inferences based on all of the available data may be more robust than those based on a subset of the data (Barrett

et al. 1991). Bootstrap comparisons indicated that combined analyses of molecular and morphological data provided the best-supported estimate of phylogeny for both the figs (Chapter 1) and the pollinators (Chapter 2). It was also argued in the preceding chapter that phylogenetic inferences based on pollinator mtDNA were potentially more accurate under parsimony than under likelihood due to the inadequacy of the available models of nucleotide substitution. For these reasons, tests of cospeciation were performed using most parsimonious (MP) trees inferred from combined molecular and morphological data sets.

Templeton tests of topological incongruence were performed to evaluate the statistical significance of conflicts between fig and pollinator phylogenies (Larson 1994). For example, the length of pollinator MP tree was compared to the length of MP trees found in heuristic searches constrained by rival fig topologies including MP, strict consensus, and bootstrap consensus trees. All searches were conducted as in Chapter 1. Templeton tests were also performed using the 50%, 70% and 90% bootstrap consensus trees. Kishino and Hasegawa (1989) tests examined the likelihood of obtaining the molecular data on alternative phylogenies, assuming the GTR+Γ model of nucleotide substitution. In the case of pollinator mtDNA, the likelihood of the pollinator MP tree based on combined data was compared to MP trees based on analyses of fig nrDNA and morphology. Reciprocal tests were also applied to the fig nrDNA data.

Global tests of incongruence, however, are not useful for evaluating particular topological conflicts (deQuerioz et al. 1995; Ferguson 1998). A local conflict between host and associate trees, for example, could represent either an instance of host switching or an inaccurate estimate for either of two cospeciating lineages. As a first step in distinguishing between these explanations, bootstrap values were assumed to reflect phylogenetic accuracy (Sanderson 1995). Levels of bootstrap support (>50%, >70% and >90%) for congruent and incongruent nodes in fig and pollinator phylogenies were then compared. Local conflicts with weak support (<50%) in both analyses were regarded as

cases in which systematic error could account for topological differences. On the other hand, conflicts with strong support (>90%) in both analyses were regarded as instances in which fig and pollinators could have different phylogenetic histories. Conflicts with strong support in one data set but weak support in the other were less clear and were evaluated on an individual basis.

Statistical tests of cospeciation were also implemented. The program TREEMAP was used to generate a reconciled tree (Page 1994a) maximizing fig and pollinator cospeciation while minimizing the number of duplicated and lost host associations under parsimony (Page 1996). A heuristic search was performed to identify the maximum number of cospeciation events in the absence of host switching. A randomization test calculated the probability of obtaining the observed number of cospeciation events solely by chance. The null distribution was obtained by computing the maximum number of cospeciations for 10,000 randomly generated trees of 42 taxa (randomizing both hosts and associate topologies). As discussed in the *Introduction*, the assumptions of the reconciled tree approach are not entirely met by the fig/pollinator mutualism and, therefore, a likelihood ratio test of cospeciation was also performed.

Fig and pollinator data sets were used to compare the hypotheses that the same or different histories underlie the mtDNA and nrDNA sequences (Huelsenbeck et al. 1997). The likelihood ratio test of heterogeneity (Huelsenbeck and Bull 1996) requires tree topologies, branch lengths and parameter estimates under a model of nucleotide substitution. For each data set, MP topologies based on combined analyses of morphological and molecular data were selected from Chapters 1 and 2. Insertions, deletions and regions of ambiguous alignment were excluded so that branch lengths could be estimated for nucleotide substitutions only. Under maximum likelihood, GTR+Γ (Yang 1993, Yang 1994b) parameters were estimated separately on each tree. For mtDNA, the rate matrix parameters were 0.6688, 3.8541, 2.8352, 2.4471 and 2.4469 with $\alpha = 0.5919$. To determine the most appropriate model for nrDNA, several nested models

were compared using lilkelihood ratio rests (Goldman 1993a) as described in Chapter 2 (Posada and Crandall 1998). The GTR+Γ model fit the data significantly better than simpler models while the addition of a parameter (I) for the proportion of invariant sites did not significantly increase the likelihood of the data (Table 2). Rate matrix parameters for fig nrDNA estimated on the MP combined Ficus tree were 0.8912, 1.9650, 1.1773, 0.6683 and 2.9758 with $\alpha = 0.5268$. ML model parameters were then used to estimate branch lengths for mtDNA and nrDNA on their respective trees.

The likelihood of the alternative hypothesis (i.e. different histories for figs and pollinators) was obtained by summing the likelihoods of the mtDNA and nrDNA given different topologies and model parameters. The likelihood of the null hypothesis (i.e. identical histories) was obtained by summing the likelihoods of the separate data sets given the same topology but different model parameters. A single MP tree resulting from a heuristic search of the four molecular and morphological data sets in combination was used for the null hypothesis. In comparing the null and alternative hypotheses, Monte Carlo simulation was used to generate a null distribution because the likelihood ratio test statistic (δ) is not X^2 distributed in this case (Goldman 1993b). One hundred pairs of nrDNA and mtDNA data sets were simulated using the program Seq-Gen (Rambaut and Grassly 1997). Each data set was simulated along the combined tree assuming a Markov process with branch length estimates and GTR+Γ model parameters based on either nrDNA or mtDNA. Heuristic searches under parsimony with 10 random addition sequence replicates were performed on the simulated data sets separately and in combination. Likelihood ratios of null and alternative hypotheses for the simulated data provided a null distribution for the observed likelihood ratio test statistic.

Branch length comparisons

An indirect comparison of evolutionary change in cospeciating lineages of figs and their pollinators was also attempted. Specifically, the hypothesis that vertically transmitted

lineages accumulate parallel changes over time was tested through the regression of congruent branch lengths in fig nrDNA and pollinator mtDNA phylogenies. Branch lengths were estimated under parsimony and maximum likelihood for pollinator mtDNA and host nrDNA using the same criteria outlined for tests of cospeciation. Only congruent branches in the fig and pollinator trees were subjected to regression analysis. Comparable branches included those subtending congruent nodes in the respective phylogenies and all terminal branches except for the outgroups (F. insipida in sect. Pharmacosycea and non-pollinating Apocryptophagus spinitarus). The outgroup branches were excluded because they were not comparable. According to this procedure, the lengths of 20 internal and 41 terminal branches were compared. In addition, the assumption of a molecular clock under maximum likelihood was used to compare the branch lengths of reconciled trees (Page 1996). In this case, only the branches of internal copaths (cospeciating lineages) were used because the lengths of terminal branches in sister lineages are equal under the clock assumption.

Correlated evolution of interacting traits

The correlated evolution of discrete morphological and behavioral traits was examined through ancestral state reconstruction using MacClade (Maddison and Maddison 1992). Under parsimony, ancestral states for fig and pollinator traits were inferred on completely resolved MP trees assuming accelerated transformation (ACCTRAN; Maddison 1994). A parsimony method for testing whether gains and losses of discrete traits are correlated on a phylogenetic tree (Maddison 1990) requires a minimum number of evolution events in order to test for statistical significance. The method was not applied due to rarity of gains and losses in the selected fig and pollinator traits. Traits were selected for comparison on the basis of published hypotheses of fig/pollinator coadaptation (e.g. Ramirez 1974, Ramirez 1980, Murray 1985, Compton and McLaren 1989). Scoring of character states followed Appendices 4 and 7.

Correlations between continuous morphological traits were also examined using the method of independent contrasts (Felsenstein 1985b). By comparing the difference in character values between sister groups, this method takes into account the non-independence of species. In the case of cospeciating lineages, it was possible to test for correlations between traits of interacting figs and pollinators. On the other hand, contrasts cannot be compared between two traits not sharing the same phylogenetic history (e.g. in the event of a host switch). Comparative analyses of fig and pollinator traits were based on the assumption of strict cospeciation. However, separate fig and pollinator phylogenies were used to explore the sensitivity of the results to alternative accounts of cospeciation.

Three sets of coadaptation hypotheses for continuous traits were tested. These included the correlated evolution of (A) fig style length and pollinator ovipositor length (Ramirez 1980, Murray 1985), (B) seed size and fig wasp body size (Nefdt and Compton 1996) and (C) fig wall thickness and parasitoid ovipositor length (Compton and Hawkins 1992). Species means and standard deviations for each trait were obtained from specimens listed in Appendices 1 and 6. All data except for fig diameter were captured using an Olympus® SZH10 Stereo Microscope and a Polaroid® Digitial Microscope Camera. Measurements to the nearest 0.01 mm were made digitially using Adobe Photoshop® version 5.0 and NIH Image 1.61 software (http://rsb.info.nih/gov/nih-image/).

For functional comparisons of style and ovipositor lengths it was necessary to consider the influence of fig developmental phase. Pollinator ovipositors penetrate the style during a brief period of receptivity (B phase; Galil and Eisikowitch 1968) while the

variance in style length increases proportionally to accommodate the enlarging florets during subsequent phases of development (Verkerke 1988). It was not feasible to measure style lengths from figs in receptive phase for each species in the phylogenetic analysis. Instead, style lengths were taken from ripe figs (D phase) after the pollinators had been reared. In dioecious species, style lengths from ripe seed figs were also measured. Although D phase measurements overestimated the style length at B phase, they at least provided a basis for relative comparisons with ovipositor length. Furthermore, studies of functional morphology typically have not appreciated the fact that the length of the style underestimates the distance from the stigmatic platform (synstigma) to the site of oviposition, which is actually located between the integument and the nucellus of the fig ovule (Cunningham 1888, Ganeshaiah et al. 1995). Measurements were taken from the first and second valvulae because the ovipositor sheaths (third valvulae) underestimate the functional length of the ovipositor (Nefdt and Compton 1996).

Thorax length was used as a relative indicator of body size because it is highly correlated with overall body length and is easier to measure (S. Compton, pers. comm.). Ovipositor length was divided by thorax length to obtain a relative measure of ovipositor size. For the seed size variable, it was necessary to distinguish between the gall ovaries containing fig wasps and viable fig "seeds". Gall ovaries tend to be larger than the viable "seeds" (achenes or drupes; Verkerke 1988). The width of mature gall ovaries was measured for comparison with fig wasp body size. In addition, ovipositor and thorax lengths were measured for 29 parasitic Sycoscapter species (Sycoryctinae: Agaoninae). Measurements from non-pollinating fig wasps tested the hypothesis that oviposition

through the fig wall by external parasitoids is coevolved with the size of the fig. (Compton and Nefdt 1988). Fig wall thickness and fig diameter are strongly correlated and measures of fig diamteter were used to test this hypothesis indirectly.

The diameters of ripe syconia were measured from fresh or alcohol-preserved species (D phase). When herbarium specimens only were available, measurements were converted to values approximating the fresh condition by multiplying by 1.67. This factor was derived from a regression of dried on fresh fig diameters (N = 240) across a broad size of species and sizes ($r^2 = 0.9$; Laman and Weiblen, In press). Several exceptions to the protocol for measurements are also noteworthy. Measurements for F. mollior and F. vasculosa were substituted for F. adenosperma and F. albipila, respectively, and paired with their actual pollinators (see also *Taxon sampling*). Measurements for F. sur and Ceratosolen capensis were taken from the literature (Baijnath and Ramcharun 1983, Nefdt and Compton 1996). Style lengths in F. variegata were adopted from Weiblen et al. (1995). Style and ovipositor lengths for F. racemosa and C. fusiceps were taken from Kathuria et al. (1995). Lastly, averages for F. botryoides were estimated from published ranges (Berg 1986).

Contrasts were calculated using the program CAIC (Purvis and Rambaut 1995). Independent contrasts were standardized in order to satisfy parametric statistical assumptions by dividing them by the standard deviation of expected change along each branch (Martins and Garland 1991, Garland et al. 1992). Assuming a model of Brownian motion for the evolution of continuous characters, the variance in the expected change along a branch is proportional to the branch length. Contrasts were standardized using ML branch lengths for fig and pollinator phylogenies estimated from nrDNA and

mtDNA. Under maximum likelihood, branch lengths were calculated separately assuming a molecular clock and a GTR+Γ model of substitution with parameter estimates as in the test of cospecation. Contrasts generated for each set of characters, trees and branch lengths were subjected to regression analysis with the line constrained to pass through the origin due to the arbitrary sign of each contrast (Garland et al. 1992). Evolutionary and statistical assumptions of this method were also tested as suggested by Purvis and Rambaut (1995).

Results

Tests of cospeciation

Phylogenies for figs and their associated pollinators are compared in Figure 2. Most parsimonious trees based on combined analyses of molecular and morphological data for each of the two lineages indicate that 20 out of 41 nodes are strictly congruent between the phylogenies. Results for Templeton tests of incongruence are summarized in Tables 3 and 4. Pollinator morphology and mtDNA rejected most rival constraint trees based on fig nrDNA and morphology (Table 3). The pollinator combined tree was significantly shorter than trees compatible with fig rival constraints except in the case of strongly supported nodes (>90%) in the separate fig nrDNA and morphological analyses. A similar pattern was detected for the reciprocal tests involving fig nrDNA and morphology (Table 4). The combined Ficus tree was significantly shorter than trees compatible with pollinator rival constraint trees except in the case of strongly supported nodes (>90%) in the pollinator morphological and combined analyses. The overall results indicated that

fig and pollinator trees were significantly incongruent except at nodes with high bootstrap support.

Kishino-Hasegawa tests showed that the pollinator mtDNA data were significantly more likely under the combined pollinator topology than under either the separate or combined fig nrDNA and morphological topologies (Table 5). Similarly, fig nrDNA was more likely under the combined fig tree than under either separate or combined pollinator mtDNA and morphological trees. The maximum likelihood results were consistent with the parsimony results in demonstrating that fig and pollinator phylogenies were significantly incongruent.

In spite of significant topological conflicts between fig and pollinator phylogenies, the observation that nearly half of the nodes were strictly congruent is indicative of cospeciation. A reconciled tree for figs and pollinators also supported the notion of cospeciation. The maximum number of cospeciation events in the reconciled tree (28) was significantly greater than expected by chance (i.e. if fig and pollinator lineages were associated at random; Figure 3A). Along with 28 cospeciations, the reconciled tree invoked 13 duplication events (i.e. pollinator speciation in the same host lineage) and 56 lineage-sorting events (i.e. extinction of one or more pollinator lineages in the same host lineage). A heuristic search allowing for host switching found 14 MP reconciled trees with 31 cospeciations, eight duplications, two host switches and 37 losses. Further manipulation of the reconciled tree showed that the assumption of up to eight host switches reduced the number of duplications and losses to three and 27, respectively. All reconstructions suggested that an ancestral pollinator of dioecious figs switched to monoecious subg. Urostigma.

Although the result of a randomization test for reconciled trees provides evidence of cospeciation, the validity of certain assumptions in the case the fig/pollinator mutualism is questionable. For example, assigning the same cost to duplications and losses under parsimony may not be appropriate because the loss of a pollinator association also implies extinction of the host lineage. In addition, the relative weights assigned to evolutionary events under parsimony (i.e. host switching versus cospeciation) have no biological meaning. The maximum likelihood test of heterogeneity, on the other hand, made a different set of assumptions based on observed patterns of nucleotide substitution. A likelihood approach rejected the null hypothesis that topological conflicts arose through sampling error in the pollinator mtDNA and host fig nrDNA sequences (Figure 3B).

Cospeciation tests were useful for summarizing the overall evolutionary pattern of fig/pollinator associations but not for identifying particular instances of incongruence. Bootstrap values in the respective host and pollinator phylogenies were examined to see whether particular conflicts were weakly or strongly supported by either data set (Figure 2). The overall pattern is summarized in Table 6, showing numbers of congruent and conflicting fig and pollinator clades with varying levels of bootstrap support. In general, conflicting clades tended to be weakly supported by either the pollinator or fig data sets and congruent clades were most often supported by bootstrap values >50% in both data sets. For example, in Table 6A, the majority of conflicting nodes in the fig phylogeny (11 out of 19 nodes) had <50% bootstrap values. On the other hand, 19 out of 21 congruent nodes were supported by >50% bootstrap and the majority of these (11 nodes) was supported by >90% bootstrap support in both trees. In several instances of conflict, well-supported host clades were not contradicted by bootstrap support in the pollinator tree (Figure 2). This pattern is illustrated by clades including sects. Malvanthera and Conosycea (100%), the monophyly of sect. Sycomorus (99%) and the sister relationship of sect. Neomorphe to sect. Sycomorus.

In the pollinator phylogeny (Table 6B) conflicting nodes with >50% bootstrap support outnumbered those without bootstrap support (13 compared to 6). Most instances involving strongly supported pollinator relationships, however, were not contradicted by bootstrap support in the host tree. Examples of this situation included: Kradibia sp. "ohuensis" plus K. sp. "salembensis" (92%), the sister relationship of this clade to K. jacobsi (83%), Ceratosolen corneri plus C.bisulcatus (99%), a clade including most of subg. Strepitus and the pollinators of sect. Adenosperma (98%) and the paraphyly of Dolichoris (68%).

There were also several conflicting fig and pollinator clades that received strong bootstrap support in each analysis. For example, the fig clade including sects. Ficus, Kalosyce, Rhizocladus and Sycidium with 94% bootstrap support was not congruent with the clade including Blastophaga, Wiebesia and the pollinators of subg. Urostigma with 89%. The fit could be improved somewhat by shifting the Kradibia-Liporrhopalum clade into position as sister to Blastophaga, Wiebesia, and the Urostigma-pollinators. Moving the pollinators of sect. Sycidium nearer to the pollinators of sects. Ficus, Kalosyce and Rhizocladus would not contradict any nodes with bootstrap support. Even so, the sister relationship of the subg. Urostigma-pollinators and Blastophaga (73%) is in direct conflict with the fig clade including sects. Ficus, Kalosyce, Rhizocladus and Sycidium. Incongruence in this case could be explained by an ancestral host switch from the dioecious lineage (Ficus, Kalosyce plus Rhizocladus) to monoecious subg. Urostigma. This scenario minimally requires that the ancestors of both host clades were in temporal and geographical proximity to the ancestral pollinator lineage. Neotropical fossil Pegoscapus (pollinating subg. Urostigma sect. Americana; Poinar 1993) suggests that such a host shift from dioecious subg. Ficus to monoecious subg. Urostigma would have occurred not less than 15-35 mya. The absence of subg. Ficus in the Neotropics also suggests that the shift from dioecious to monoecious Ficus would have occurred in the Paleotropics. However, host switching is not the only explanation for fig/pollinator

phylogenetic conflicts. Particular instances of incongruence can also result from systematic error, lineage sorting or hybridization in either the host or pollinator phylogeny. Additional sampling is needed to evaluate the hypothesis that pollinators of dioecious figs may have given rise to the pollinators of the monoecious strangler figs.

An additional conflict involved the monophyletic sect. Sycomorus with 99% bootstrap support and the sister relationship of Ceratosolen blommersi and C. vissali with 72% support. As suggested in Chapter 2, the apparent relationship between widely separated endemics from Madagascar and the Solomon Islands may instead reflect the attraction of long branches under parsimony (Felsenstein 1978). Ceratosolen vissali belongs to subg Strepitus and, along with other members of this group, pollinates species in sect. Sycocarpus. Ceratosolen blommersi, on the other hand, is a member of subg. Ceratosolen associated with African sect. Sycomorus. Mitochondrial DNA sequences from these two species were among the most divergent included in the study. They are placed near the Rothropus pollinators of subsect. Sycocarpus and the branch leading to the Rothropus clade is the longest internal branch in the mtDNA phylogeny. Unequal rates of substitution in different lineages combined with extreme A-T bias could result in the erroneous placement of C. blommersi and C. vissali. In the combined analysis of fig and pollinator data sets (not shown), C. blommersi groups with the pollinators of sect. Sycomorus but C. vissali remains in the position as sister to the Rothropus clade. The available data do not favor a host switching explanation, but additional sampling would allow for more powerful tests of Ceratosolen relationships.

Branch length comparisons

Branch lengths under ACCTRAN parsimony optimization for host fig nrDNA and pollinator mtDNA were significantly positively correlated ($p < 0.01$), but only a fraction of the variance in branch length was explained by a linear relationship ($r^2 = 0.12$; Figure 4A). Some of the scatter around the line could be explained by the failure of flat-

weighted parsimony to take into account biases in base composition and in the frequency of nucleotide substitutions, particularly for transitions at third positions in pollinator mtDNA. Maximum likelihood branch lengths correcting for these biases showed a higher positive correlation ($r^2 = 0.22$; Figure 4B). However, much of the scatter about the line was due to disproportionate rates of substitution in the associated lineages. For example, the Rothropus lineage showed a substantial increase in the rate of substitution relative to other Ceratosolen, but there was no proportional increase in host subsect. Sycocarpus (Figure 4B). Outlying points in Figure 4 represent extremely divergent sequences from Dolichoris vasculosae, C. vissali, C. appendiculatus and C. abnormis. Except for C. vissali, each of these pollinators is associated with a long terminal branch in host fig nrDNA.

Branch lengths in cospeciating lineages were also compared assuming a molecular clock (Page 1996). First, likelihood ratio tests compared the fit of pollinator mtDNA and fig nrDNA sequences to separate GTR+Γ models with and without the constraint of a molecular clock. In each case, the data rejected the molecular clock assumption (mtDNA: $-\ln L_0 = 30595.3$; $-\ln L_1 = 30836.7$; $X^2 = 482.8$; $p < 0.0001$; nrDNA: $-\ln L_0 = 4253.0$; $-\ln L_1 = 4310.5$; $X^2 = 115.0$; $p < 0.001$). Due to heterogeneity in the rate of substitution across both lineages, it is inappropriate to use a molecular clock to test the hypothesis of simultaneous fig and pollinator speciation under maximum likelihood (Huelsenbeck et al. 1997). However, the assumption of a molecular clock was used to test a prediction of contemporaneous speciation, namely, whether fig and pollinator branch lengths are proportional in ultrametric trees. Ultrametric trees are constrained such that the distance between any two descendants and their common ancestor is equal. Figure 5A depicts the branch length correlations for cospeciating lineages in the reconciled tree. In contrast to Figure 4, terminal branches are omitted because sister tips were constrained to be equidistant. The correlation between fig and pollinator branch lengths was substantially increased by the assumption of molecular

clock ($r^2 = 0.62$). A randomization test also indicated that the correlation of fig nrDNA and pollinator mtDNA branch lengths was significantly higher than expected by chance (Figure 5B). Although there was evidence for proportional change in fig and pollinator lineages, rates of nucleotide substitution were higher in pollinators than in host figs. For example, substitutions per site in pollinator mtDNA were 5-6 times greater on average than in fig nrDNA.

Coevolution in discrete characters

The evolution of fig breeding systems was associated with ancestral changes in pollinator morphology (Figure 6), although the rarity of inferred changes did not meet the assumptions of a statistical test for correlated change (Maddison 1990). Three or four shifts in breeding system were reconstructed (depending on the host MP tree; Chapter 1) compared to five shifts in the relative length of pollinator ovipositors. Ovipositors are either shorter or longer than the abdomen and the shift from monoecy to dioecy in figs was accompanied by a reduction in the length of the ovipositor relative to the abdomen. Both reversals from dioecy to monoecy within the Ceratosolen-pollinated clade were associated with shifts to longer ovipositors. The pollinators of the monoecious Sycomorus clade were not monophyletic, suggesting that the elongation of ovipositors occurred two times independently (in C. blommersi, as well as in the C. capensis plus C. fusciceps lineages). Short ovipositors were always associated with dioecy except in the case of Pleistodontes and monoecious sect. Malvanthera. One interpretation is that the host shift from dioecious figs to monoecious subg. Urostigma occurred prior to ovipositor elongation in the switching pollinator lineage. The position of Pleistodontes as sister to the pollinators of subg. Urostigma supported this notion but the derived position of sect. Malvanthera did not.

The general pattern of correlated change in fig breeding systems and pollinator ovipositors based on discrete traits was also supported by patterns of variation in

continuous characters. Correlated shifts in Ceratosolen and their host figs serve to illustrate this point. In the Ceratosolen-pollinated clade, two unambiguous reversals to monoecy were evident from the phylogenetic analysis (Figure 6) and species like monoecious F. prichardii and C. marshalli in Fiji, representing a third possible reversal were not sampled. Six species pairs were selected for three independent contrasts. Dioecious F. itoana, monoecious F. microdictya, and their pollinators represent an unambiguous cospeciation event in montane New Guinea. Dioecious sect. Neomorphe and monoecious sect. Sycomorus are widespread sister groups from which Papuasian F. nodosa, African F. sur and their respective pollinators were selected for comparison. Ficus pungens and C. nanus were also contrasted with F. prichardii and C. marshalli, respectively, based on morphology, geography and host associations (Wiebes 1963b).

Comparison of style lengths between representatives of these dioecious and monoecious sister groups showed multiple shifts from a bimodal distribution to a unimodal distribution of style lengths in Ceratosolen-pollinated figs (Figure 9). Each shift represents the loss of heterostyly. In each case, a doubling of ovipositor length was correlated with the loss of heterostyly. It is also noteworthy that the variance in ovipositor length is less than the variance of the corresponding style lengths in all six species pairs. Ovipositor lengths in monoecious fig pollinators closely match the mean style length of their host figs. By contrast, ovipositor lengths in dioecious fig pollinators match the length of short-styled florets in gall figs (hatched bars in Figure 7) but long-styled florets in seed figs (open bars) are beyond reach. For this reason, independent contrasts between mean style and ovipositor lengths in dioecious species were limited to gall fig measurements.

Two more coadaptation hypotheses were evaluated with pairs of discrete traits. Figure 8 depicts the positioning of staminate florets in relation to pollinator escape from figs. At anthesis, male fig wasps chew an opening either through the ostiolar bracts or the fig wall. Female fig wasps that collect pollen on exiting the fig and also ostiolar

stamens are, for the most part, associated with escape by the ostiole. The ancestral position of staminate florets was unresolved due to polymorphism in subg. Pharmacosycea. Staminate florets in the ostiolar position characterize the dioecious clade except for F. punctata, with stamens interspersed among the pistillate florets. Wiebesia punctatae, the pollinator of F. punctata, also emerges through the fig wall, not the ostiole. Wiebesia is unique among the dioecious fig pollinators in emerging through the fig wall, but not all Wiebesia-pollinated figs have dispersed stamens, examples being F. bauerlenii and F. odoardi. The clade including sects. Conosycea and Malvanthera is also marked by dispersed stamens and pollinators that emerge through the fig wall. By contrast, sect. Urostigma has ostiolar stamens and a pollinator (Platyscapa) that escapes through the ostiole. The ancestral condition for pollinators of the monoecious strangling clade (subg. Urostigma) was equivocal. However, ancestral state reconstruction provided some evidence of an association between stamen position and the location of pollinator emergence. Additional sampling and more detailed observations of behavior (e.g. Frank 1984) might permit statistical tests of this relationship (Maddison 1990, Pagel 1994).

Figure 9 illustrates the evolution of fluid-filled figs and enlarged spiracular peritremata in the associated pollinators. Ceratosolen-pollinated figs are characterized by syconia that fill with fluid between pollination and fig maturity and the abdominal respiratory openings (spiracular peritremata of the eighth urotergite) are grossly enlarged in the associated fig wasps. Compton and McLaren (1989) showed that the peritremata are lined with hairs capable of repelling fluid. The hypothesis that spiracular enlargement is an adaptation to a semi-aquatic environment is supported by the overall phylogenetic pattern. The enlargement of peritremata in female pollinators was associated with a shift from dry to fluid-filled figs. However, there were also two secondary reductions of peritremata in Ceratosolen and enlarged spiracles also evolved in some members of Blastophaga, Kradibia, and Waterstoniella.

Coevolution in continuous characters

Measurements of continuous fig and pollinator traits for 42 pairs of species are summarized in Tables 7 and 8, respectively. Style lengths are reported separately for gall and seed figs due to the bimodal distribution in dioecious species (Figure 7). Ripe seed figs were unavailable in three cases but the pattern for 24 dioecious species was clearly bimodal. Ovipositor and thorax measurements were gathered for 29 parasitic Sycoscapter species (Sycoryctinae; mostly undescribed) associated with 42 pollinator species. Twenty host species had a single Sycoscapter species and four host species had two; three Sycoscapter species were reared from F. microcarpa (see Chapter 4). In cases of multiple parasitism, a single species was selected at random for calculation of independent contrasts, limiting the number of parasitoid species in the analysis to 24 (Table 8). Although no phylogeny was available for Sycoscapter, molecular phylogenies suggest that the non-pollinators have cospeciated with their hosts (Machado et al. 1996). Based on this suggestion, fig and pollinator phylogenies were used to calculate independent contrasts for Sycoscapter.

Pairwise correlations of traits within the same lineage (e.g. fig diameter and style length) were generally not as strong as the correlations of interacting traits in different lineages (e.g. style length and ovipositor length). Comparison of ahistorical (AC) and contrast correlations (CC) indicated that taking fig or pollinator phylogeny into account did not have a dramatic affect on pairwise relationships between traits (Table 9). There was no significant relationship between fig diameter and style length. However, gall width was correlated with fig diameter, and to a lesser extent, with style length. Ovipositor length showed a significant relationship with body size (as estimated by

thorax length) in parasitoids, but not in pollinators. Similar results were obtained assuming either the fig or pollinator phylogeny assuming equal branch lengths.

Fig style and pollinator ovipositor lengths were highly correlated (Figure 10A). Although this pattern might be explained by heterostyly in dioecious figs, it is important to note that the correlation remains strong even in separate analyses of monoecious and dioecious figs (Murray 1985, Ganeshaiah et al. 1995, Nefdt and Compton 1996). A pair of monoecious and dioecious clades might reflect a spurious correlation between variables arising from the clustering of two sets of non-independent data points (Felsenstein 1985b, Harvey and Pagel 1991). However, monoecious and dioecious figs are not monophyletic and the contrast correlation for style and pollinator ovipositor lengths is also significant (Figure 10B). It is possible that a third variable (i.e. body size) could be responsible for the correlation between style and ovipositor lengths. However, relative ovipositor length (standardized for body size) is significantly correlated with style length, although the relationship is less pronounced. Supportive evidence for the coadaptation of pollinator ovipositors and style lengths is provided by parasitic Sycoscapter ovipositors that do not penetrate the style and their ovipositor lengths show no correlation with style length (Table 9C).

There were significant relationships between gall size and body size for both pollinating and non-pollinating fig wasps (Figure 10C; Table 9C). Comparative analyses support the coevolution of gall size and wasp size that could be involved in the regulation of resource conflicts in the fig/pollinator mutualism (Herre and West 1997). In the case of pollinators, the relationship between gall size and body size was strengthed using independent contrasts compared to the ahistorical correlation. The relationship was

weaker for parasitoids, where the allometry of extra-long ovipositors may have an additional impact on body size (Compton and Nefdt 1988). Parasitic Sycoscapter do not enter syconia as adults but oviposit externally through the syconium wall. Although fig diameter is not a direct measure of fig wall thickness, it was highly correlated with parasitoid ovipositor length (Figure 10E). By contrast, there was no relationship between fig diameter and pollinator ovipositors that interact with style length as opposed to the fig wall (Table 10C). Again, the strength of the correlation between parasitoid ovipositors and fig diameter increased upon consideration of fig and pollinator phylogeny (Figure 10F). The highest contrast correlation was obtained for the relative ovipositor length of parasitoids in relation to fig diameter. Overall, these results provide compelling evidence for the correlated evolution of interacting traits that will be discussed in terms of coadaptation.

Discussion

Phylogenetic analyses indicate that cospeciation has occurred in the history of the fig/pollinator mutualism despite some significiant differences in the fig and pollinator phylogenies. In general, phylogenetic comparisons are consistent with the predictions based on species specificity (Ramirez 1970) and the interdependence of lifecycles (Wiebes 1979a). Phylogenies of the dioecious figs and their pollinators also contribute to the emerging picture of cospeciation based on earlier phylogenetic studies (Yokoyama 1995, Herre et al. 1996). The evolutionary conservatism of host associations (Chapters 1 and 2) is further confirmed by direct comparisons of interacting clades (Figure 2). For example, some matching monophyletic groups of figs and pollinators include: (A) sect. Urostigma and Platyscapa, (B) sect. Conosycea and Eupristina plus Waterstoniella, (C) sect. Malvanthera and Pleistodontes, (D) sect. Ficus and Blastophaga, (E) sects. Kalosyce

plus Rhizocladus and Wiebesia, (F) sect. Sycidium and Kradibia plus Liporrhopalum, and (G) subsect. Sycocarpus and Ceratosolen subg. Rothropus. The congruence between fig and pollinator phylogenies, however incomplete, was more than expected by chance and this is further evidence of cospeciation. A maximum likelihood test also indicated that conflict between the phylogenies is not due to chance differences in the pattern of nucleotide substitution in pollinator mtDNA and fig nrDNA.

There can be little doubt that phylogeny provides new and powerful insights into coevolutionary processes (Huelsenbeck and Rannala 1997). With regard to parallel diversification, a phylogenetic analysis of pairwise associations is preferred to the comparison of fig and pollinator classifications given the potential for taxonomic artifacts (Chapters 1 and 2). However, phylogenetic approaches have problems and limitations that are worthy of consideration. These issues can be divided into those regarding the accuracy of phylogeny estimates (Hillis et al. 1994, Hillis 1998) and those of ancestral state reconstruction (see *Character evolution*; Ree and Donoghue 1998; Cunningham et al. 1998).

The major question concerning the evolution of interactions is whether or not two lineages share a parallel phylogenetic history (i.e. cospeciation) arising from the vertical transmission of associations through time (Herre et al. 1999). The problem is similar to the question of whether two genes share a common species history or whether two species have the same biogeographic history (Page 1994a). In the case of cospeciating lineages, topological incongruence can be due to inaccurate phylogeny estimates (Huelsenbeck and Bull 1996), gene tree versus species tree conflicts (Maddison 1997) or host switching (Page 1994b). Statistical tests can aid in distinguishing between sampling error and separate histories (Huelsenbeck and Bull 1996, Huelsenbeck et al. 1996) but how to attribute different histories to the effects of lineage sorting, hybridization and host switching is less clear.

Hybridization does not appear to have played a major role in the evolution of fig and pollinator lineages (reviewed in Chapters 1 and 2). Furthermore, the potential for lineage sorting in pollinator mtDNA should be low due to inbreeding and small effective population size (Herre 1985, Hoelzer 1997). Having rejected the hypothesis that incongruence between fig and pollinator phylogenies is due to sampling error, it is tempting to attribute particular conflicts to host switching. However, inaccuracy (i.e. systematic error) can account for the majority of these conflicts. If bootstrap support is taken as a measure of accuracy (cf Sanderson 1995) then the majority of weakly supported conflicts are potentially inaccurate. Strongly supported conflicts can provide evidence of host switching, but it is also important to consider the circumstances in which bootstrapping may be inconsistent (Chapter 2; Felsenstein 1978, Chang and Campbell In prep). In the case of Ceratosolen vissali and C. blommersi, for example, it was argued on the basis of sequence divergence, geography and host associations that these taxa are not likely to be sister species in spite of 72% bootstrap support. On the other hand, the pollinators of subg. Urostigma could represent an ancestral shift from dioecious to monoecious figs. The later possibility requires more exploration with additional sampling of genes and taxa.

The rarity of host switching in the fig/pollinator mutualism is possibly a consequence of extreme specialization and life cycle interdependence. Mating within the syconium reduces the effective population size of pollinators and levels of genetic variation within species (Machado 1998). Loss of genetic variation in traits pertaining to host selection and performance can act as a constraint on the evolution of host use (Futuyma et al. 1993, Bush and Smith 1997, Bush and Smith 1998). In addition, the linkage of fig and pollinator life cycles implies that a foundress invading a new host would compete for resources with an established pollinator population. Local adaptation in the resident pollinator would favor its competitive ability against invaders. A rare but fascinating possibility is the evolution of cheating in conjunction with a pollinator host

shift. Machado (1998) showed that pollinating Ceratosolen arabicus and cheating C. galili in F. sycomorus are not sister species (Galil and Eisikowich 1968b). The origin of cheating within a mutualistic lineage has also been shown in yucca moths (Pellmyr et al. 1996a) and future studies should explore this possibility in the fig/pollinator mutualism. The extent of cospeciation between non-pollinating fig wasps and hosts is another area for additional study (Machado et al. 1996; Machado 1998).

Molecular evolution and cospeciation

Molecular evolution differs substantially between plants and insects (cf Soltis et al. 1992, Brower and DeSalle 1994) and there is no reason to expect correlated patterns of DNA substitution in plant/insect interactions unless specialized host associations were conserved deep in time. Vertical transmission in parasitic and mutualistic interactions invites comparison of evolutionary rates between distantly related organisms (Thompson 1994a). For instance, the rate of molecular evolution in pocket gophers appears to be slower than in their parasitic lice (Huelsenbeck et al. 1997) and endosymbiotic bacteria show higher rates compared to free living relatives (Moran and Baumann 1994). However, differences in relative rates do not preclude the accumulation of correlated change through time in vertically transmitted lineages (Herre et al. 1999). Correlated branch lengths in fig and pollinator molecular phylogenies, for example, could reflect the ages of deeply conserved associations (Pellmyr et al. 1998). On the other hand, host switching, rate heterogeneity within either fig or pollinators lineages, and sampling error in phylogenetic analysis (Huelsenbeck and Bull 1996) could obscure correlations resulting from vertical transmission.

Given that different gene loci within species may evolve at different rates, it would be most appropriate to compare the same gene region in fig and pollinator lineages. For example, Huelsenbeck et al. (1997) compared mitochondrial cytochrome oxidase I (COI) between pocket gophers and their parasitic lice and concluded that the

relative rate of substitution was higher in the parasitic lineage. This pattern may also hold for obligate plant/insect interactions although direct comparisons are not available at the present time. For instance, sequence divergence in the internal transcribed spacer (ITS) region of nuclear ribosomal DNA can be phylogenetically informative in plant genera (Baldwin et al. 1995), but extreme ITS divergence within insect populations provides no phylogenetic signal at comparable taxonomic levels (e.g. O'Grady et al. 1998). Rapid divergence in insect mitochondrial DNA is an asset to phylogenetic studies (Simon et al. 1994) but major rearrangements in the plant mitochondrial genome have limited its use in phylogenetic studies (Palmer 1992). However, indirect comparisons of evolutionary change in cospeciating fig and pollinator lineages are possible. The hypothesis that vertically transmitted lineages accumulate parallel changes over time was tested through a regression analysis of congruent branch lengths in fig nrDNA and pollinator mtDNA phylogenies.

The increased correlation of branch lengths under maximum likelihood compared to unweighted parsimony suggests that an explicit model of evolution improves the pattern of correlated change. In spite of a large difference in the relative rate of molecular evolution between fig and pollinator lineages, a positive correlation of comparable branch lengths was suggestive of proportional change through time. Molecular data confirm the supposition based on taxonomic congruence that less divergent pollinators are associated with less divergent host figs (Ramirez 1974, Wiebes 1979a, Corner 1985). Although the molecular clock assumption was rejected by both data sets, the correlation between fig and pollinator branch lengths was further strengthened under the assumption of a clock. There is no reason to expect proportional changes in nrDNA and mtDNA from plant and insect lineages except under conditions of extreme specialization and host conservatism.

Indirect comparison shows that rates of nucleotide substitution are several times faster in pollinator mtDNA than in fig nrDNA. The direction and magnitude of the

difference is consistent with an overall trend toward faster evolutionary rates in associates than in hosts (Hafner et al. 1994, Page 1996, Huelsenbeck et al. 1997). Interestingly, this trend contradicts Manter's Rule that evolution proceeds faster in hosts than in parasites (Mitter and Books 1983). However, confirmation of the pattern for figs and pollinators requires a direct comparison of the same gene region (e.g. genes for conserved metabolic enzymes with similar inheritance in both lineages; Page 1996, Huelsenbeck et al. 1997). An obvious explanation for this pattern is generation time. Fig trees reproduce over a period of decades while their pollinators can pass through several generations per year depending on the phenology of the host species (Janzen 1979b). Dioecious F. fistulosa and F. variegata, for example, produce four crops per year on average (Corlett 1987, Spencer et al. 1996) and the minimum time to reproduction is probably on the order of five to ten years. This would imply a minimum difference in generation time between figs and their pollinators of twenty to forty-fold. Comparison of fig and pollinator sequence divergence across a range of fig species with different generation times (i.e. short-lived pioneer figs versus long-lived strangler figs) could take a first step toward evaluating the generation time hypothesis.

Inbreeding is another possible explanation for the difference between rates of evolution in figs and pollinators. There is a strong possibility of mating between sibling pollinators given that foundresses are few in number and that mating is restricted to the natal fig (Herre 1985). Machado (1998) showed that inbreeding, as estimated by the proportion of single foundress broods, is negatively correlated with levels of intraspecific variability in mtDNA sequences. Inbreeding reduces effective population size and the loss of genetic variability within pollinator populations through drift could contribute to the genetic divergence of pollinator species. On the other hand, the mating system of host fig species is obligately outcrossing due to protogyny and synchronous reproductive phenology within individuals (Spencer et al. 1996). Estimates of genetic diversity in fig populations based on allozymes are high and suggest that the effective population sizes of

fig species are large (Nason et al. 1996, Nason et al. 1998). Outbreeding combined with large effective population size could maintain neutral genetic variability within fig species. Whether differences in the breeding structure of fig and pollinator populations could contribute to differences in evolutionary rates is an intriguing area for future research. However, in addition to effective population size and generation time effects, there may also be differences in the rate of mutation and the intensity of selection on particular loci. The most promising avenue for testing such hypotheses in the fig and pollinator mutualism is to examine rates of change at a comparable locus (e.g. alcohol dehydrogenase genes in cospeciating lineages).

Asymmetry in the evolutionary rates of fig and pollinator lineages has several implications for specialization, reciprocal adaptation, and the maintenance of evolutionary conflicts. Hafner and Page (1995) suggested that the relative timing of speciation in hosts and parasites could be inferred from the y-intercept of sequence divergence plots. Branch length comparisons in Figure 4 suggest that speciation in pollinators preceeds speciation in figs, possibly due to higher rates of local adaptation in pollinators compared to hosts. Michaloud et al. (1996) based their hypotheses on modes of speciation in the fig/pollinator mutualism on the deviations from one-to-one specificity in natural populations. The most common departure from specificity involves the geographic isolation of two pollinator species across the range of a single host species. For example, there are 21 documented cases of multiple allopatric pollinator species or subspecies on individual host species distributed throughout Indo-Australia (Wiebes 1977, Chen and Chou 1997, Rasplus 1994).

The reproductive isolation of fig and pollinator populations depends primarily on host choice. Studies have shown that species-specific fig volatiles attract pollinators and elicit a sequence of behaviors including entry to the syconium, oviposition and pollination (van Noort et al. 1989, Ware et al. 1993, Hossaert-McKey et al. 1994, Ware and Compton 1994b). Further experiments have indicated that pollinator species avoid

entering the syconia of related host species in sympatry (Chapter 4). Local adaptation in host choice by allopatric populations of rapidly evolving pollinators could lead to the reproductive isolation and subsequent divergence of more slowly evolving fig populations. An alternative explanation, namely that preemptive speciation is due to differences in dispersal rates (Rasplus 1994), is not supported by genetic studies indicating that pollinators are capable of traveling great distances (Nason et al. 1996, Nason et al. 1998). In any event, the overall phylogenetic patterns are consistent with a geographical model of diversifying coevolution (Thompson 1994a). It seems logical to suppose that the mode of cospeciation could be related to differences in rates of genetic change between pollinator and host lineages. Testing this hypothesis will require more detailed studies of genetic variation throughout the geographical range of particular fig and pollinator species.

Morphological evolution and coadaptation

Phylogenetic analyses provided new evidence for the correlated evolution of morphological traits pertaining to resource conflicts and the stability of the fig/pollinator mutualism (Pellmyr and Huth 1994, Herre et al. 1999). Although the fitness consequences of resource trade-offs between mutualists were not examined directly (Pellmyr et al. 1996a, Herre and West 1997), specific hypotheses of adaptation were tested with comparative methods (Coddington 1988, Baum and Larson 1991). In particular, the role of style lengths and pollinator ovipositors in mitigating the conflict over seed resources was supported by phylogenetic evidence for correlated morphological traits (Figure 10B). That style lengths select for optimal ovipositor lengths (Ganeshaiah et al. 1995) or that the fitness costs of seed predation select for optimal style lengths (Bronstein 1988b) are not mutually exclusive hypotheses. In any event, the correlated evolution of traits across interacting lineages agrees with the predictions of coevolutionary models (Kiester et al. 1984, Ganeshaiah et al. 1995) and

provides compelling evidence for interspecific adaptation of figs and pollinators. A common problem for historical and ahistorical studies (Murray 1985, Nefdt and Compton 1996), however, is that correlations do not separate causes and effects. Definitive proof of coevolution in the strict sense of reciprocal adaptation is the domain of experimental biology (Thompson 1994a). Coevolutionary models supported by phylogeny help to pinpoint the experiments that test for the role of coadaptation in maintaining mutualism stability (Chapter 4).

The impact of unequal evolutionary rates on the stability of the mutualism is also worthy of discussion. In a simple model of gene-for-gene coevolution, change in a gene for host exploitation will select for a response in a resistance gene (Thompson 1994a). In the case of figs and pollinators, unequal rates of change in the genes involved in resource conflicts could lead to extinction or a shift from mutualism to parasitism. For example, suppose that the resource tradeoff is regulated by the coadaptation of a pollinator gene for ovipositor length and a fig gene for style length. Evolution of longer ovipositors would increase pollinator fitness but a corresponding reduction in fig fitness would select for longer styles. Countering the expectation of runaway evolution, style and ovipositor lengths are constrained by the efficiency of floret packing within syconia and the time-efficiency of oviposition (Verkerke 1988, Nefdt and Compton 1996). In any event, unequal rates imply that fine-tuning in a host fig population could be overturned by a rapidly evolving pollinator population. The consequences of such rate asymmetry should be incorporated in models of coadaptation (Kiester et al. 1984).

Phylogeny also provides new insights with regard to evolutionary conflicts in dioecious figs (Kjellberg et al. 1987a, Grafen and Godfray 1991, Anstett et al. 1997). The results showed that the evolution of dioecy was accompanied by a reduction in pollinator ovipositor length and multiple reversals to monoecy were associated with ovipositor elongation. A possible explanation relates to the fundamental difference between fig breeding systems; homostyly in monoecious figs and heterostyly in dioecious

figs (Kathuria et al. 1995). Empirical studies in monoecious figs support the model prediction that effective ovipositor lengths are slightly less than mean style lengths and that florets with longer styles tend not to be consumed by pollinator larvae (Ganeshaiah et al. 1995, Nefdt and Compton 1996). On the other hand, the ovipositors of dioecious fig pollinators closely match the mean style lengths of gall figs (Figure 7). The inability of dioecious fig pollinators to distinguish between gall and seed figs prior to passage through the ostiole (Patel et al. 1995, Anstett et al. 1998) and low variability in ovipositor length (Table 8) may constrain the optimization of ovipositors to short-styled gall figs.

A mutation for increased variability in ovipositor length allowing for successful oviposition in long-styled seed figs would result in the breakdown of functional dioecy. Pollinator escape from gall figs could result in extinction of the host population, or alternatively, it could select for a shift from dioecy to monoecy through loss of heterostyly. However, the lack of pollen in seed figs prevents any offspring of a long-ovipositor mutant from producing an F2 generation. This is precisely why the linkage of genes for heterostyly and staminate abortion is required for stable dioecy in Ficus. The possibility of an increased rate of extinction in dioecious fig lineages would be difficult to examine with diversification rate tests (Sanderson and Donoghue 1994). On the other hand, the rare occurrence of monoecious reversals in dioecious lineages could reflect changes in the two linked loci responsible for functional dioecy (Storey 1955). It would be informative to evaluate the sequence of changes in breeding system and ovipositor lengths (Donoghue 1989), but this is complicated when the inferred changes are apparently coincident (as in Ceratosolen). In one case, pollinator phylogeny suggests that a shift from a dioecious to a monoecious host occurred prior to ovipositor elongation (Figure 6). The ovipositor is short in Pleistodontes, the sister group to the rest of the pollinators of monoecious subg. Urostigma. However, Pleistodontes' host lineage, sect. Malvanthera, is not sister to the rest of subg. Urostigma.

Such situations point to several limits on tests of adaptation (Frumhoff and Reeve 1994, Leroi et al. 1994). Statistical tests of adaptation are limited by the numbers of evolutionary events (Maddison 1990) and by the extent of taxon sampling (Sillen-Tullberg 1993). Accuracy of the phylogenetic trees underlying these tests is also a concern and phylogenetic uncertainties should be explored using sensitivity analyses (Donoghue and Ackerly 1996). In addition, considerable uncertainty is associated with the reconstruction of ancestral states (Ree and Donoghue 1998; Cunningham et al. 1998). Although the general patterns of correlated morphological evolution in figs and pollinators do not seem especially sensitive, uncertainty should be explored in more depth in the future. An especially demanding problem is how to test for the correlated evolution of traits in less specialized lineages that do not share the same phylogenetic history.

The overall correlation of fig breeding systems and pollinator ovipositor lengths (Ramirez 1980) has not been appreciated in the discussion of resource conflicts for particular species (Kathuria et al. 1995, Anstett et al. 1996, Nefdt and Compton 1996, Herre and West 1997). Explanations for the origin and stability of dioecious fig pollination should also consider the possibility of coadaptation in style and ovipositor lengths. Furthermore, there are alternative hypotheses for the evolution of dioecy based on mimicry, seasonality, vicarious selection and escape from parasitoids (Chapter 4; Kjellberg et al. 1987a, Kjellberg et al. 1987b, Grafen and Godfray 1991, Kerdelhue and Rasplus 1996a, Anstett et al. 1997). In general, the mitigation of ecological and evolutionary conflicts may involve the interaction of multiple factors (Herre and West 1997). Kathuria et al. (1995) argued, for example, that style and ovipositor lengths alone do not account for mutualism stability and the remainder of the discussion concerns possible roles of additional traits.

Phylogenetic evidence for correlated evolution of body size in fig wasps and gall size in figs suggests the role of resource limitation in the stability of mutualism. Herre

(1989) proposed that gall size could constrain pollinator egg load and play a role in stabilizing levels of seed and pollinator production. Consumption of seed resources by pollinators could also be regulated by changes in the configuration of the ostiole affecting foundress sizes and numbers (Ware and Compton 1994a, Nefdt and Compton 1996). Correlated evolution of foundress morphology and the ostiole bears directly on this possibility and should be tested using comparative methods (van Noort and Compton 1996). Another intriguing adaptive scenario involves the correlation between the respiratory apparatus in Ceratosolen and fluid-filled figs (Compton and McLaren 1989). With regard to the evolution of pollinator behavior (Frank 1984), there was an association between pollinator emergence from syconia and the position of the pollen-bearing florets in some clades but the evidence was equivocal in other instances. The evolution of pollen pockets in relation to pollen:ovule ratios also deserves further attention (Ramirez 1978).

Overall, the strongest evidence of morphological adaptation is the relationship between ovipositor length and fig wasp life history. In contrast to pollinators, parasitic Sycoscapter oviposit externally through the syconium wall. Ovipositor lengths in pollinators and parasitoids were highly correlated with style lengths and fig diameters, respectively. Sycoscapter ovipositors were not correlated with style length and pollinator ovipositors were not correlated with fig diameter. These examples illustrate how phylogeny and comparative methods can test coevolutionary hypotheses in the fig/pollinator mutualism. Dioecious fig pollination, in particular, can serve as a model system for exploring other facets of coevolution, including the phylogenetic relationships and evolutionary impacts of non-pollinating fig wasps.

Table 1: Ficus species and associated Agaoninae selected for phylogenetic studies of coevolution in dioecious fig pollination [see Methods for exceptions]. Abbreviations follow Chapter 2.

Ficus section	Ficus species	Pollinator
Adenosperma	adenosperma	[Ceratosolen (C.) medlerianus Wiebes]
	ochrochlora	Ceratosolen (C.) sp. "riparianus"
Conosycea	microcarpa	Eupristina (P.) verticillata Waterson
	pellucidopunctata	Waterstoniella brevigena Wiebes
Ficus	grossularioides	Blastophaga (V.) malayana Wiebes
	padana	Blastophaga (V.) intermedia Grandi
Kalosyce	punctata	Wiebesia punctatae Wiebes
Malvanthera	destruens	Pleistodontes rigisamos Wiebes
	hesperidiiformis	Pleistodontes plebejus Wiebes
	xylosycia	Pleistodontes rieki Wiebes
Neomorphe	auriculata	Ceratosolen (C.) emarginatus Mayr
	nodosa	Ceratosolen (C.) nexilis Wiebes
	robusta	Ceratosolen (C.) cf nexilis Wiebes
	semivestita	Ceratosolen (C.) grandii Wiebes
	variegata	Ceratosolen (C.) appendiculatus Mayr
Oreosycea	albipila	[Dolichoris vasculosae Hill]
	edelfeltii	Dolichoris inornata Wiebes
	hombroniana	Dolichoris sp. "hombronianae"
Pharmacosycea	insipida	[Apocryptophagus spinitarsus Mayr]
Rhizocladus	baeuerlenii	Wiebesia sp. "brusi"
	odoardi	Wiebesia sp. "frustrata"
Sycidium	conocephalifolia	Kradibia jacobsi (Wiebes)
	copiosa	Kradibia copiosae (Wiebes)
	phaeosyce	Kradibia sp. "salembensis"
	pungens	Ceratosolen (C.) nanus Wiebes
	tinctoria	Liporrhopalum cf. gibbosae Hill
	trachypison	Kradibia sp. "ohuensis"
	virgata	Liporrhopalum virgatae Hill
	wassa	Kradibia wassae (Wiebes)
Sycocarpus	bernaysii	Ceratosolen (R.) hooglandi Wiebes
	botryocarpa	Ceratosolen (R.) corneri Wiebes
	dammaropsis	Ceratosolen (S.) abnormis (Wiebes)
	hispidioides	Ceratosolen (R.) dentifer Wiebes
	itoana	Ceratosolen (S.) armipes Wiebes
	microdictya	Ceratosolen (S.) sp. "kaironkensis"
	septica	Ceratosolen (C.) bisulcatus (Mayr)
	theophrastoides	Ceratosolen (S.) vissali Wiebes
Sycomorus	botryoides	Ceratosolen (C.) blommersi Wiebes
	racemosa	Ceratosolen (C.) fusciceps (Mayr)
	sur	Ceratosolen (C.) capensis Grandi
Urostigma	prasinicarpa	Platyscapa fischeri Wiebes
	superba	Platyscapa corneri Wiebes

Table 2: Log likelihood ratio tests comparing models of molecular evolution for the ITS region of nrDNA in Ficus. Results are listed for models including JC (Jukes and Cantor 1969), F81 (Felsenstein 1981), HKY85 (Hasegawa et al. 1985), and GTR (Rodriguez et al. 1990). The addition of parameters for heterogeneity of substitutions across sites (Γ; Yang 1994) and for the proportion of invariant sites (I) were also tested. Other models assuming equal base frequencies (Kimura 1980 and Zharkikh 1994) were rejected and are not listed. Significance was evaluated at $\alpha = 0.01$ following sequential Bonferroni correction for multiple tests.

H_0	H_1	$-\ln L_0$	$-\ln L_1$	df	X^2	p
JC	F81	4453.14	4430.42	1	45.44	<0.0001
F81	HKY	4430.42	4365.59	1	129.66	<0.0001
HKY	GTR	4365.59	4358.38	4	14.42	< 0.01
GTR	GTR+Γ	4358.38	4352.10	1	212.56	<0.0001
GTR+Γ	GTR+Γ+I	4352.10	4251.37	1	1.46	n.s.

Table 3: Templeton test results for incongruence between fig and pollinator phylogenies based on the combined pollinator mtDNA and morphological data sets. The MP tree from the combined pollinator data sets (Chapter 2) was compared to MP trees found in searches constrained by rival fig phylogenies based on nrDNA, morphology and combined analyses (Chapter 1).

combined data (MP tree) vs.	L	rank sum	N	z	p
fig nrDNA MP tree	7185	-20342	404	-9.3541	<0.0001
fig nrDNA strict consensus	7146	-18410	372	-8.3821	<0.0001
fig nrDNA 50% bootstrap	7094	-20201.5	361	-6.749	<0.0001
fig nrDNA 70% bootstrap	7018	-9814	241	-4.8342	<0.0001
fig nrDNA 90% bootstrap	6958	-11627.5	230	-1.8224	n. s.
fig morphology MP tree	7536	-14365.5	506	-15.6496	<0.0001
fig morphology strict cons.	7462	-13838	486	-15.2537	<0.0001
fig morphology 50% bootstrap	6991	-10243.5	232	-3.5273	<0.001
fig morphology 70% bootstrap	6943	-10439.5	212	-1.0657	n. s.
fig morphology 90% bootstrap	6943	-9937	207	-1.0724	n. s.
fig combined MP tree	7184	-21600	416	-9.5236	<0.0001
fig combined strict consensus	7058	-11186	276	-6.5556	<0.0001
fig combined 50% bootstrap	7018	-9814	241	-4.8342	<0.0001
fig combined 70% bootstrap	7000	-7981	214	-4.2941	<0.001
fig combined 90% bootstrap	6983	-13044	255	-3.1209	<0.005

Table 4: Templeton test results for incongruence between fig and pollinator phylogenies based on the combined host fig nrDNA and morphological data sets. The MP tree from the combined Ficus data sets (Chapter 1) was compared to MP trees found in searches constrained by rival pollinator phylogenies based on mtDNA, morphology and combined analyses (Chapter 2).

combined data (MP tree) vs.	L	rank sum	N	z	p
pol mtDNA MP tree	885	-204	94	-8.027	<0.0001
pol mtDNA ML tree	900	-76.5	86	-7.9423	<0.0001
pol mtDNA 50% bootstrap	832	-334	70	-5.7567	<0.0001
pol mtDNA 70% bootstrap	804	-238	48	-3.9224	0.0001
pol mtDNA 90% bootstrap	792	-161	36	-2.9122	<0.005
pol morphology MP tree	941	-210	120	-9.3538	<0.0001
pol morphology consensus	850	-451.5	85	-6.4161	<0.0001
pol morphology 50% bootstrap	785	-315.5	43	-2.1387	<0.05
pol morphology 70% bootstrap	784	-492	51	-1.7947	n.s.
pol morphology 90% bootstrap	770	-1.5	2	0	n.s.
pol combined MP tree	856	-127.5	72	-6.9691	<0.0001
pol combined strict consensus	855	-125	71	-6.9099	<0.0001
pol combined 50% bootstrap	821	-331.5	65	-5.3564	<0.0001
pol combined 70% bootstrap	802	-250	47	-3.603	<0.005
pol combined 90% bootstrap	779	-166	30	-1.4879	n.s.

Table 5: Kishino-Hasegawa test results for topological incongruence between fig and pollinator phylogenies. (A) This test compared the likelihood of observing the mtDNA data under alternative phylogenies and the GTR+Γ model of nucleotide substitution. The likelihood of the pollinator MP tree based on combined mtDNA and morphological datasets was compared to MP trees based on separate and combined analyses of host fig nrDNA and morphology. (B) The likelihood of nrDNA data given the combined Ficus tree was also compared with the separate and combined pollinator trees.

(A) pollinator MP tree vs.	-ln L	-ln L diff	sd diff	T	p
pol combined MP tree	30596.037	(best)			
fig nrDNA MP tree	31080.016	483.98	46.21	10.4734	<0.0001
fig morphology MP tree	31740.73	1144.69	77.15	14.8374	<0.0001
fig combined MP tree	30873.61	277.57	36.39	7.6271	<0.0001

(B) fig MP tree vs.	-ln L	-ln L diff	sd diff	T	p
fig combined MP tree	4256.37	(best)			
pol mtDNA MP tree	4428.40	172.03	29.91	5.7506	<0.0001
pol mtDNA ML tree	4427.55	171.18	31.80	5.3833	<0.0001
pl morphology MP tree	4554.98	298.61	40.71	7.335	<0.0001
pol combined MP tree	4380.70	124.33	27.10	4.5878	<0.0001

Table 6: Comparison of bootstrap support in respective host and pollinator phylogenies. **(A)** Nodes in the host tree with varying levels of support were counted as either congruent or incongruent with respect to the pollinator tree. **(B)** Nodes in the pollinator tree were compared in relation to the host tree.

(A)	pollinator tree					
	incongruent	congruent nodes				
host tree		<50%	>50%	>70%	>90%	tot
<50%	11	1	0	0	0	12
>50%	2	1	0	0	1	4
>70%	1	0	2	0	2	5
>90%	5	1	2	0	11	19
total nodes	19	2	5	0	14	40

(B)	host tree					
	incongruent	congruent nodes				
pollinator		<50%	>50%	>70%	>90%	tot
<50%	6	1	0	0	1	9
>50%	3	0	1	2	2	8
>70%	6	0	0	0	0	5
>90%	4	0	1	2	11	18
total nodes	19	1	2	4	14	40

Table 7: Continuous host traits for comparative analyses of coevolution (see Methods).

	fig diameter (cm)		style ln (mm)		style ln (mm) [2]		gall width (mm)	
Ficus	X (±SD)	N	X (±SD)	N	X (±SD)	N	X (±SD)	N
auriculata	5.01 (0.83)	3	0.39 (0.06)	4	1.59 (0.22)	5	1.20 (0.02)	4
baeuerlenii	2.45 (0.06)	3	0.47 (0.10)	5	?		0.97 (0.09)	5
bernaysii	1.35 (0.37)	17	0.37 (0.05)	5	1.09 (0.15)	5	1.05 (0.07)	5
botryocarpa	2.92 (0.50)	33	0.50 (0.12)	5	1.55 (0.17)	7	0.96 (0.12)	5
botryoides	3.08 (1.53)	2	1.25 (1.06)	2	-		1.70 (0.49)	2
conoceph.	3.19 (0.48)	25	0.70 (0.14)	5	2.24 (0.65)	5	1.45 (0.11)	5
copiosa	3.96 (0.62)	32	0.65 (0.11)	5	2.14 (0.12)	5	1.18 (0.06)	5
dammaropsis	5.84 (0.99)	17	1.24 (0.09)	5	2.22 (0.33)	6	1.82 (0.15)	5
destruens	1.92 (0.27)	4	0.92 (0.39)	12	-		0.96 (0.04)	5
edelfeltii	3.14 (0.14	3	1.76 (0.70)	12	-		1.25 (0.09)	5
grossular.	1.25 (0.21)	4	0.40 (0.10)	5	0.94 (0.08)	5	1.14 (0.11)	5
hesperidii.	3.60 (0.02)	3	1.32 (0.65)	11	-		1.98 (0.12)	5
hispidioides	4.99 (0.88)	66	0.35 (0.08)	180	1.05 (0.22)	190	1.28 (0.08)	5
hombroniana	1.60 (0.06)	3	1.32 (0.46)	12	-		0.74 (0.06)	5
insipida	5.29 (0.71)	3	1.94 (0.79)	11	-		1.37 (0.18)	3
itoana	5.12 (0.57)	5	0.82 (0.25)	25	1.66 (0.29)	18	1.38 (0.10)	5
microcarpa	0.86 (0.25)	12	0.99 (0.34)	12	-		0.53 (0.07)	5
microdictya	2.38 (0.06)	3	0.98 (0.37)	129	-		1.29 (0.08)	10
mollior [1]	1.37 (0.08)	10	0.42 (0.03)	5	?		0.87 (0.04)	5
nodosa	3.49 (0.39)	35	0.54 (0.07)	59	1.22 (0.31)	64	1.19 (0.09)	5
ochrochlora	3.23 (0.08)	3	0.50 (0.07)	5	1.34 (0.25)	7	1.06 (0.12)	5
odoardi	3.53 (0.24)	3	0.80 (0.09)	5	2.13 (0.21)	5	1.19 (0.09)	5
padana	2.99 (0.37)	3	0.40 (0.10)	5	0.95 (0.19)	5	1.10 (0.10)	5
pellucido.	1.36 (0.04)	3	1.56 (0.35)	11	-		1.16 (0.09)	6
phaeosyce	0.64 (0.09)	25	0.41 (0.15)	6	1.22 (0.25)	8	0.62 (0.02)	5
prasinicarpa	0.47 (0.06)	3	0.90 (0.39)	18	-		0.70 (0.05)	5
punctata	7.48 (3.12)	3	0.71 (0.21)	12	2.03 (0.39)	4	1.30 (0.07)	7
pungens	0.48 (0.09)	35	0.39 (0.12)	48	0.68 (0.12)	63	0.77 (0.08)	10
racemosa	2.99 (0.31)	6	1.02 (0.22)	61	-		1.32 (0.18)	5
robusta	4.14 (0.18)	3	0.50 (0.10)	5	1.28 (0.35)	5	0.95 (0.10)	5
semivestita	4.12 (0.16)	3	0.66 (0.09)	6	1.63 (0.39)	6	1.21 (0.06)	6
septica	2.35 (0.46)	36	0.42 (0.07)	5	1.86 (0.39)	5	1.03 (0.05)	5
superba	1.42 (0.11)	6	1.13 (0.44)	11	-		0.95 (0.09)	5
sur	3.25 (0.35)	2	1.75 (0.44)	99	-		1.63 (0.21)	3
theophrast.	2.39 (0.18)	3	0.59 (0.11)	6	1.42 (0.25)	7	1.60 (0.14)	5
tinctoria	1.25 (0.15)	24	0.36 (0.04	5	1.35 (0.09)	5	0.81 (0.06)	5
trachypison	0.78 (0.12)	25	0.40 (0.10)	5	1.06 (0.19)	5	0.96 (0.12)	5
variegata	3.05 (0.71)	43	0.30 (0.10)	10	1.70 (0.20)	10	0.99 (0.05)	5
vasculosa [1]	1.25 (0.07)	3	1.11 (0.34)	12	-		0.69 (0.03)	5
virgata	1.43 (0.05)	4	0.33 (0.08)	5	?		0.68 (0.12)	5
wassa	1.26 (0.14)	31	0.47 (0.09)	5	2.34 (0.26)	5	0.91 (0.05)	5
xylosycia	1.42 (0.08)	5	1.29 (0.63)	14	-		0.86 (0.10)	5

[1] Species not sampled in Chapter 1. [2] Style lengths for seed figs in dioecious species.

Table 8: Continuous fig wasp traits for comparative analyses of coevolution. Scale is in mm.

	pollinator			parasitoid [2]		
Agaoninae species	ovip ln X (±SD)	thorax ln X (±SD)	N	ovip ln X (±SD)	thorax ln X (±SD)	N
B. intermedia	0.05 (-)	0.54 (-)	1	2.72 (0.16)	0.41 (0.01)	3
B. malayana	0.12 (0.04)	0.54 (0.03)	5	3.26 (0.61)	0.48 (0.05)	5
C. appendiculatus	0.34 (0.02)	0.57 (0.09)	5	3.54 (0.14)	0.45 (0.03)	5
C. bisulcatus	0.17 (0.02)	0.56 (0.07)	5	4.17 (0.33)	0.47 (0.09)	4
C. blommersi	1.01 (0.04)	0.69 (0.04)	5	-	-	-
C. capensis	1.17 (0.11)	0.64 (0.03)	5	-	-	-
C. emarginatus	0.37 (0.05)	0.69 (0.06)	5	-	-	-
C. fusciceps	0.99 (0.05)	0.62 (0.02)	5	-	-	-
C. grandii	0.33 (0.04)	0.63 (0.08)	5	8.44 (0.55)	0.70 (0.03)	5
C. medlerianus	0.24 (0.03)	0.52 (0.05)	5	-	-	-
C. nanus	0.25 (0.04)	0.43 (0.03)	33	2.95 (0.30)	0.41 (0.01)	3
C. nexilis	0.58 (0.04)	0.54 (0.05)	59	7.16 (0.37)	0.60 (0.13)	5
C. "riparianus"	0.31 (0.04)	0.49 (0.04)	3	-	-	-
C. cf. nexilis	0.50 (0.05)	0.54 (0.05)	5	-	-	-
C. corneri	0.26 (0.02)	0.59 (0.03)	5	7.23 (0.18)	0.55 (0.03)	2
C. dentifer	0.25 (0.04)	0.75 (0.03)	5	10.93 (0.73)	0.69 (0.07)	5
C. hooglandi	0.19 (0.09)	0.68 (0.10)	5	5.69 (0.45)	0.68 (0.04)	3
C. abnormis	0.59 (0.01)	1.10 (0.07)	5	15.25 (1.02)	0.94 (0.02)	5
C. armipes	0.46 (0.05)	0.95 (0.06)	20	-	-	-
C. "kaironkensis"	0.89 (0.05)	0.65 (0.07)	14	5.91 (0.71)	0.52 (0.09)	3
C. vissali	0.27 (0.03)	1.00 (0.05)	5	-	-	-
D. "hombronianae"	0.68 (0.06)	0.41 (0.02)	5	-	-	-
D. inornata	1.20 (0.10)	0.64 (0.08)	5	-	-	-
D. vasculosae	0.67 (0.06)	0.60 (0.02)	3	-	-	-
E. verticillata	0.83 (0.13)	0.40 (0.02)	5	3.19 (0.09)	0.44 (0.03)	5
K. copiosae	0.56 (0.06)	0.74 (0.03)	5	10.63 (0.52)	0.82 (0.06)	5
K. jacobsi	0.21 (0.05)	0.78 (0.04)	5	8.23 (0.64)	0.97 (0.07)	5
K. "ohuensis"	0.29 (0.08)	0.41 (0.05)	5	5.08 (0.33)	0.52 (0.05)	4
K. "salembensis"	0.18 (0.01)	0.39 (0.05)	5	1.78 (0.14)	0.48 (0.02)	5
K. wassae	0.29 (0.06)	0.55 (0.05)	5	5.49 (0.17)	0.57 (0.06)	4
L. cf. gibbosae	0.14 (0.02)	0.49 (0.04)	5	3.41 (0.16)	0.53 (0.06)	5
L. virgatae	0.17 (0.02)	0.37 (0.05)	5	2.73 (0.29)	0.44 (0.06)	4
Pla. corneri	0.74 (0.03)	0.48 (0.05)	5	-	-	-
Pla. fischeri	0.56 (0.02)	0.36 (0.04)	5	-	-	-
Ple. plebejus	1.14 (0.16)	1.41 (0.14)	5	7.46 (0.52)	0.66 (0.07)	5
Ple. rieki	0.70 (0.02)	0.86 (0.13)	5	4.71 (0.39)	0.68 (0.03)	5
Ple. rigisamos	0.34 (0.05)	0.55 (0.10)	5	2.80 (0.43)	0.58 (0.07)	3
T. costaricanus [1]	1.15 (0.23)	0.76 (0.07)	3	-	-	-
Wat. brevigena	1.12 (0.07)	0.65 (0.03)	5	5.74 (0.26)	0.68 (0.05)	5
Wie. "brusi"	0.18 (0.04)	0.48 (0.04)	5	-	-	-
Wie. "frustrata"	0.24 (0.02)	0.55 (0.07)	5	-	-	-
Wie. punctatae	0.36 (0.05)	0.69 (0.01)	2	-	-	-

[1] Species not sampled in Chapter 2. [2] Parasitic Sycoscapter (Sycoryctinae).

Table 9: Testing for correlated evolution of continuous morphological traits in figs and fig wasps. Ahistorical correlations (AC) correspond to Pearson's product-moment correlation while contrast correlations (CC) refer to the coefficient of correspondence for independent contrasts based on alternative fig and pollinator phylogenies assuming equal branch lengths. Regressions of standardized contrasts were constrained to pass through the origin due to the arbitrary sign of each contrast (Garland et al. 1992). P-values are based on sequential Bonferroni correction (Rice 1989). Abbreviations: (ovip)ositor; (pol)inator; (par)asitoid; (l)ength; (w)idth.

			fig phylogeny		pollinator phylogeny	
trait 1	trait 2	AC	CC	P	CC	P
(A) host plant traits						
fig diameter	style l	0.1462	0.3308	n.s.	0.2109	n.s.
fig diameter	gall w	0.6385	0.6396	<0.001	0.6147	<0.001
style l	gall w	0.3743	0.6184	<0.001	0.4948	<0.05
(B) fig wasp traits						
pol ovip l	pol thorax l	0.3235	0.4736	<0.05	0.3705	n.s.
par ovip l	par thorax l	0.8003	0.6611	<0.05	0.8094	<0.001
(C) interacting traits						
style l	pol ovip l	0.8976	0.7567	<0.0001	0.8339	<0.0001
gall w	pol thorax w	0.8093	0.8629	<0.0001	0.8359	<0.0001
style l	pol rel ovip l	0.7645	0.5124	<0.05	0.5884	<0.01
fig diameter	pol ovip l	0.1301	0.3166	n.s.	0.1646	n.s.
style l	par ovip l	0.2169	0.4065	n.s.	0.4734	n.s.
gall wid	par thorax w	0.6961	0.6597	<0.05	0.6743	<0.01
fig diameter	par ovip l	0.6491	0.881	<0.0001	0.8501	<0.0001
fig diameter	par rel ovip l	0.8344	0.8904	<0.0001	0.7864	<0.001

Figure 1: Life cycles of dioecious and monoecious figs. **(A)** In dioecious species, the maturation of seed and pollinator larvae is segregated in two types of figs on separate plants. Each type requires pollination by adult female fig wasps. Seed figs contain long-styled pistillate florets that are unharmed by the ovipositing females while gall figs contain short-styled florets that are consumed by pollinator larvae. **(B)** The bimodal distribution of style lengths in dioecious figs divides the maturation of pollinator larvae and seeds into gall figs (open) and seed figs (closed). **(C)** In monoecious species, seeds and pollinator larvae reach maturity in the same fig. **(D)** The distribution of style lengths in monoecious figs is unimodal, but seeds in shorter-styled florets (open) tend to be consumed by pollinators while seeds in longer-styled florets tend to be unharmed (closed).

Figure 2: Phylogenies for figs (at left) and their host-specific pollinators (at right). Topologies are based on the combined analyses of molecular and morphological data (Chapters 1 and 2) with missing taxa pruned; three exceptions being enclosed in brackets. Bootstrap values greater than 50% from combined analyses in the previous chapters are listed above or below the branches. Host associations between pairs of taxa are indicated by connecting lines. Closed circles indicate 20 nodes that are strictly congruent between the fig and pollinator phylogenies.

Figure 3: Results of statistical tests for cospeciation in fig and pollinator lineages. **(A)** Null distribution of the maximum number of cospeciation events for 10,000 pairs of randomized trees with 42 species-specific associations. The maximum number of

cospeciation events was based on a reconciled tree allowing for the duplication and loss of host associations but disallowing host switching (Page 1994b). The maximum number of fig/pollinator cospeciations (28) was unlikely to have arisen by chance ($p < 0.0001$). **(B)** Monte-Carlo distribution of the -log likelihood ratio test statistic (Δ) for differences between fig/pollinator phylogenies. One hundred pairs of data sets were simulated on separate fig and pollinator phylogenies using model parameters estimated independently from nrDNA and mtDNA under maximum likelihood (Rambaut and Grassly 1997). The null hypothesis that incongruence between fig and pollinator phylogenies is due to sampling error was rejected ($p < 0.01$).

Figure 4: Correlated branch lengths of host fig nrDNA and pollinator mtDNA. **(A)** Linear regression of congruent fig and pollinator branch lengths estimated under parsimony ($y = 0.0392x + 2.1130$). There was a weak positive correlation between nrDNA and mtDNA branch lengths in the associated lineages ($r^2 = 0.12$; $p < 0.01$). **(B)** Regression of congruent branch lengths estimated under maximum likelihood ($y = 0.0682x + 0.0052$). The strength of the correlation between fig nrDNA and pollinator mtDNA branch lengths increased under maximum likelihood ($r^2 = 0.22$; $p < 0.0001$).

Figure 5: Correlated branch lengths of fig nrDNA and pollinator mtDNA assuming a molecular clock. **(A)** Linear regression of copaths in the fig and pollinator phylogenies under maximum likelihood. Copaths are the equivalent paths between two successive cospeciations (Page 1996). **(B)** Null distribution for the coeffficient of correlation based on 1000 randomizations of branch lengths for 28 copaths.

Figure 6: The evolution of fig breeding systems and pollinator ovipositor lengths.

Figure 7: Phylogenetic contrasts in Ceratosolen-pollinated figs. Length distributions of styles (shaded or open bars) and ovipositors (closed bars) are compared between representatives of monoecious and dioecious sister groups. Open bars indicate the distribution of style lengths in seed figs of dioecious species. (**A**) Dioecious F. itoana and monoecious F. microdictya in sect. Papuasyce (**B**) Dioecious F. nodosa (sect. Neomorphe) and monoecious F. sur (sect. Sycomorus). (**C**) Dioecious F. pungens and monoecious F. pritchardii are putative sister species based on the close similarity of their pollinators, C. marshalli and C. nanus (Wiebes 1963b).

Figure 8: The position of staminate florets in relation to pollinator emergence from figs.

Figure 9: The evolution of fluid-filled figs and enlarged spiracular peritremata in the female pollinators of figs.

Figure 10: Pairwise correlations between continuous fig and pollinator traits. (**A**) Ahistorical correlation of mean style and ovipositor lengths for 42 paired species associations. Open and closed circles indicate dioecious gall figs and monoecious figs, respectively. (**B**) Contrast correlation of mean style and ovipositor length. (**C**) Ahistorical correlation of seed size (gall width) and fig wasp body size (thorax length). Open and closed circles denote pollinators (Agaoninae) and parasitoids (Sycoryctinae),

respectively. (**D**) Contrast correlation of seed size and pollinator body size. (**E**) Ahistorical correlation of fig diameter and fig wasp ovipositor length. Open and closed circles denote pollinators (Agaoninae) and parasitoids (Sycoryctinae), respectively. (**F**) Contrast correlation of fig diameter and parasitoid ovipositor length. Abbreviations are as in Table 9.

Figure 1A: Life cycles of dioecious figs and their pollinators

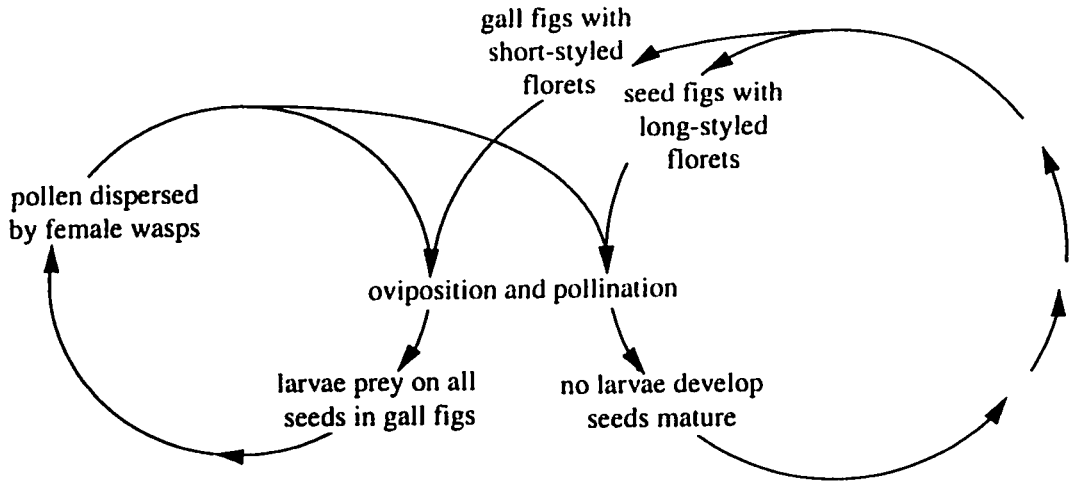


Figure 1B: Style lengths in dioecious figs

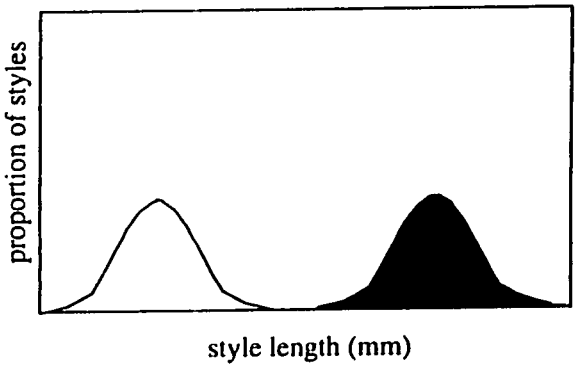


Figure 1C: Life cycles of monoecious figs and their pollinators

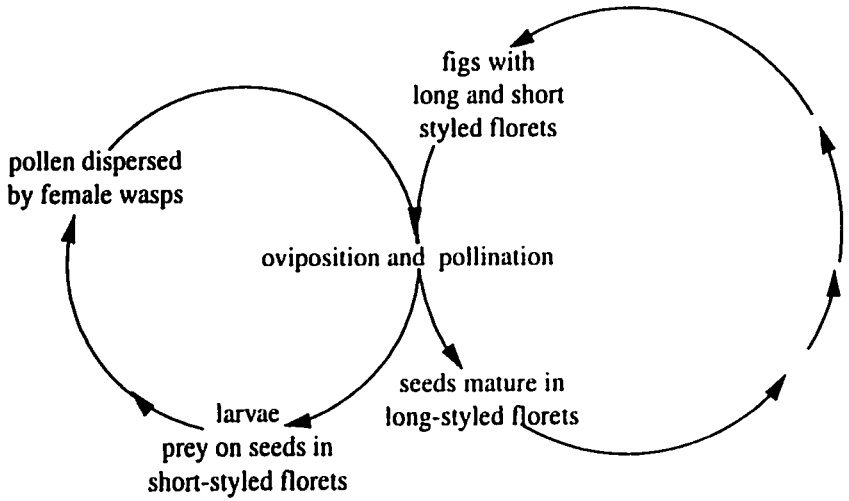


Figure 1D: Style lengths in monoecious figs

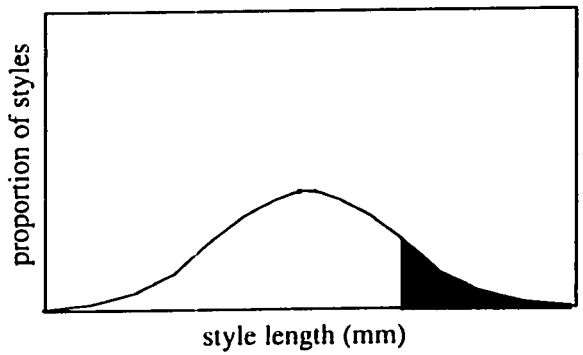
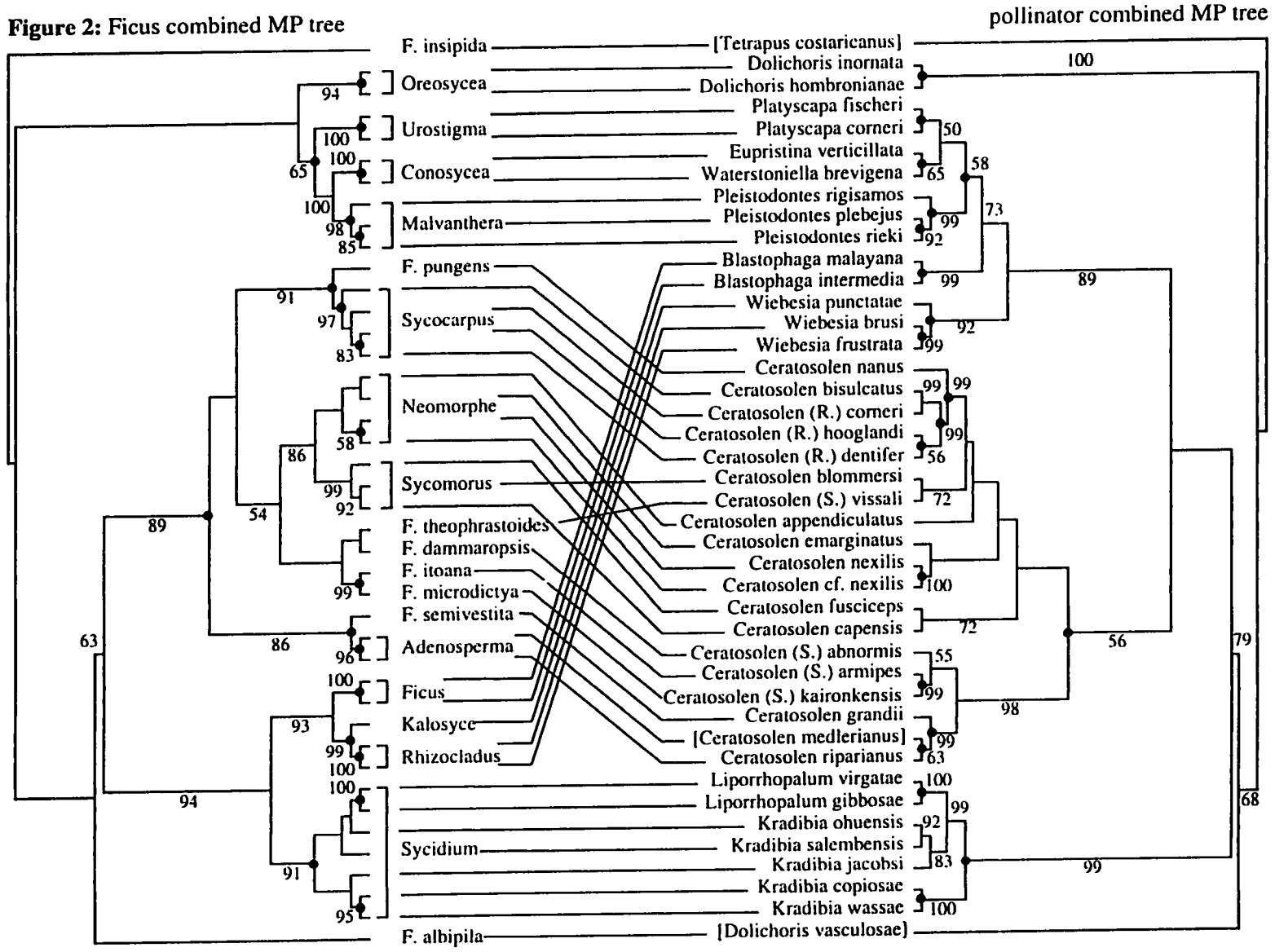


Figure 2: Ficus combined MP tree



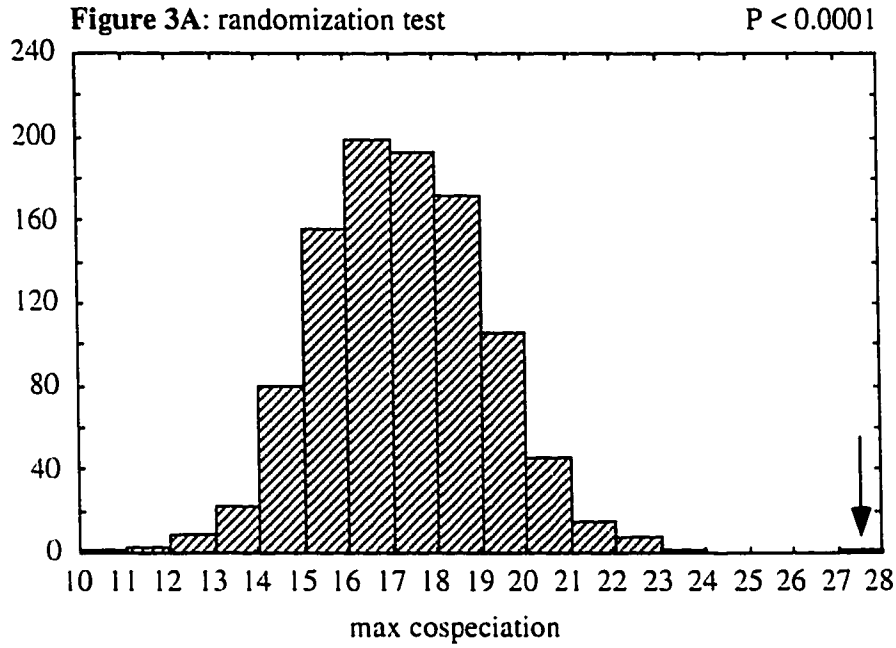


Figure 3A: randomization test
P < 0.0001
240
200
160
120
80
40
0
10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28
max cospeciation

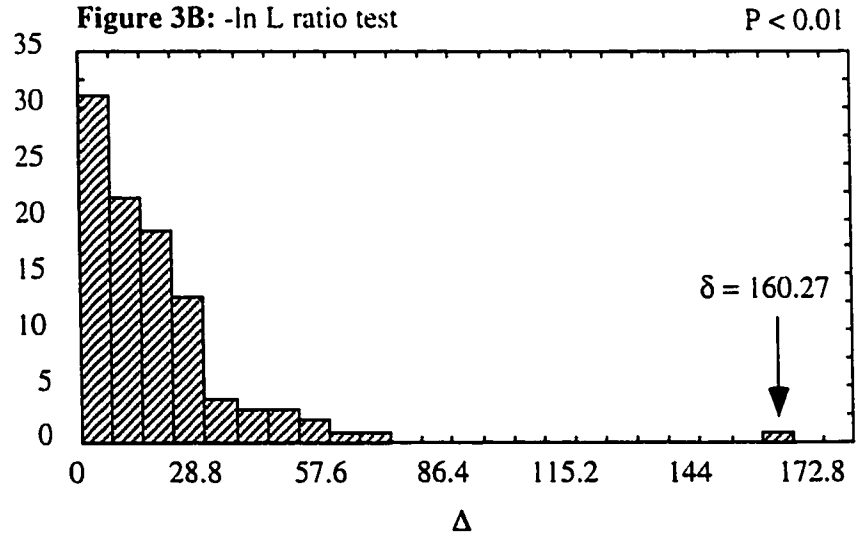


Figure 3B: -ln L ratio test
P < 0.01
35
30
25
20
15
10
5
0
δ = 160.27
0
28.8
57.6
86.4
115.2
144
172.8
Δ

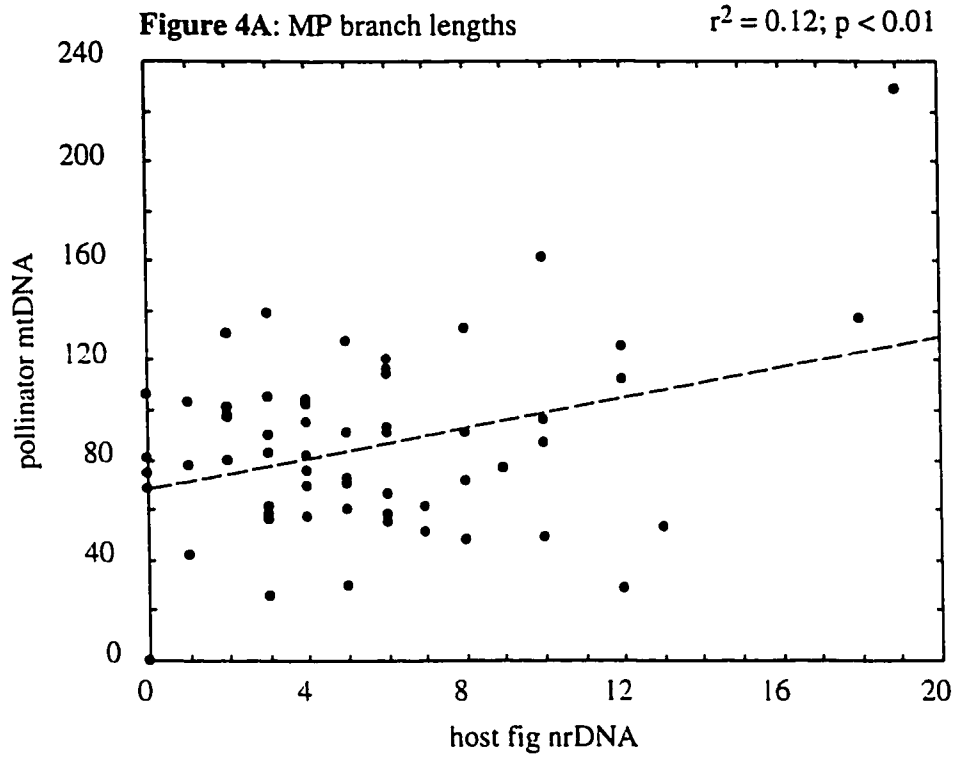


Figure 4A: MP branch lengths
$r^2 = 0.12$; $p < 0.01$
240
200
160
120
80
40
0
pollinator mtDNA
0
4
8
12
16
20
host fig nrDNA

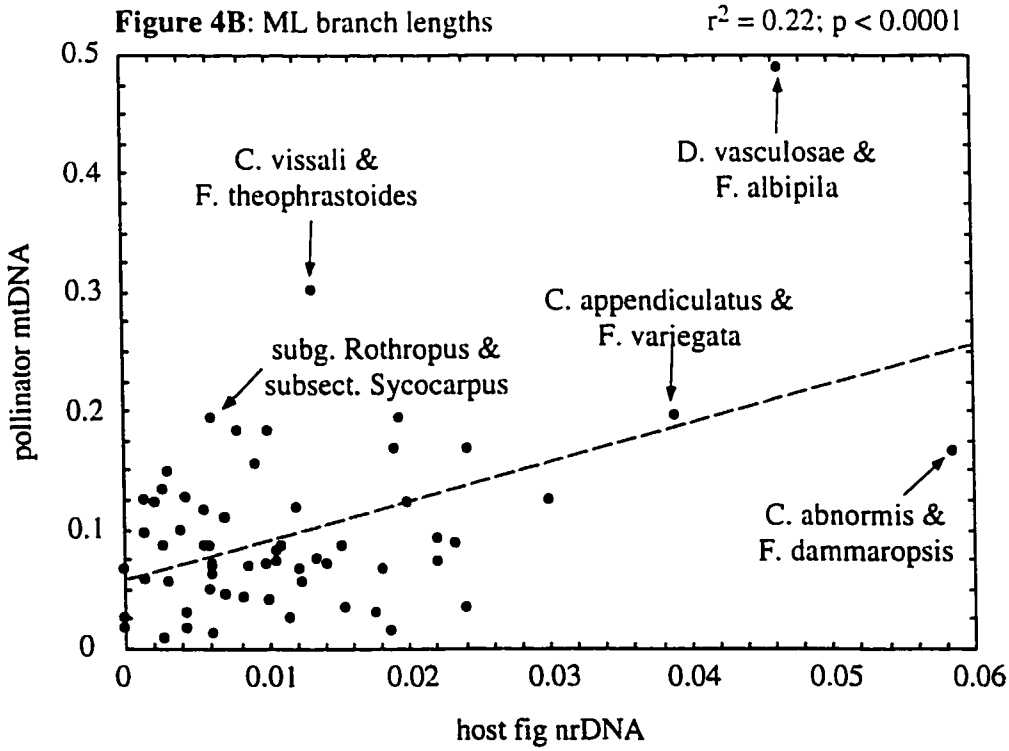


Figure 4B: ML branch lengths
$r^2 = 0.22$; $p < 0.0001$
0.5
0.4
0.3
0.2
0.1
0
pollinator mtDNA
C. vissali &
F. theophrastoides
D. vasculosae &
F. albipila
C. appendiculatus &
F. varjegata
subg. Rothropus &
subsect. Sycocarpus
C. abnormis &
F. dammaropsis
0
0.01
0.02
0.03
0.04
0.05
0.06
host fig nrDNA

Figure 5A: ML branch lengths (molecular clock) $r^2 = 0.62$; $p < 0.001$

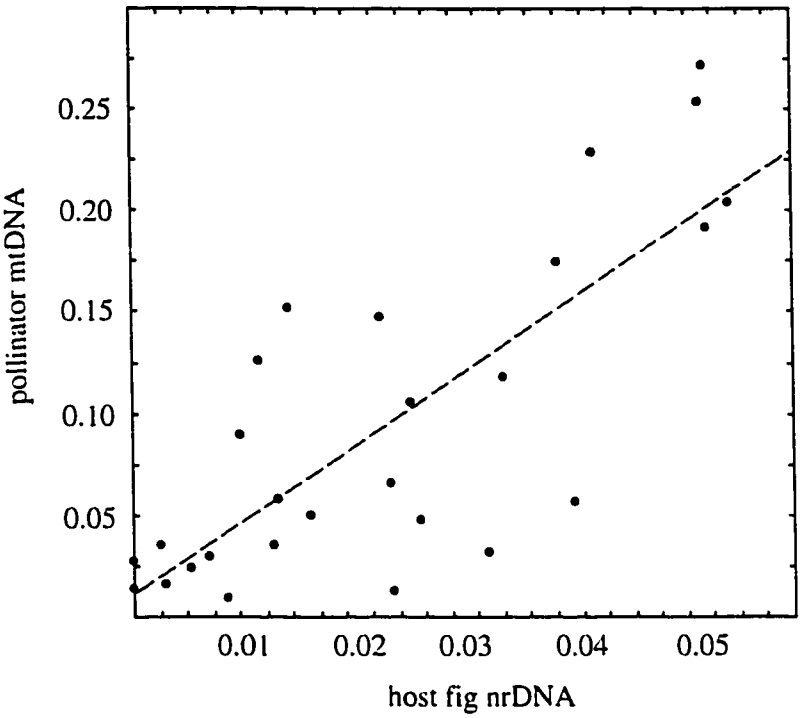


Figure 5B: randomization test $p = 0.006$

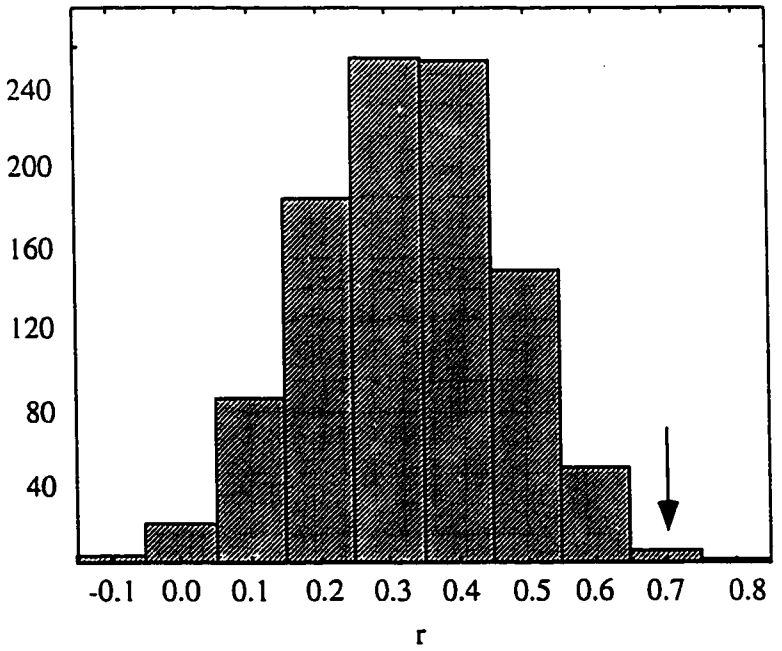


Figure 6: Evolution of fig breeding systems and pollinator ovipositor lengths

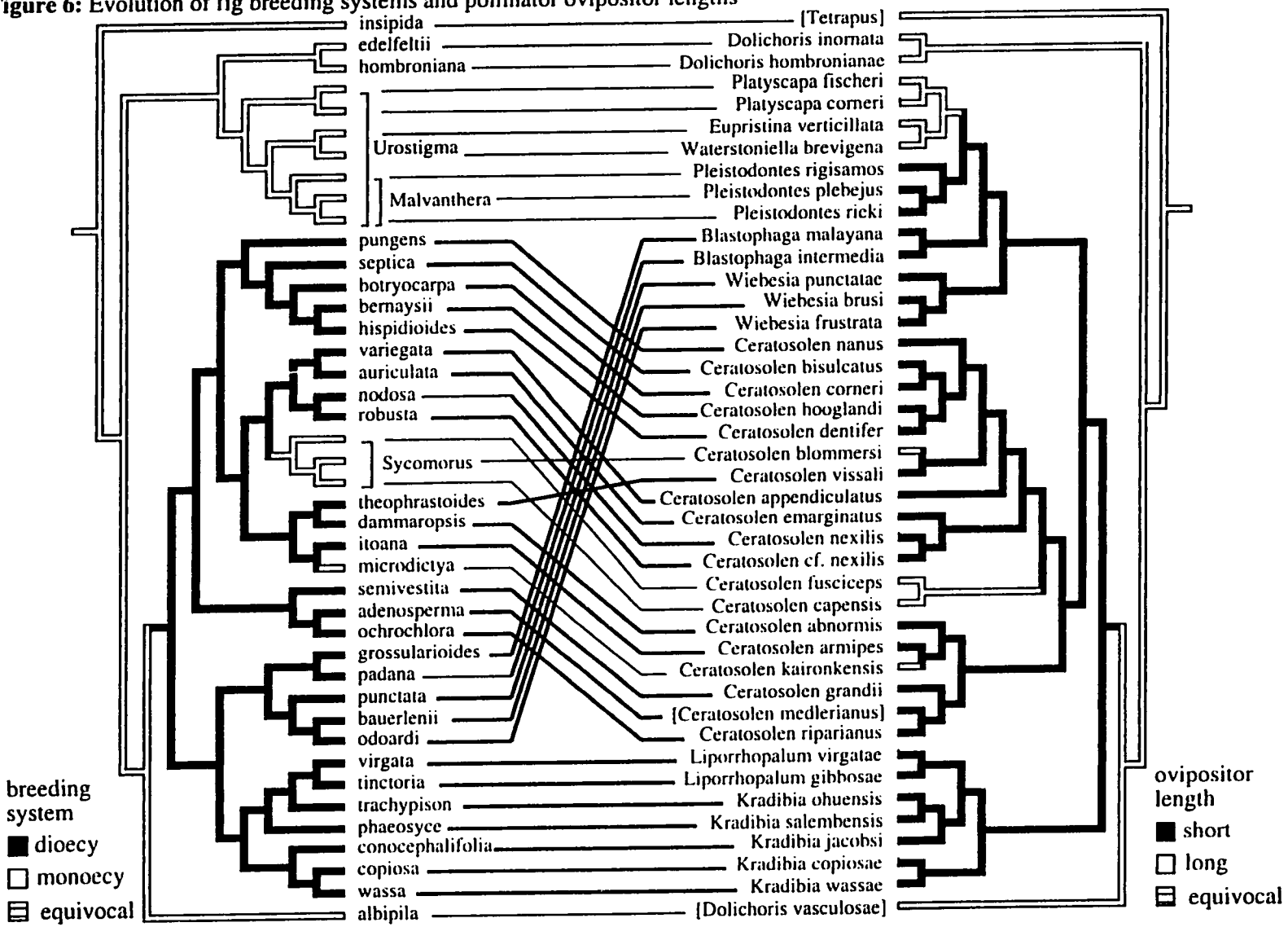
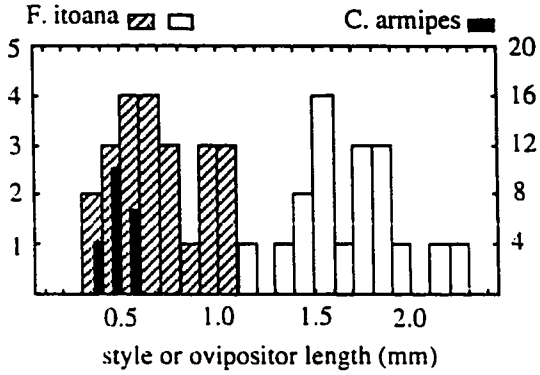


Figure 7A



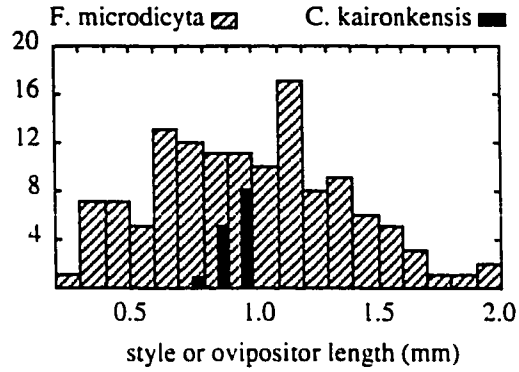
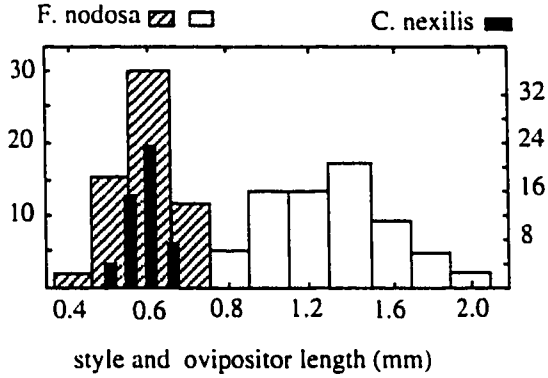


Figure 7B



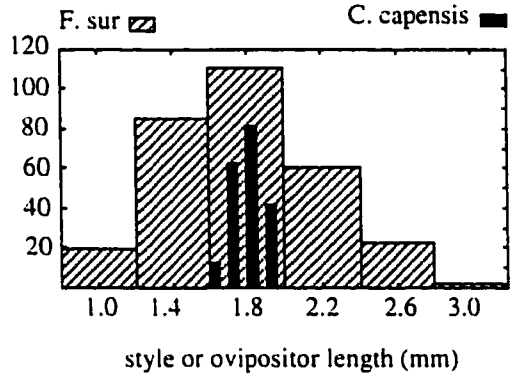
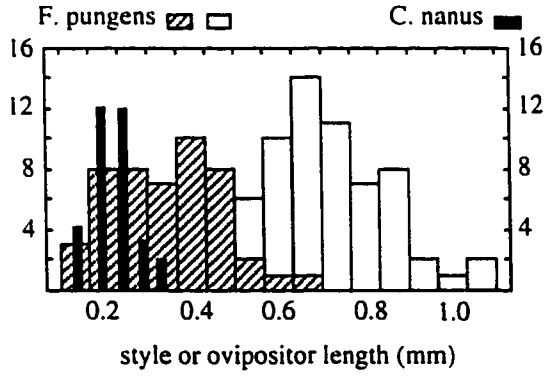


Figure 7C



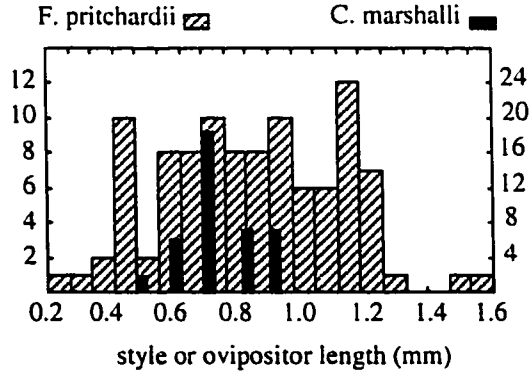


Figure 8: Positioning of staminate florets in relation to pollinator emergence

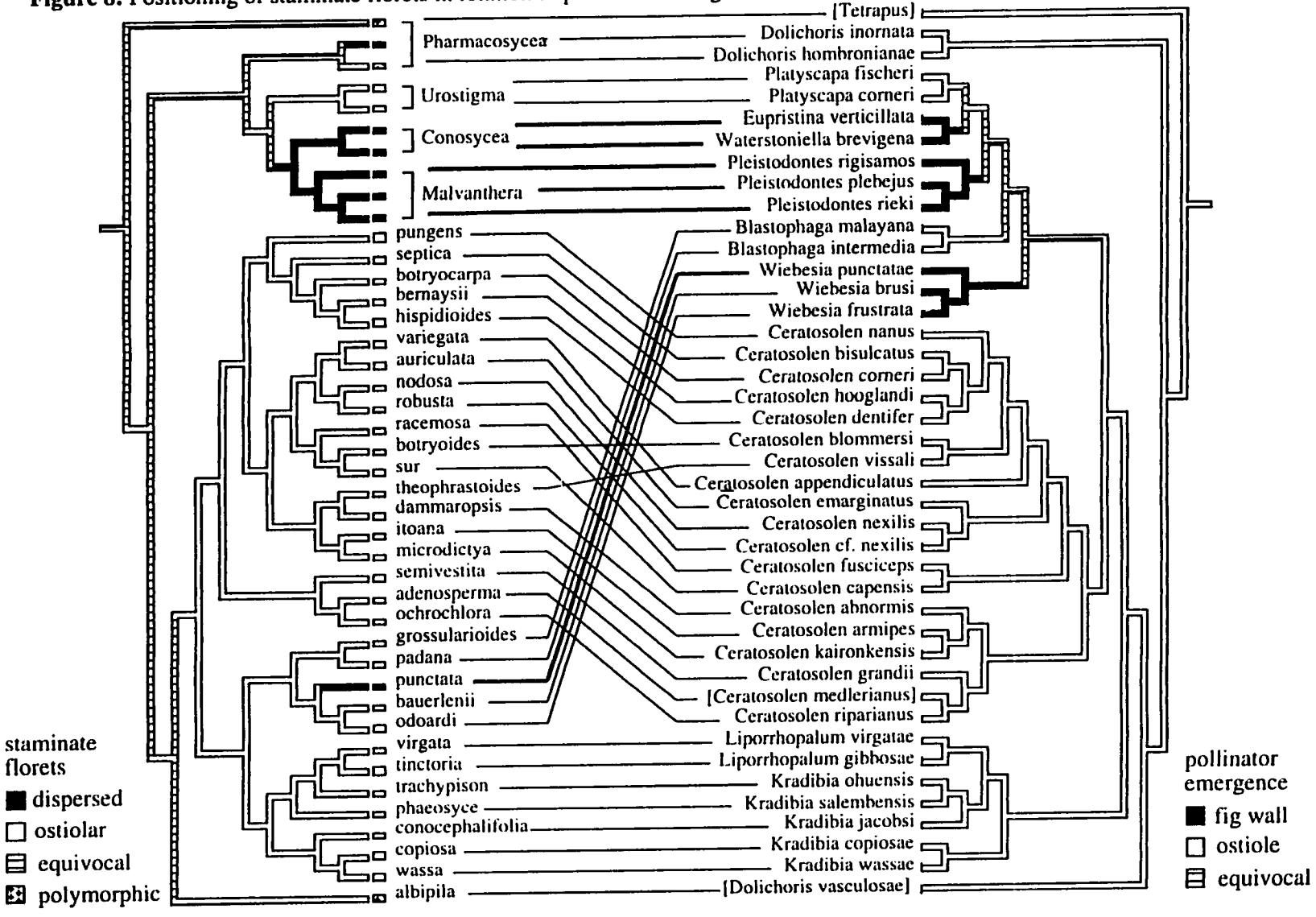


Figure 9: Evolution of fluid-filled syconia and enlarged spiracular peritremata

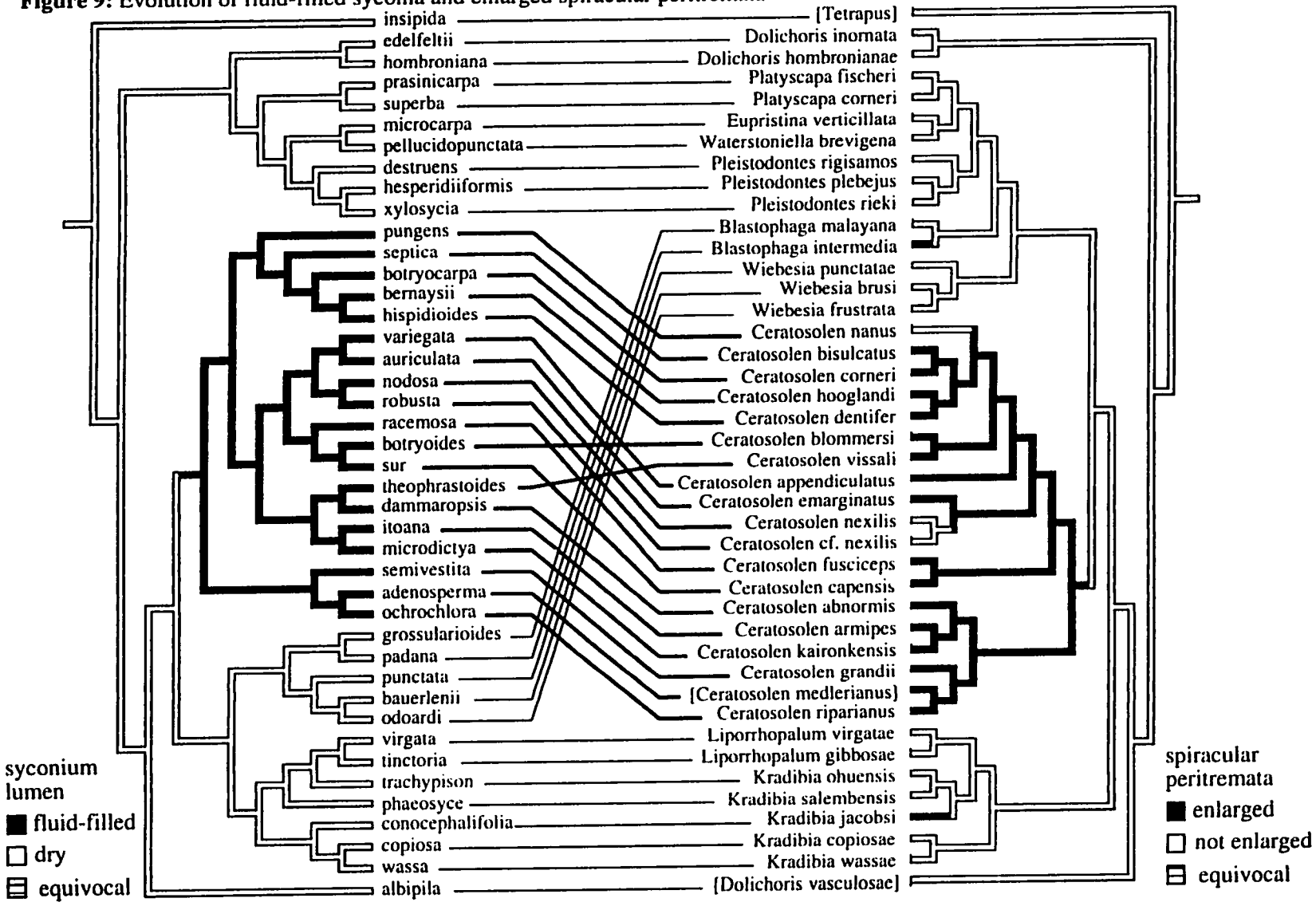


Figure 10A: dio (○); mono (●)

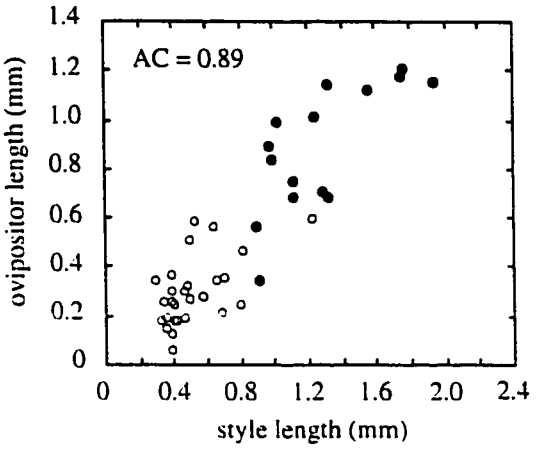


Figure 10B

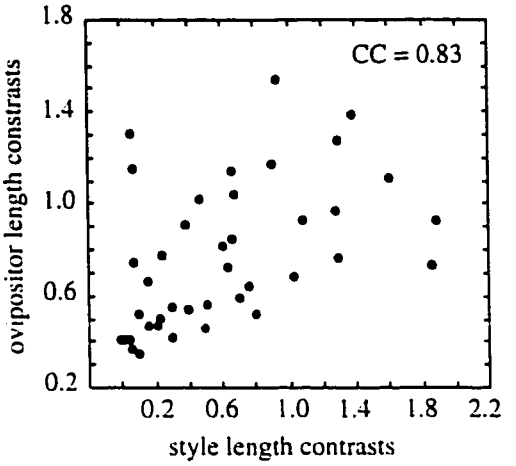


Figure 10C: pol (○); par (●)

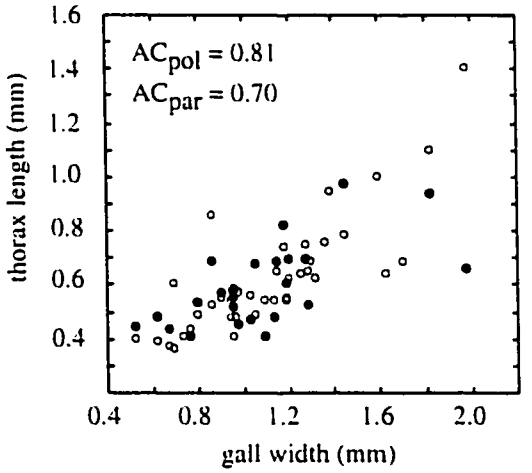


Figure 10D: pol (●)

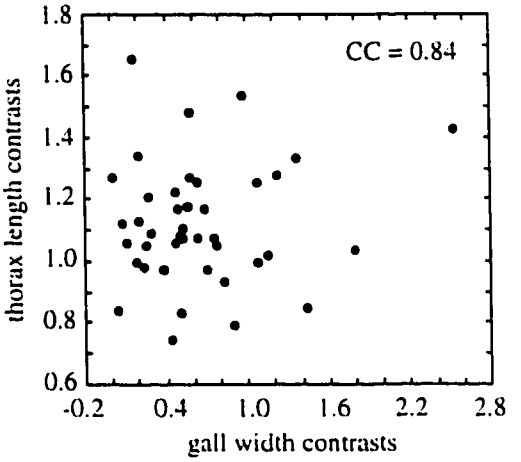


Figure 10E: pol (○); par (●)

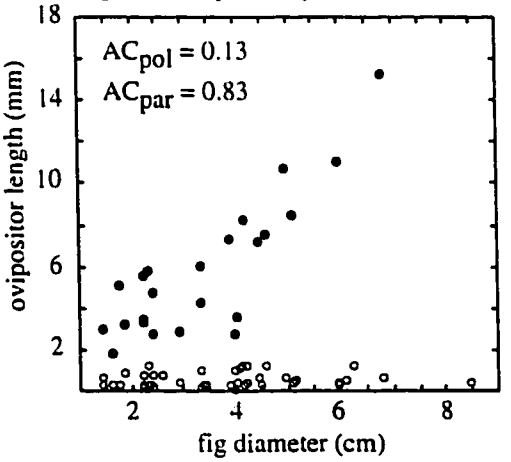
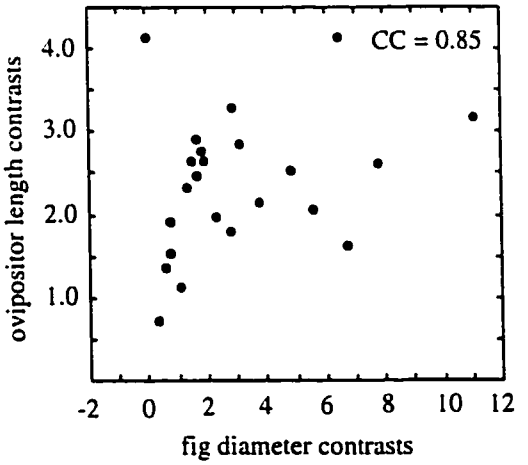


Figure 10F: par (●)



CHAPTER 4

Ecology of pollination and parasitism in New Guinea dioecious figs

"...there is such a close interaction between all the fig-insects, the flowers and the atmosphere inside the fig, that its interior appears as a micro-habitat, and no detail of construction or chemistry can be neglected."

(Corner 1940), p. 527

Introduction

The preceding chapters have provided phylogenetic evidence for coevolution between the dioecious figs and their pollinators. Patterns of cospeciation and reciprocal adaptation suggest that dioecious fig pollination is ancient and has been relatively stable through evolutionary time. In this regard, the mutualism between dioecious figs and their pollinators seems paradoxical due to apparent conflicts that threaten the stability of the interaction (Grafen and Godfray 1991, Bronstein 1992, Anstett et al. 1996). In general, mutualisms involving pollinating seed predators are characterized by conflicts over seed resources (Pellmyr 1997b). Analogous resource tradeoffs have been observed in yucca moths and yuccas (Pellmyr and Huth 1994), weevils and cycads (Norstog and Nicholls 1997), Anthomyiid flies and buttercups (Pellmyr 1992), gall midges and Monimiaceae (Feil 1992), and in beetles and palms (Henderson 1986). In the mutualistic exchange of pollination services for larval food, seeds can serve as a common currency for measuring the success of subsequent generations of mutualists (Janzen 1979a). The consumption of too many or too few seeds by pollinators could threaten to destabilize the mutualism, leading to extinction or to parasitism. However, opposing selection on the participants in an obligate mutualism might maintain equilibrium between the allocation of seed resources by the host plant and resource exploitation by the pollinator (Kiester et al. 1984). In this regard, dioecious fig pollination poses a unique evolutionary conflict (Kjellberg et al. 1987a, Grafen and Godfray 1991).

Seed and pollinator production are segregated on different plants in dioecious Ficus, due to the interaction of pollinator ovipositor and style lengths (Chapter 3). The pollinators of seed figs are entombed inside of the syconia and do not reproduce, while the pollinators of gall figs have relatively higher fitness. It would be advantageous for pollinators to avoid seed figs, however, this has not been observed. Pollinators do not prefer gall figs in dioecious Ficus (Patel et al. 1995). Kjelberg et al. (1987) argued that seasonal reproductive phenology could reduce or eliminate the opportunity for pollinators

to choose between gall and seed figs (but see Spencer et al. 1996). Alternatively, Grafen and Godfray (1991) supposed that vicarious selection could maintain the stability of the pollination mutualism in dioecious Ficus. The term "vicarious selection" was coined to describe the situation in which seed figs act as the agent of selection on wasps in gall figs. Grafen and Godfray argued that the external similarities of gall and seed figs maintain stability but few ecological data are available on the stability of dioecious fig pollination in New Guinea, where a substantial radiation of dioecious figs has occured (see Chapter 1; Wiebes 1982, Godfray 1988).

Another evolutionary conflict in dioecious figs involves the impact of parasitism on pollination. Non-pollinating fig wasps, including parasitoids and gallers, are abundant and species-rich components of fig wasp assemblages (Bronstein 1991, Compton et al. 1994a). Non-pollinators have a negative impact on the pollination mutualism through predation of pollinator larvae or through competition with pollinators for seed resources (Pellmyr et al. 1996a). Kerdelhue and Rasplus (1996a) argued that the evolution of dioecy in figs might reduce the incidence of non-pollinators. Assuming that non-pollinators compete for fig ovaries and that competitive displacement favors the partitioning of resources according to the position of fig ovaries, Kerdelhue and Rasplus (1996a) attributed lower numbers of non-pollinating species in dioecious figs compared to monoecious figs to the absence of multiple ovary layers in gall figs. However, competitive displacement has not been demonstrated in non-pollinating fig wasps and parasites are also abundant in dioecious Ficus (Godfray 1988). The direct and indirect effects of parasitism, combined with resource conflicts and the segregation of seed resources on separate plants, would seem to weaken the stability of dioecious fig pollination at several levels.

This chapter addresses the ecology of pollination and parasitism in New Guinea dioecious figs and summarizies ecological data pertinent to the question of mutualism stability. First, the natural history of dioecious fig pollination is reviewed in general.

Second, the stability of dioecious pollination is investigated by examining fig wasp behavior in relation to the traits of seed and gall figs. Third, an experiment with dioecious figs is described, examining whether sympatric pollinator preferences are consistent with patterns of host specificity and phylogenetic evidence for cospeciation (Chapter 3). Fourth, fig wasp assemblages of dioecious figs in sympatry are identified and the impact of non-pollinators on the mutualism is estimated. Lastly, the abundance of pollinators, parasites and gallers is compared in sympatric Ficus and the population dynamics of fig wasps is examined in a dioecious species.

Pollination in dioecious figs

It has been known for over a century that pollination in dioecious figs required the presence of fig wasps (Agaoninae: Hymenoptera; King 1887, Cunningham 1888), although the mechanics of pollen dispersal in dioecious species were not elucidated until forty years later (Williams 1928). Dioecious figs are morphologically gynodioecious but functionally dioecious due to their interaction with pollinators (Berg 1989, Weiblen et al. 1995). The dioecious figs are exclusively pollinated by female fig wasps, which actively pollinate the pistillate florets while laying eggs in a fraction of fig ovaries (Galil and Eisikowich 1968a). The fate of the florets is determined in part by the interaction of style and fig wasp ovipositor lengths (Galil and Eisikowitch 1971, Ganeshaiah et al. 1995). In seed figs, pistillate florets are pollinated by fig wasps but the ovules are unharmed because the pollinators fail to fully penetrate the long styled florets with their ovipositors (see Chapter 3). Gall figs are hermaphroditic, containing staminate florets and short-styled pistillate florets with ovules that are accessible to pollinators. Only fig wasp eggs deposited between the integument and nucellus of fig ovules will hatch (Grover and Chopra 1971), where the larvae generally feed on the developing endosperm (Cunningham 1888). In gall figs, each larva feeds on endopserm in a single ovary per floret. Hermaphroditic gall figs are functionally staminate because wasp larvae destory

all of the fig ovaries (Weiblen et al. 1995). At adulthood, male wasps emerge from their galls and use their telescopic genitalia to mate with the females. Males also use their specialized mouth parts to chew exits from the galls and from the fig cavity. Fertilized females groom pollen released from staminate florets into specialized mesosternal pockets and fly from the fig cavity to other figs in receptive phase. The obligate mutualism between dioecious figs and their pollinators is also impacted by non-pollinating fig wasps (Agaonidae: Hymenoptera), including parasitoids and gallers (Bronstein 1991, Compton et al. 1994a).

Although a general picture of dioecious fig pollination has emerged from independent studies (Galil 1973, Nair and Abdurahiman 1984, Beck and Lord 1988b, Corlett et al. 1990, Weiblen et al. 1995), relatively little is known about fig pollination in New Guinea, where a considerable radiation of dioecious figs has occurred (Wiebes 1982a, Godfray 1988, Basset et al. 1997). With approximately 15 percent of the global total, more Ficus species occur in New Guinea than in Africa, the Neotropics, or Borneo (Corner 1958). Ecological studies near Madang, Papua New Guinea documented the pollination biology of dioecious figs through field observations and experiments.

Methods

Study area

The study area was located in the Madang district of Papua New Guinea, in moderately disturbed lowland rain forest and coastal forest (0-400 m above sea level). Collections and experiments were made in primary and secondary lowland forests near Baitabag (145°047' E, 5°08' S, ca. 50 m), Ohu (145°41' E, 5°14' S, ca. 100 m) and Riwo villages (145°48'E, 5°09' S, 0 m). Average rainfall in the Madang area amounts to 3500 mm annually with a dry season from July to September and an average temperature of 26.5°C (Bowman et al. 1990). The lowland and coastal habitats are described in Bowman et al. (1990) and Jebb and Lowry (1995), respectively.

Pollinator observations

During June through August in 1996 and 1997, reproductive phenology of fig crops in dioecious F. hispidioides (Figure 1.1) was monitored in the Kau Wildlife Area near Baitabag village and on the grounds of the Christensen Research Institute (CRI). Seven cauliflorous trees (four with gall figs and three with seed figs) were selected prior to inception (i.e. with bracts enclosing fig receptacles in bud). From 12-50 days after inception, the diameter of approximately 20 figs per tree was measured to the nearest 0.1 mm at 48-96 hr intervals. Measurements declined to 5-10 figs per tree as figs aborted apparently due to low rates of pollination, high rates of parasitism, or changing patterns of resource allocation. Data were pooled from the seven trees to obtain averages of fig diameter with respect to days from inception. The arrival of fig wasps at each tree was recorded through direct observation and through sticky traps fastened to the cauliflorous branchlets. Sticky traps consisted of petri dishes (18.2 cm^2) lined with Tanglefoot™ and oriented in a vertical position to prevent rain from wetting the adhesive surface. A rolling census of the traps provided counts of fig wasp arrivals per 48-96 hrs. Pollination observations on cut figs were made with a dissecting microscope in the CRI laboratory.

Pollination in gall and seed figs

To examine the stability of pollination in F. hispidioides, traits of gall and seed figs were compared with respect to pollinator behavior. Measurements included a tree census to estimate the ratio of gall and seed plants in the study population. The estimated sex ratio was compared to a 1:1 expectation with a G-test for goodness of fit. Pollinators and non-pollinators trapped outside of figs and foundresses trapped inside of figs were also counted. Pollinator visitation rates and foundress numbers in seed and gall figs were compared with Kruskal-Wallis tests. In addition, size and weight of fresh figs were measured when ripe (D phase; Galil and Eisikowich 1968a). The abundance of pistillate

and staminate florets per fig, seed set, and the percentage of ovules occupied by fig wasps were also estimated. Due to 3000-4000 pistillate florets per fig in F. hispidioides, it was not feasible to make absolute counts. Instead, figs were cut into longitudinal sections (2 mm in width from the base of the fig to the apex) and the florets in one half section per fig were counted. Pistillate florets were scored as containing either (1) mature seeds, (2) aborted ovules, or (3) galls, as indicated by conspicuous exit holes in the apical end of the seed coat (Weiblen et al. 1995). To obtain estimates for whole figs, each count was multiplied by a factor consisting of fig dry weight divided by the dry weight of the longitudinal section. Style lengths were measured in pistillate florets using a Polaroid Digital Microscope Camera® with Adobe Photoshop® and NIH Image software (see Chapter 3). Measurements of fig diameter, mass, ovule occupancy and style length from seed and gall figs were compared using nested analyses of variance with unequal sample sizes.

Pollination experiment

A pollination experiment in F. hispidioides was carried out at the Kau Wildlife Area and at CRI during July-August, 1997. Four treatments included: (1) open pollination, without any experimental manipulation; (2) pollinator exclusion; (3) intraspecific pollination by Ceratosolen dentifer, the obligate pollinator, and (4) interspecific pollination by C. hooglandi, the pollinator of F. bernaysii. In treatments (2)-(4), pollinators were excluded from figs by sealing the ostiole with 1.5 mL screw-cap microcentifuge tubes (Sarstedt®) during A phase (see Results for summary of developmental phases). A hole was drilled in the cap of each tube and tubes were attached to the fig with Liquid Nails® contact cement so that the ostiolar bracts were sealed within a removable container but were not obstructed by glue (Figure 1.12). It was also necessary to replace the closed end of the tubes with 0.1 mm nylon mesh to allow the escape of fluid from the ostiole during A

phase. Some replicates were discarded from the experiment after expanding figs ruptured the contact cement seal, allowing uncontrolled access to pollinators.

Pollination was carried out during B phase. On the day prior to pollination, D phase gall figs from F. hispidioides and F. bernaysii were collected at the Ohu Conservation Area and pollinators were reared overnight in the CRI laboratory. Live pollinators were sealed in Eppendorf tubes (6-25 pollinators per tube), briefly cooled (less than 1 hr) in an ice chest, and assigned to specific treatments. Introductions were made by replacing the mesh-covered tubes with tubes containing the pollinators. Pollinators revived on reaching the ambient temperature and, after 48 hr, had either penetrated the ostiole or died in the tubes. The number of foundresses per fig could be estimated by subtracting pollinators remaining in tubes from initial counts.

Rearing of local fig wasp assemblages

The composition of local fig wasp assemblages, including 14 Ficus species in sympatry with F. hispidioides, was examined through a series of rearing experiments. Sixty-eight Ficus species have been recorded in forests near Madang (Weiblen 1999), which is comparable to the most speciose localities in the world (Corner 1962b, Laman and Weiblen in prep.). Target species for rearing experiments included fourteen dioecious Ficus and monoecious F. microcarpa, selected on the basis of local abundance (Table 1). The species differed in growth form, architecture, and regeneration niche but all were essentially sympatric within the study area (Basset et al. 1997). In choosing these species, easy recognition in the field was an important consideration for village assistants who collected figs and reared most of the insects. Ficus species names were verified by C. C. Berg.

Fig wasps were reared from 1995 to 1997. Samples were collected from an average of ten trees representing each target species. Several figs were removed from each tree at approximately 6-36 hr prior to the emergence of the fig wasps. Figs were

stored in plastic bags or mesh-covered vials. After emergence, adult wasps were preserved in 70% alcohol and selected individuals from each of 207 fig crops were sorted into morphospecies using a dissecting microscope. Slide-mounted vouchers of pollinators were keyed to species and compared against the collections of J. T. Wiebes at the Leiden Natural History Museum, Naturalis (see also Chapter 2). All agaonids reared and identified in this manner are listed in Table 1.

Samples of 5-10 figs per crop were pooled to examine variation in agaonid species occurrence among crops and among host species. In addition, 25 figs per host species were reared separately (from five separate crops per species), providing estimates of variation among figs and among crops. During 1996-1998, the population dynamics of fig wasps in F. trachypison were examined at the Ohu Conservation Area near Madang. Individual crops were sampled from fifteen trees. Sampling of five crops in 1996 included an average of five figs per crop. Samples of ten additional crops in 1997-1998 included approximately 25 figs per crop. Wasps were reared separately from each fig, sorted into morphospecies, and the abundance of pollinators, parasites and gallers was measured.

Results

Pollination in dioecious Ficus hispidioides

The timing of wasp arrivals at figs in F. hispidioides was synchronized with reproductive phenology (Figure 2). The period of development from inception to pollination (A phase; Galil and Eisikowich 1968a) lasted about 2-3 weeks. During the second week following inception, female Apocryptophagus were observed probing the exterior surface of figs with their ovipositors (Figure 1.2). Two Apocryptophagus species reared from F. hispidioides differed in ovipositor length (Figure 3). Kerdelhue and Rasplus (1996b) suggested that such a difference might reflect a difference in oviposition timing due to resource partitioning or interspecific competition for oviposition sites within figs.

Indeed, the diameter of F. hispidioides figs changed rapidly during the peak period of Apocryptophagus visitation (Figure 2). By the third week following inception, some pistillate florets in both seed and gall figs were relatively enlarged (Figure 1.4). These observations, combined with the rearing of adults from both seed and gall figs, confirm that Apocryptophagus does not require the presence of pollinator larvae to induce galls.

Female Ceratosolen dentifer were trapped at figs between 14-28 days, with a peak visitation rate at 20-26 days (Figure 2). Females entering the ostiole lost their wings and the apical segments of their antennae while penetrating the ostiolar bracts (Figure 1.3). Pollination (B phase) mostly occurred at three or four weeks following inception. Inside the fig cavity, multiple females per fig were observed while engaged in the repetitive behavior of probing pistillate florets with their ovipositors and brushing their fore legs past the mesothoracic pollen pockets to the stigmatic surface (Figure 1.4). In these details, active pollination by C. dentifer is similar to other species of Ceratosolen (Baker 1913, Galil and Eisikowich 1968b, Galil 1973, Joseph and Abdurahiman 1981, Kerdelhue et al. 1997). There was no significant difference between gall and seed figs in rates of pollinator visitation, suggesting that C. dentifer does not distinguish between the two types (Table 2) . Also, average numbers of foundresses in gall and seed figs were not significantly different in dioecious F. hispidioides, but they were substantially higher than in neotropical monoecious figs (Herre 1989). Within days of pollination, the fig cavity became filled with fluid.

Nonpollinating fig wasps were also trapped at figs 26-42 days following inception (Figure 2). Female Sycoscapter, Philotrypesis and Apocrypta spp. (Sycoryctinae) were observed probing the syconium exterior, each with a specially modified ovipositor. In Sycoscapter, a sheath covering the entire length of the ovipositor was fully inserted into the syconium wall. In Philotrypesis, a sheath covering only half the length of the ovipositor was bent like a hairpin when inserted into the syconium (Figure 1.5). In Apocrypta, the v-shaped abdominal tergites were contracted to form an erect support for

the ovipositor, which was held above the abdomen in a whip-like posture during oviposition. Although female Philotrypesis and Sycoscapter were observed at both gall and seed figs, none were reared from seed figs. Observations on the timing of oviposition are also consistent with the notion that Philotrypesis and Sycoscapter are parasitoids and are dependent on the presence of pollinator larvae for successful development. Philotrypesis, in particular, is known to feed on fig endosperm only after killing pollinator larvae (Kuttamathiathu 1959). Ulenberg (1985) suggested that Apocrypta may be a hyperparasitoid of Apocryptophagus and the relative body sizes of the F. hispidioides-associated wasps are consistent with this view. Trophic interactions within the syconia of F. hispidioides are summarized in Figure 3.

The interval between pollination and fig ripening (C phase) lasted 4-6 weeks, and during this period, the fig cavity remained fluid-filled. The cavity dried out during eclosure of the adult wasps in gall figs (D phase). Male pollinators (Figure 1.6) were first to emerge into the cavity, where they clasped galls with their enlarged hind tarsi, chewed circular openings, inserted their telescopic genitalia, and mated with the females (Figure 1.7). Male C. dentifer also chewed the ostiolar bracts to create an exit from the fig cavity (Figure 1.10). Female C. dentifer did not actively collect pollen from staminate florets, rather they were dusted with pollen on passing through the ostiolar tunnel created by the males. Prior to flight, the pollinators groomed themselves with their front coxae, delivering the pollen to the mesosternal pockets (Figures 1.8-1.9). In addition, weaver ants (Oecophila smaragdina) preyed on both pollinating and non-pollinating fig wasps during their arrival and departure from figs, often poised near the ostiole with mandibles reflexed, ready to attack pollinators and non-pollinators alike (Figure 1.11).

Gall and seed figs in Ficus hispidioides

The ratio of gall and seed figs in F. hispidioides was not significantly different from one (Table 2). In addition, approximately equal numbers of pollinators were trapped at figs of

both types. Non-pollinators were trapped in lower numbers than pollinators, but similar to pollinators, there was no significant difference in the rate of non-pollinator visitation to gall and seed figs. There was no significant difference in foundress number between gall and seed figs. Foundress numbers in dioecious F. hispidioides were higher on average than in monoecious species (cf 5.0-5.7 and 1.0-4.5; Herre 1989) although such a comparison does not take historical or ecological factors into account. It was not possible to control for the effect of foundress number on seed set and pollinator production (e.g. West et al. 1996) because C. dentifer foundresses could not be counted accurately after C phase owing to their deterioration in fluid-filled syconia. Ripe gall figs were significantly larger in diameter than seed figs although the difference in fresh weight was not significant.

As in other dioecious species, functional staminate florets were restricted to gall figs. There was high variance in the estimates of pistillate florets per fig and the difference between gall and seed figs was not significant. On the other hand, a significantly higher percentage of ovules developed into seeds than were occupied by pollinators (cf 86% and 52% in seed and gall figs, respectively). A bias toward higher levels of seed set than wasp production in dioecious figs has often observed but a convincing explanation has not yet been suggested (Corlett et al. 1990, Compton et al. 1994b, Weiblen et al. 1995). Similar to other dioecious species, style length in F. hispidioides was bimodally distributed between gall figs and seed figs (Figure 3). Styles in seed figs were longer than the ovipositors of C. dentifer. Mean style lengths in gall figs were slightly longer than the mean ovipositor length of the pollinator. However, the difference could be explained by the fact that, although oviposition occured in B phase, style length measurements were made in D phase. After correcting for a post-pollination increase in style length (Verkerke 1987), it appeared that ovaries in gall figs were accessible to pollinators while ovaries in seed figs were not. No pollinators were ever

reared from seed figs. Also, style length did not vary significantly among figs of the same sex ($F = 2.066$, $p = 0.383$).

Pollination experiment

Pollinator specificity in F. hispidioides was tested experimentally and attempts to introduce sympatric C. hooglandi, the pollinator of closely related F. bernaysii, to figs of F. hispidioides were unsuccessful. Table 3 shows that C. hooglandi was not induced to enter figs of F. hispidioides. Seven of the 102 introduced females of C. dentifer entered figs of F. hispidioides. By contrast, none of the 75 females of C. hooglandi entered F. hispidioides figs. Comparison of open-pollinated and intraspecific-pollinated figs showed a strong effect of the experimental treatment. The mean foundress number in intraspecific-pollinated figs (1.41) was much reduced compared to open-pollinated figs (5.22). Due to low numbers of experimental foundresses, Monte Carlo simulation was used in place of Williams' correction to evaluate the goodness-of-fit and, by this procedure, C. dentifer foundresses significantly outnumbered C. hooglandi foundresses ($p < 0.02$).

Local fig wasp assemblages

A species accumulation curve for the fig wasp assemblage of 15 sympatric Ficus species, including F. hispidioides, is shown in Figure 5. A total of 214 crops were collected from separate trees and 74 fig wasp morphospecies were reared during the study (Table 1). The species accumulation curve suggested that sampling of the local fig wasp assemblage for the 15 host species was exhaustive. For example, random sampling of 100 trees would have yielded 95% of the assemblage. Similar to the findings of Hawkins and Compton (1992) for monoecious figs in Africa, sampling of a single crop per fig species was sufficient to detect more than half of the associated wasp species.

The abundance of wasps per fig varied by three orders of magnitude according to fig size and according to the abundance of flowers per fig. It was not feasible to count individuals within large samples, however, it was evident that unique pollinator species were consistently associated with each fig species (Wiebes 1994c). A large assemblage of undescribed non-pollinating Agaonidae was also found (Boucek 1988). On average, there were between three and four non-pollinating species per host, ranging from one in F. dammaropsis to 13 in F. microcarpa. The total of 75 morphospecies associated with the 15 host species was based on an assumption of one-to-one host specificity of non-pollinators (Ulenberg 1985, Machado et al. 1996).

The non-pollinating Agaonidae appeared to be monophagous, although more taxonomic revisions and phylogenetic analyses are needed to make a stronger assessment of host range. The larval biology of the non-pollinating fig wasps is also not well understood but the there were at least two feeding modes: gall-makers that competed with pollinator larvae for fig ovules and parasitoids that killed pollinator larvae and fed on fig ovaries. All of the non-pollinators in this study appeared to oviposit externally through the wall of the fig. Sycoscapter and Philotrypesis (Sycoryctinae) oviposited after the pollinators, apparently feeding on fig endosperm after killing pollinator larvae (Kuttamathiathu 1959). Apocryptophagus (Sycophaginae) galled both seed and gall figs and it is possible that Apocryptophagus larvae feed on the proliferating nucellus, as in other Sycophaginae (Galil et al. 1980). Parasitic Apocrypta (Sycoryctinae) were reared from both types of figs, apparently attacking Apocryptophagus (Ulenberg 1985). Having relatively short ovipositors, Grandiana (Otitesellinae), Epichrysomalla, and Neosycophila (Epichrysomallinae) were restricted to smaller figs (Boucek 1988). These genera are thought to be phytophagous gallers, but unlike Apocryptophagus, they were not reared from the seed figs of dioecious species. Micranisa (Otitesellinae) Walkerella (Otitesellinae), Orymus (Orymidae), and three species of Odontofrogattia

(Epichrysomallinae) were gallers restricted to monoecious F. microcarpa (Beardsley 1998).

Gallers and/or parasites impacted most figs. The incidence of pollinators, gallers and parasites in crops from the 15 host species is shown in Table 4. In dioecious species, pollinators occurred in 93-100% of the crops. However, in monoecious F. microcarpa, pollinators were present in only 56% of the crops. There were also more non-pollinating fig wasps associated with F. microcarpa (13 morphospecies) than with any dioecious species (1-5 morphospecies). These results are consistent with the observation that dioecious figs generally have a lower incidence of non-pollinators than monoecious species (Kerdelhue and Rasplus 1996a). Alternative interpretations of these results will be discussed. The overall incidence of non-pollinators at the crop level varied 47-100% for parasites and 0-100% for gallers, and on average, non-pollinators occurred in more than half of the crops. The incidence of parasites was generally higher than that of gallers. At the crop level, the frequency of parasites and gallers was negatively but not significantly correlated across the 15 host species (Spearman correlation = -0.25).

The occurrence of each fig wasp species in crops and in figs of the 15 host species is summarized in Table 5. Comparing the 15 host species, most non-pollinator species were present in less than half of the fig crops but some specialist gallers and parasites occurred in all or nearly all crops. In F. nodosa, for example, Apocryptophagus sp. A occurred in 94% of crops and in 85% of figs within a crop, while Apocryptophagus sp. B was less common (29% among crops and 30% within crops). Some parasites were ubiquitous across crops but less so within crops, such as Philotrypesis sp. in F. trachypison (100% among crops and 53% within crops). Overall, there was a strong positive correlation between the incidence of fig wasp species in crops and in figs (Spearmann correlation = 0.81), indicating comparable distribution patterns at these levels.

In several host species with small figs, the abundance of fig wasps within syconia was recorded (i.e. F. microcarpa, F. phaeosyce, F. trachypison, and F. wassa). Monoecious F. microcarpa was unique in showing similar abundance of pollinator and non-pollinator species. For example, the mean abundance of the pollinator, Eupristina verticillata, (3.4 wasps per fig) was even exceeded by the mean abundance of Odontofroggatia sp. B (5.4 wasps per fig). In the dioecious fig species, by contrast, average pollinator abundance consistently surpassed the combined abundance of all non-pollinator species. Population dynamics of fig wasps in F. trachypison were examined in more detail.

Parasitism in Ficus trachypison

In figs of F. trachypison, pollinators (Kradibia sp. "ohuensis") outnumbered parasites (Philotrypesis sp.) and gallers (Grandiana sp.) by a factor of ten, on average (Table 6). Pollinators were found in all 288 samples, while parasites and gallers were found in 154 and 84 samples, respectively. Non-pollinator abundance was relatively low, with averages of eight and six individuals per fig for parasites and gallers, respectively, compared to an average of 89 pollinators per fig. This amounted to a mean non-pollinator frequency of less than 10% per fig. Pollinator sex ratio (male/female: 0.08) was substantially lower than in non-pollinators (0.68 in parasites and 0.63 in gallers). A highly female-biased sex ratio in the pollinator of F. trachypison is consistent with the theory of local mate competition (Hamilton 1967). Kradibia sp. "ohuensis" appeared to have only single foundress broods. If single foundresses oviposit in each gall fig, then all matings occur between siblings. This contrasts sharply with the externally ovipositing non-pollinators, where oviposition by more than one female per fig reduces levels of inbreeding and the strength of local mate competition. Consequently, perhaps, sex ratios in gallers and parasites were less female-biased than in pollinators.

There was significant variation among F. trachypison crops in pollinator abundance, pollinator sex ratio, and the overall abundance of wasps per fig (Table 7). The average number of pollinators per fig ranged from 63 ± 33 in a crop at Hogoli to 137 ± 43 in another nearby crop. The overall frequency of non-pollinators per fig was consistently low at the crop level (i.e. less than 20% in all crops). The incidence of parasitism per fig was not significantly different among crops; ranging from two to nine percent on average (Table 7). Also, the incidence of parasitism was not significantly different among crops after excluding 128 samples that lacked parasites ($H = 16.440$; $p = 0.0583$). The frequency of gallers per fig ranged from 0.4% to 15%, on average, and there was significant among-crop variation in galler abundance (Table 7). Among-crop variation in the incidence of gallers per fig remained significant after excluding 188 samples that lacked gallers ($H = 26.497$; $p = 0.002$).

Pollinator abundance was normally distributed while non-pollinator abundance was Poisson-distributed in 288 syconia (Figure 6). In addition, there were negative associations between pollinator, galler, and parasitoid abundance (Figure 7A). For example, figs with the highest abundance of gallers tended to have low abundance of parasites. Conversely, figs with high pollinator abundance tended to have low galler and parasitoid abundance. Also, the relationship between the number of pollinators per fig and the rate of parasitism was one of inverse density dependence (Figure 7B). Contrary to intuition, figs with the highest host density had the lowest rates of parasitism while figs with the lowest host density suffered the highest rates of parasitism.

Discussion

Stability of dioecious fig pollination

Pollination in F. hispidioides was similar to other dioecious species (Baker 1913, Galil 1973, Nair and Abdurahiman 1984, Beck and Lord 1988b, Corlett et al. 1990, Weiblen et al. 1995) in suggesting a paradoxical resource conflict between the mutualists (Kjellberg

et al. 1987a, Grafen and Godfray 1991). In particular, Ceratosolen dentifer showed no preference for gall figs in spite of the failure of foundresses to reproduce in seed figs. The simplest explanation for seed fig pollination may be pollinator deception. Pollinators of dioecious figs are capable of selecting a unique host from a range of closely related species in sympatry, presumably through olfactory cues released from figs during the period of receptivity (Hossaert-McKey et al. 1994). However, pollinators may not be capable of distinguishing between the two types of figs because selection favors seed figs that mimic gall figs in attractiveness (Grafen and Godfray 1991). Patel et al. (1995) pointed out that it would be difficult to detect less stable interactions because the evolution of a gall fig preference in pollinators would rapidly drive hosts to extinction. However, dioecious fig lineages are no less diverse than monoecious lineages (Chapter 1), perhaps indicating that dioecious figs are no less extinction prone than their monoecious relatives.

Results from F. hispidioides are in agreement with genetic data that suggest an evolutionary scenario for the evolution of dioecious fig pollination. Ficus hispidioides is heterostylous and the ratio of seed and gall figs in the study population was approximately one. This observation is consistent with the genetic system of sex determination in the edible fig, F. carica (Storey 1955, 1975). As outlined in Chapter 1, gynodioecious morphology in F. carica involves linked genes affecting style length and male-sterility. Seed figs are homozygous (ggaa) and gall figs are heterozygous (GgAa; G is dominant for short-styled florets, g is recessive for long-styled florets and a is recessive for male-sterility). Generally, mutations for male sterility in plants are favored by selection if the genetic contribution of female mutants through seed production is at least twice that of hermaphrodites (Lewis 1942). Along these lines, Valdeyron and Lloyd (1979) suggested that inbreeding depression favored the evolution of male sterility in figs. However, functional dioecy results from the interaction of pollinators and heterostylous florets, effectively separating the male and female components of fitness

between gall and seed plants, respectively. In theory, male sterility in seed figs would ensure that any offspring of a seed fig pollinator (e.g. a long ovipositor mutant) cannot produce a second generation because pollen is absent from their natal fig. In this scenario, the evolution of heterostyly as a means of separating male and female function could precede the evolution of male sterility but male sterility is required to maintain the stability of mutualism in dioecious figs. Under what circumstances would the separation of seed and pollinator production have been advantageous? Selection favoring the separation of sexes in figs has been attributed to environmental seasonality (Kjellberg and Maurice 1989) and parasitism (Kerdelhue and Rasplus 1996a). Aspects of these hypotheses will be integrated with data from F. hispidioides to outline a new hypothesis for the selective advantage of dioecious fig pollination.

Dioecious figs also pose an interesting problem related to pollination and seed dispersal (Laman and Weiblen, in review). Wasps are required for seed fig pollination but there is an obvious fitness cost to pollinators that become entombed in seed figs and do not reproduce themselves. The similar external appearance of both sexes of figs prior to pollination may prevent pollinator avoidance of seed figs, but after pollination, it is advantageous for seed figs to be dispersed by frugivores and for gall figs to protect developing pollinators from being eaten. Although gall and seed figs appear similar prior to pollination, they ultimately differ in size, coloration and palatability when ripe (Table 2; Lambert 1992, Weiblen et al. 1995). Indeed, ripe gall figs are often ignored by frugivores that prefer to eat seed figs of the same species, even though gall figs tend to be larger when ripe (Table 2; Lambert 1992, Laman and Weiblen, in review). Selection favoring gall and seed fig similarity during the pollination phase could be opposed by selection favoring dissimilarity during the dispersal phase. Examining the possibility of stabilizing selection on traits of dioecious figs is a challenging avenue for additional study.

Specificity of dioecious fig pollination

Pollination experiments in F. hispidioides were consistent with inferences of host specificity based from rearing experiments (Table 1) and from phylogenetic patterns (Chapter 3). Pollinators could not be induced to switch between closely related host species. Results of the pollination experiment are also consistent with earlier attempts at fig breeding. Intraspecific crosses of F. carica were made by introducing the obligate pollinator, Blastophaga psenes, to cultivars of the edible fig (Condit 1928). However, crosses between F. carica and F. pumila using the same technique failed because B. psenes could not be induced to enter figs of F. pumila (Condit 1950). F. aurea x religiosa hybrid seedlings were reported in Florida, where Pegoscapus mexicanus, the local pollinator of F. aurea was observed visiting exotic Ficus (Ramirez 1994). A similar breakdown of specificity, involving a local pollinator (Ceratosolen capensis) and an exotic fig (F. lutea) in Africa produced hybrids (Compton 1990, Ware and Compton 1992). Artificial hybrids have also been produced by blowing pollen into figs (Condit 1950, Ramirez 1986), suggesting that pollinator preference is the primary mechanism of pre-mating reproductive isolation, and in natural populations, pollinators rarely make mistakes (Bronstein 1987). Furthermore, the absence of fertile intermediates in nature also suggests that hybridization has not played a major role in speciation, and the overall results of pollination experiments are consistent with phylogenetic evidence in this regard (Chapter 3).

Stability of parasitism in dioecious figs

The impact of non-pollinators on dioecious fig pollination was widespread in New Guinea (Tables 4-5). Inferences on the costs and benefits of interactions within non-pollinating assemblages were based on field observations and rearing experiments with 15 species. Non-pollinators appeared to have direct negative impacts on pollinators and indirect negative impacts on dioecious figs. Non-pollinating fig wasps were assigned to

two guilds: gallers directly competing with pollinators for seed resources and parasitoids attacking pollinator larvae (Yu 1997). Most dioecious fig gallers apart from Apocryptophagus were restricted to gall figs but it is not clear why some gallers that oviposit externally should be limited to the same flowers as the internally-ovipositing pollinators (West et al. 1996).

The incidence of parasitism in New Guinea figs was consistent with overall patterns for monoecious and dioecious species. For example, species richness of the non-pollinating fig wasp fauna was lower in 14 dioecious species than in monoecious F. microcarpa (Table 1). Lower non-pollinator species diversity in dioecious figs compared to monoecious figs has been observed in other species (Kerdelhue and Raspuls 1996a). In addition, the incidence of pollinators at the crop level and the abundance of pollinators per fig were lower in Eupristina verticillata than in the pollinators of dioecious figs (Tables 4-5). These observations, however, do not suggest an explanation for the stability of parasitism in dioecious figs. The question remains as to what factors promote the coexistence of parasites and hosts in dioecious fig wasp assemblages.

A key factor in stabilizing host-parasite interactions is parasitoid aggregation in space and time (Hassell and Pacala 1990, Pacala et al. 1990). If hosts are distributed in patches and the incidence of parasitism varies from patch to patch, then increasing parasitoid density reduces parasitoid search efficiency (Pacala et al. 1990). In addition, heterogeneity in the rate of parasitism can be divided into components that are dependent on host density (HDD) and independent of host density (HDI; Taylor 1993). Both HHD and HDI components can contribute to the stability of an interaction, and a simple framework has been developed for testing whether heterogeneity is sufficient to stabilize a host-parasitoid system (Hassell and Pacala 1990). West et al. (1996) applied this test to a case of parasitism in a monoecious fig species and reported that HHD heterogeneity was sufficient to stabilize the interaction between parasitic Physothorax and Aepocerus.

Data on the population dynamics of Philotrypesis and Kradibia in F. trachypison could similarly test whether heterogeneity is sufficient to stabilize the host-parasite interaction in a dioecious fig. In contrast to the findings of West and Herre (1996), however, there was an inverse relationship between the rate of parasitism and host density in F. trachypison (Figure 7B). Hassell et al. (1985) showed that inverse density dependence may result from a low limit on the rate of parasitism per patch and little or no aggregation of parasitoids within patches. Results suggest that both of these processes may be operating at the crop level in F. trachypison. The frequency of parasitism per fig never exceeded 10% on average and there was no significant variation in the frequency of parasitism among ten crops (Table 7). What ecological factors could limit the rate of parasitism within patches or explain the non-aggregation of parasites at the crop level? Possible explanations include parasitoid egg limitation, failure by parasitoids to detect patches of high pollinator density, or failure to determine where parasitoid eggs have been laid. Ant predation was observed while Philotrypesis probed syconia for oviposition sites (e.g. Figures 1.5 and 1.11) and this could impose a limit on parasitoid search time (e.g. Figure 1.11). In addition, the efficiency of parasitoid searching may also be reduced by the presence of seed figs, which could represent an adaptive benefit of dioecy.

Adaptive benefit of dioecious fig pollination

Field observations suggest an alternative hypothesis concerning the evolution of dioecious fig pollination. The fact that non-pollinating fig wasps waste time probing seed figs suggests a more direct advantage of dioecy than an earlier hypothesis based on competition and partitioning of ovary layers among non-pollinators (Kerdelhue and Rasplus 1996a). Trapping data from F. hispidioides (Table 2) and F. congesta (H. Spencer, pers. comm.) show that non-pollinators do not distinguish between gall and seed figs in the search for oviposition sites. Time wasted by parasites on seed figs could

reduce levels of parasitism in gall figs. Seed figs that serve as ecological sinks for parasitoids could represent a novel adaptive benefit of dioecy.

The parasitoid-sink hypothesis is illustrated through a simple model of pollinator production (D. Yu, pers. comm). Suppose that in a fig population, X equals the number of pollinator-producing trees, Y equals the number of pollinators produced per tree, and Z equals pollinator survivorship. Pollinator production in a monoecious population is then defined as:

$$P_M = (X)(Y)(Z)$$

In a dioecious population, pollinator production is defined as:

$$P_D = (X/2)(2Y)(aZ)$$

where half of the trees have gall figs but pollinator production is doubled in gall figs relative to monoecious figs due to foundresses having access to twice as many ovaries. Reduced parasitism in gall figs also increases pollinator survivorship relative to monoecious figs (a). The model predicts that pollinator production in a dioecious population will exceed that of a monoecious population ($P_D > P_M$). The doubling of pollinator production in gall figs relative to monoecious figs is a direct consequence of heterostyly because oviposition is more efficient in short-styled florets (Nefdt and Compton 1996). However, the increase in pollinator survivorship (a) resulting from search time wasted by parasitoids on seed figs is less obvious. This adaptive hypothesis differs from that proposed by Kerdelhue and Rasplus (1996a), in which a reduction in non-pollinator species diversity was viewed as the main advantage of dioecy. An increase in pollinator production (Y) would be especially advantageous in situations where pollinators are limiting, as in seasonal environments (Kjellberg and Maurice 1989, Spencer et al. 1996).

It is important to validate the assumptions underlying the model prediction before discussing further evolutionary implications. The assumption of an equal sex ratio in dioecious figs is supported by population census data from F. hispidioides (Table 2), F.

variegata (G. Weiblen, unpublished) and by progeny arrays from F. carica (Storey 1955). The assumption of increased pollinator production in gall figs relative to monoecious figs is also supported by census data (Bronstein 1988b, Corlett 1993, Weiblen et al. 1995, Kerdelhue et al. 1997). In monoecious F. pertusa, for example, 11% of ovaries were occupied by pollinators on average (Bronstein 1988b), compared to 22% in gall figs of dioecious F. fistulosa (Corlett et al. 1990). However, such a comparison does control for historical or ecological factors affecting the production of pollinators. The magnitude of the increase in pollinator survivorship in gall figs depends on how much time parasitoids spend on seed figs and more empirical data are needed to address this point. If parasitoids spend equal time searching gall and seed figs, then a equals two and a dioecious population would produce twice as many pollinators as a monoecious population.

The model suggests that, with the evolution of dioecy, the male component of plant fitness could rise through increased pollinator production. A model of the monoecious fig/pollinator resource conflict suggest that gains in male fitness through pollinator production are offset by losses in female fitness through the consumption of seeds by pollinators (Janzen 1979a). However, a reduction in parasitism through dioecy is advantageous for the pollinator and for the host plant in both fitness components. Parasitism, therefore, could have played a key role in the origin and maintenance of dioecious fig pollination. Unlike the hypothesis of Kerdelhue and Rasplus (1996), the new hypothesis provides a direct benefit of dioecy compared to monoecy. Sister-group comparison of monoecious and dioecious species under similar ecological conditions would aid in testing this idea but more data on seed set, pollinator production, and rates of parasitism are needed.

Table 1: Agaonidae associated with 15 Ficus species at Madang, Papua New Guinea.

Ficus	pollinators (Agaoninae)	gallers (Epichrysomallinae, Otitesellinae, Sycophaginae)	parasitoids (Sycoryctinae)
bernaysii	Ceratosolen hooglandi	2 Apocryptophagus spp.	Apocrypta meromassa Ulenberg Sycoscapter sp.
botryocarpa	Ceratosolen corneri	Apocryptophagus sp.	Philotrypesis sp. Sycoscapter sp.
conocephalifolia	Kradibia jacobsi		Philotrypesis sp. Sycoscapter conocephalus Wiebes
copiosa	Kradibia copiosae	Grandiana sp.	Sycoscapter sp. Tenka percaudata Boucek
dammaropsis	Ceratosolen abnormis		
hispidioides	Ceratosolen dentifer	2 Apocryptophagus spp.	Apocrypta sp. Philotrypesis sp. Sycoscapter sp.
microcarpa	Eupristina verticillata	Orymus silvae Girault 3 Odontofroggatia spp. 2 Epichrysomalla spp. Walkerella microcarpae Boucek Micranisa sp.	2 Philotrypesis sp. 3 Sycoscapter sp.
nodosa	Ceratosolen nexilis	2 Apocrytophagus spp.	Apocrypta sp. Sycoscapter sp.
phaeosyce	Kradibia sp. "salembensis"	Grandiana cf. wassae Wiebes	Philotrypesis sp. Sycoscapter sp.
pungens	Ceratosolen nanus		Philotrypesis sp. 2 Sycoscapter spp.
septica	Ceratosolen bisulcatus	Apocryptophagus sp.	Philotrypesis sp. 2 Sycoscapter sp.
tinctoria	Liporrhopalum gibbosae	Epichrysomalla sp. Neosycophila sp.	2 Philotrypesis spp. Sycoscapter sp.
trachypison	Kradibia sp. "ohuensis"	Grandiana sp. Epichrysomalla sp.	Philotrypesis sp. Sycoscapter sp.
variegata	Ceratosolen appendiculatus	Apocrytophagus spinitarus Mayr	Apocrypta caudata (Girault) 2 Sycoscapter spp.
wassa	Kradibia wassae	Epichrysomalla sp. Grandiana wassae Wiebes Neosycophila sp.	Sycoscapter sp. Philotrypesis sp.

Table 2: Comparisons of gall and seed fig traits in Ficus hispidoides. The frequency of gall and seed trees at the Kau Wildlife Area (Madang, Papua New Guinea) was compared against an even sex ratio with a G-test for goodness of fit. Mean pollinator visits and foundresses per fig in gall and seed figs were compared with Kruskal-Wallis tests (ts = test statistic). Nested analyses of variance compared fig diameter, fig mass, ovule occupancy and style length between gall and seed figs. Diameter (cm) and mass (g) are reported for fresh figs in D phase.

	gall figs		seed figs			
	X (SD)	N	X (SD)	N	ts	p
census	18 trees		21 trees		0.231	ns
pollinators/trap	22.6 (16.4)	3	22.6 (8.1)	8	0.041	ns
nonpollinators/trap	0.9 (1.6)	3	1.0 (1.0)	8	0.503	ns
foundresses/fig	5.0 (4.3)	6	5.7 (2.5)	3	0.610	ns
fig diameter (cm)	4.1 (0.4)	41	3.6 (0.4)	38	20.48	<0.001
fig mass (g)	51.9 (15.9)	41	46.4 (11.4)	38	3.092	ns
pistillate florets/fig	3749 (1399)	20	4125 (2074)	11	3.719	ns
staminate florelts/fig	100 (45)	20	-	11	-	-
% ovule occupancy	52.3 (19.1)	20	85.6 (10.5)	11	20.91	<0.0001
style length (mm)	0.35	170	1.05	190	1539	<0.0001

Table 3: Results of experimental pollination in Ficus hispidioides at Madang, Papua New Guinea. Ceratosolen dentifer, the obligate pollinator of F. hispidioides, was introduced to receptive figs in the intraspecific pollination treatment. Ceratosolen hooglandi, the pollinator of sympatric and allied F. bernaysii, was introduced to figs of F. hispidioides in the interspecific treatment.

pollination treatment	figs per treatment	average introductions per fig	total introduced	total foundresses	average foundresses per fig
C. dentifer	5	20	**102**	**7**	1.41
C. hooglandi	7	11	**75**	**0**	0
exclusion	6	0	0	0	0
open	9	-	-	47	5.22

Table 4: Summary of the incidence of fig wasp pollinators (pol), parasites (par) and gallers (gal) in samples of fig crops from 15 Ficus species at Madang, PNG. Crops refer to figs collected from the same plant. The total assemblage included an estimated 74 Agaonid species reared from 214 crops.

	wasp spp.	crops	figs	crops with			percent crops with		
host spp.	per host	sampled	sampled	pol	par	gal	pol	par	gal
bernaysii	5	15	13	15	12	12	100	80	80
botryocarpa	4	14	16	13	13	5	93	93	36
conoceph.	3	15	21	15	14	0	100	93	0
copiosa	3	19	21	19	9	10	100	47	53
dammaropsis	2	16	13	16	10	0	100	63	0
hispidioides	6	15	16	15	13	9	100	87	60
microcarpa	14	9	55	5	6	9	56	67	100
nodosa	6	17	20	17	11	15	100	65	88
phaeosyce	3	10	23	10	9	2	100	90	20
pungens	4	17	25	17	13	0	100	76	0
septica	5	14	20	14	13	2	100	93	14
tinctoria	6	8	-	8	8	2	100	100	25
trachypison	5	15	288	15	15	14	100	100	93
variegata	5	14	25	14	8	13	100	57	93
wassa	6	14	25	14	8	12	100	57	86

Table 5: Incidence of pollinating and non-pollinating fig wasp species in crops and in figs from 15 Ficus species at Madang, PNG (with three letter abbreviations). For host species with small figs, fig wasp abundance was averaged across figs sampled individually.

Ficus	associate species	guild	no. crops	percent crops	no. figs	percent figs	wasps per fig X (SD)
BER.	Ceratosolen hooglandi	pol	15	100	13	100	-
	Sycoscapter sp.	par	11	73	4	31	-
	Apocrypta meromassa	par	5	33	0	0	-
	Apocryptophagus sp. A	gal	9	60	10	77	-
	Apocryptophagus sp. B	gal	5	33	2	15	-
BOT	Ceratosolen corneri	pol	13	93	8	50	-
	Sycoscapter sp.	par	6	43	1	6	-
	Philotrypesis sp.	par	13	93	11	69	-
	Apocryptophagus sp.	gal	5	33	8	50	-
CON.	Kradibia jacobsi	pol	16	100	21	100	-
	Sycoscapter conocephalus	par	14	88	15	71	-
	Philotrypesis sp.	par	2	15	0	0	-
COP	Kradibia copiosae	pol	19	100	21	100	-
	Sycoscapter sp.	par	9	47	2	10	-
	Grandiana copiosae	gal	10	53	12	57	-
DAM	Ceratosolen abnormis	pol	16	100	12	92	-
	Tenka percaudata	par	12	80	11	85	-
HIS	Ceratosolen dentifer	pol	15	100	16	100	-
	Sycoscapter sp.	par	12	80	9	56	-
	Philotrypesis sp.	par	7	47	9	56	-
	Apocrypta sp.	par	2	13	2	13	-
	Apocryptophagus sp. A	gal	11	73	8	50	-
	Apocryptophagus sp. B	gal	1	7	0	0	-
MIC	Eupristina verticillata	pol	4	44	17	33	3.4 (11.4)
	Philotrypesis sp. A	par	4	44	32	58	2.8 (4.5)
	Philotrypesis sp. B	par	1	11	4	7	0.4 (2.3)
	Sycoscapter sp. A	par	2	22	14	25	2.5 (5.3)
	Sycoscapter sp. B	par	1	11	1	2	0.2 (1.6)
	Sycoscapter sp. C	par	1	11	0	0	0 (-)
	Orymus sp.	gal	2	22	0	0	0 (-)
	Walkerella microcarpae	gal	7	78	25	45	2.8 (5.2)
	Odontofroggatia sp. A	gal	-	-	34	62	5.4 (6.5)
	Odontofroggatia sp. B	gal	-	-	31	56	2.5 (2.7)
	Odontofroggatia sp. C	gal	-	-	25	45	3.0 (4.5)
	Epichrysomalla sp. A	gal	-	-	13	24	0.8 (1.6)
	Epichrysomalla sp. B	gal	-	-	0	0	0 (-)
	Micranisa sp.	gal	1	11	24	44	1.2 (1.8)

Table 5 (continued): Incidence of pollinating and non-pollinating fig wasp species.

Ficus	associate species	guild	no. crops	percent crops	no. figs	percent figs	wasps per fig X (SD)
NOD	Ceratosolen nexilis	pol	17	100	17	85	-
	Sycoscapter sp.	par	8	47	3	15	-
	Apocrypta sp.	par	9	53	8	40	-
	Apocryptophagus sp. A	gal	16	94	17	85	-
	Apocryptophagus sp. B	gal	5	29	6	30	-
	Epicrysomalla sp.	gal	1	6	1	5	-
PHA	Kradibia sp. "salembensis"	pol	12	100	88	100	81 (43)
	Philotrypesis sp.	par	11	92	49	56	6 (9)
	Sycoscapter sp.	par	2	17	1	1	0 (-)
	Grandiana sp.	gal	2	17	41	47	2 (5)
PUN	Ceratosolen nanus	pol	17	100	19	76	29 (21)
	Sycoscapter sp. A	par	13	76	14	56	6 (7)
	Sycoscapter sp. B	par	4	24	1	4	0 (-)
	Philotrypesis sp.	par	1	9	0	0	0 (-)
SEP	Ceratosolen bisulcatus	pol	14	100	20	100	-
	Sycoscapter sp. A	par	8	57	5	25	-
	Sycoscapter sp. B	par	12	86	9	45	-
	Philotrypesis sp.	par	1	7	15	75	-
	Apocryptophagus sp.	gal	2	14	2	10	-
TIN	Liporrhopalum gibbosae	pol	8	100	-	-	-
	Philotrypesis sp. A	par	7	88	-	-	-
	Philotrypesis sp. B	par	8	100	-	-	-
	Sycoscapter sp.	par	7	88	-	-	-
	Neosycophila sp.	gal	2	25	-	-	-
	Epicrysomalla sp.	gal	1	12	-	-	-
TRA	Kradibia sp. "ohuensis"	pol	15	100	288	100	89 (42)
	Philotrypesis sp.	par	15	100	153	53	4 (7)
	Sycoscapter sp.	par	3	20	4	1	0 (-)
	Grandiana sp.	gal	14	93	84	29	2 (6)
	Epichrysomalla sp.	gal	1	7	1	0	0 (-)
VAR	Ceratosolen appendiculatus	pol	14	100	25	100	-
	Sycoscapter sp. A	par	6	43	9	36	-
	Sycoscapter sp. B	par	5	36	17	68	-
	Apocrypta caudata	par	2	14	6	24	-
	Apocryptophagus spinitars.	gal	13	93	25	100	-
WAS	Kradibia wassae	pol	13	100	25	100	133 (87)
	Sycoscapter sp.	par	5	38	0	0	0 (-)
	Philotrypesis sp.	par	6	46	4	16	2 (6)
	Grandiana wassae	gal	12	92	25	100	24 (20)
	Epichrysomalla atricorpus	gal	2	15	0	0	0 (-)
	Neosycophila sp.	gal	1	8	0	0	0 (-)

Table 6: Abundances, sex ratios and frequencies of pollinators, parasites and gallers per fig in F. trachypison at Madang, Papua New Guinea.

	X (SD)	N	min.	max.
pollinators per fig	89.3 (42.2)	288	1	209
parasities per fig	8.2 (8.0)	154	1	36
gallers per fig	6.4 (7.9)	84	1	40
total wasps per fig	95.6 (39.8)	288	20	215
pollinator sex ratio	0.08 (0.24)	288	0	4
parasite sex ratio	0.68 (0.89)	92	0.04	8
galler sex ratio	0.63 (0.42)	46	0.08	2
frequency of parasites per fig	0.12 (0.16)	154	0.005	0.92
frequency of gallers per fig	0.09 (0.11)	84	0.008	0.46
frequency of non-pollinators	0.09 (0.15)	288	0	0.96

Table 7: Variability among ten fig crops in F. trachypison in pollinator abundance and rates of parasitism at Madang, Papua New Guinea. Only crops with more than 20 figs sampled were included. Analyses of variance compared numbers of pollinators per fig, pollinator sex ratios, and total numbers of wasps per fig. Non-parametric Kruskal-Wallis tests compared variability among fig crops in the frequency of parasites and gallers per fig.

crop location	N	pol per fig X (SD)	pol sex ratio X (SD)	wasps per fig X (SD)	freq par per fig X (SD)	freq gal per fig X (SD)
Gola 10	35	100 (29)	0.079 (0.07)	103 (28)	0.026 (0.04)	0.004 (0.02)
Hogoli 7	25	108 (45)	0.029 (0.03)	112 (44)	0.035 (0.06)	0.008 (0.00)
Hologi 8	24	137 (43)	0.084 (0.05)	140 (43)	0.018 (0.03)	0.004 (0.01)
Odubal 14	25	76 (27)	0.083 (0.09)	83 (24)	0.091 (0.12)	0.006 (0.02)
Selabab 9	25	73 (27)	0.079 (0.08)	79 (24)	0.068 (0.13)	0.018 (0.04)
Talihu 11	29	82 (33)	0.056 (0.05)	87 (32)	0.046 (0.06)	0.006 (0.03)
Hogoli 1	25	63 (34)	0.085 (0.09)	75 (33)	0.031 (0.06)	0.156 (0.16)
Gola 16	25	79 (38)	0.045 (0.05)	86 (36)	0.079 (0.12)	0.022 (0.03)
Wa-ama 5	25	91 (33)	0.102 (0.12)	97 (33)	0.045 (0.09)	0.026 (0.05)
Wa-ama 6	24	91 (44)	0.048 (0.05)	98 (42)	0.065 (0.10)	0.021 (0.08)
F (H)		8.764	2.564	7.714	(9.580)	(74.149)
P		<0.0001	0.008	<0.0001	0.385	<0.0001

Figure 1: Illustrations of pollination and parasitism in dioecious figs. (1) Cauliflorous Ficus hispidioides at Madang, Papua New Guinea. (2) Non-pollinating Apocryptophagus sp. (Sycophaginae) on a seed fig of F. hispidioides. (3) Drawing from Cunningham (1888) showing the arrival of Ceratosolen at the ostiole in F. auriculata. (4) Five C. dentifer foundresses ovipositing in a gall fig of F. hispidioides. Red pistillate florets and white Apocryptophagus galls are also visible. (5) Parasitic Philotrypesis sp. ovipositing through the syconium wall in F. congesta, a close relative of F. hispidioides in New Guinea. (6) Male C. hooglandi, showing elongate hind tarsi, a diagnostic feature of Ceratosolen subg. Rothropus. (7) Female C. appendiculatus emerging from a gall in F. variegata. (8) Habitus of female C. armipes. (9) Mesothoracic pocket in C. sp. "kaironkensis" containing F. microdictya pollen grains. (10) Male and female C. dentifer exiting through the ostiole of F. hispidioides. (11) Predatory ants (Oecophylla smaragdina) attacking C. dentifer on F. hispidioides. (12) Removable tubes excluded pollinators from the ostiole in F. hispidioides.

Figure 2: Timing of fig wasp arrivals at Ficus hispidioides at Madang, Papua New Guinea. Numbers of pollinators (open bars) and non-pollinators (closed bars) trapped at figs during 48 hr intervals are plotted against fig age (days after inception) and mean fig diameter (error bars indicate standard deviations). Gallers and parasites (indicated in brackets) arrived prior to and after the peak in pollinator visitation, respectively. Measurements were pooled from seven trees.

Figure 3: Trophic interactions in figs of dioecious Ficus hispidioides at Madang, Papua New Guinea. Arrows indicate the feeding relationships between pollinating, gall-inducing, and parasitic fig wasp species. Illustrations are scaled to show relative differences in ovipositor length and body size among the different guilds.

Figure 4: Heterostyly in dioecious Ficus hispidioides at Madang, Papua New Guinea. Gall figs contained only short-styled pistillate florets (0.1-0.5 mm) while seed figs contained only long-styled florets (0.6-1.6 mm). Measurements from gall figs (closed bars) and seed figs (open bars) were significantly different and there was no difference between fig crops of the same sex.

Figure 5: Species accumulation curve for the fig wasp assemblage of 15 sympatric Ficus species at Madang, Papua New Guinea. A total of 75 putative fig wasp species were reared from 214 fig crops collected from separate trees. The sampling order of trees was randomized following the proceedure of Colwell and Coddington (1994) for estimating species richness by extrapolation.

Figure 6: Abundance of (**A**) pollinators, (**B**) parasites and (**C**) gallers per fig in Ficus trachypison at Madang, Papua New Guinea. Numbers of pollinators per fig were distributed normally in 288 samples from 15 different crops. Numbers of parasites and gallers per fig were Poisson distributed in 154 and 84 out of 288 samples, respectively.

Figure 7: Abundance relationships among pollinators, parasites and gallers in Ficus trachypison at Madang, Papua New Guinea. (**A**) Scatterplot of pollinator, parasite and galler abundance per fig. (**B**) The relationship between pollinator abundance and the frequency of parasitism in F. trachypison. The relationship reflects inverse density dependence.

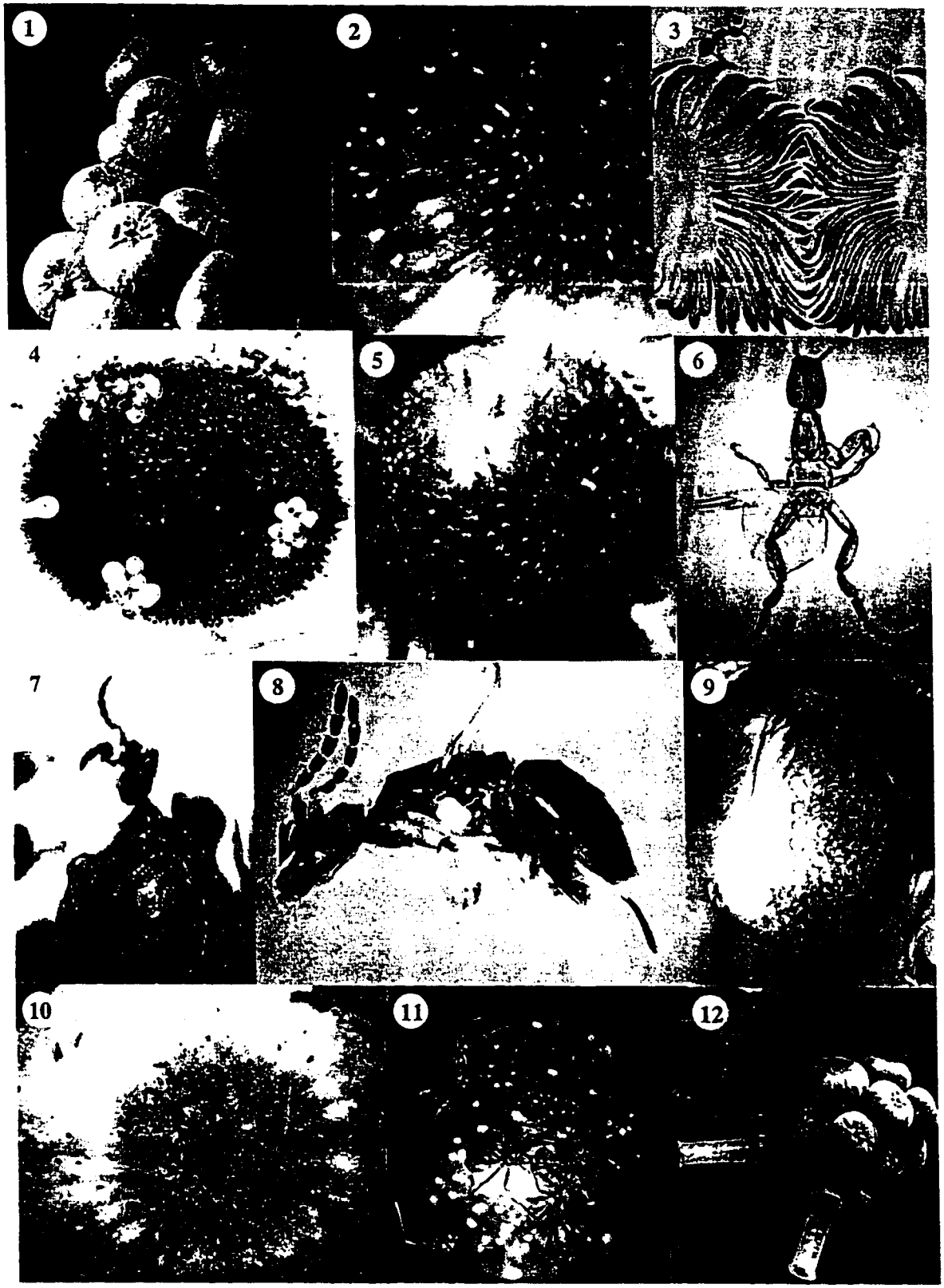

1
2
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Figure 2

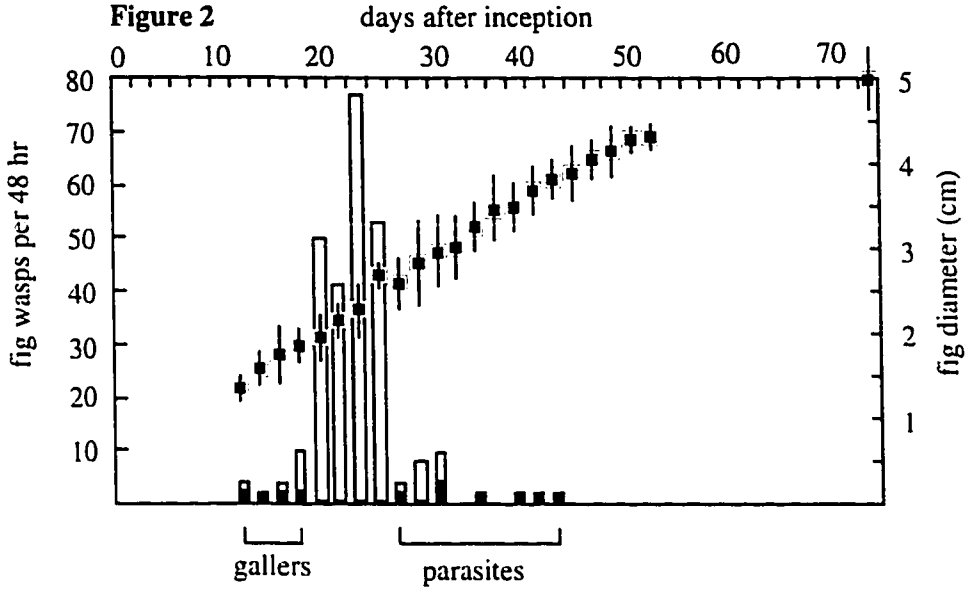

days after inception
0
10
20
30
40
50
60
70
80
70
60
50
40
30
20
10
5
4
3
2
1
fig wasps per 48 hr
fig diameter (cm)
gallers
parasites

Figure 3

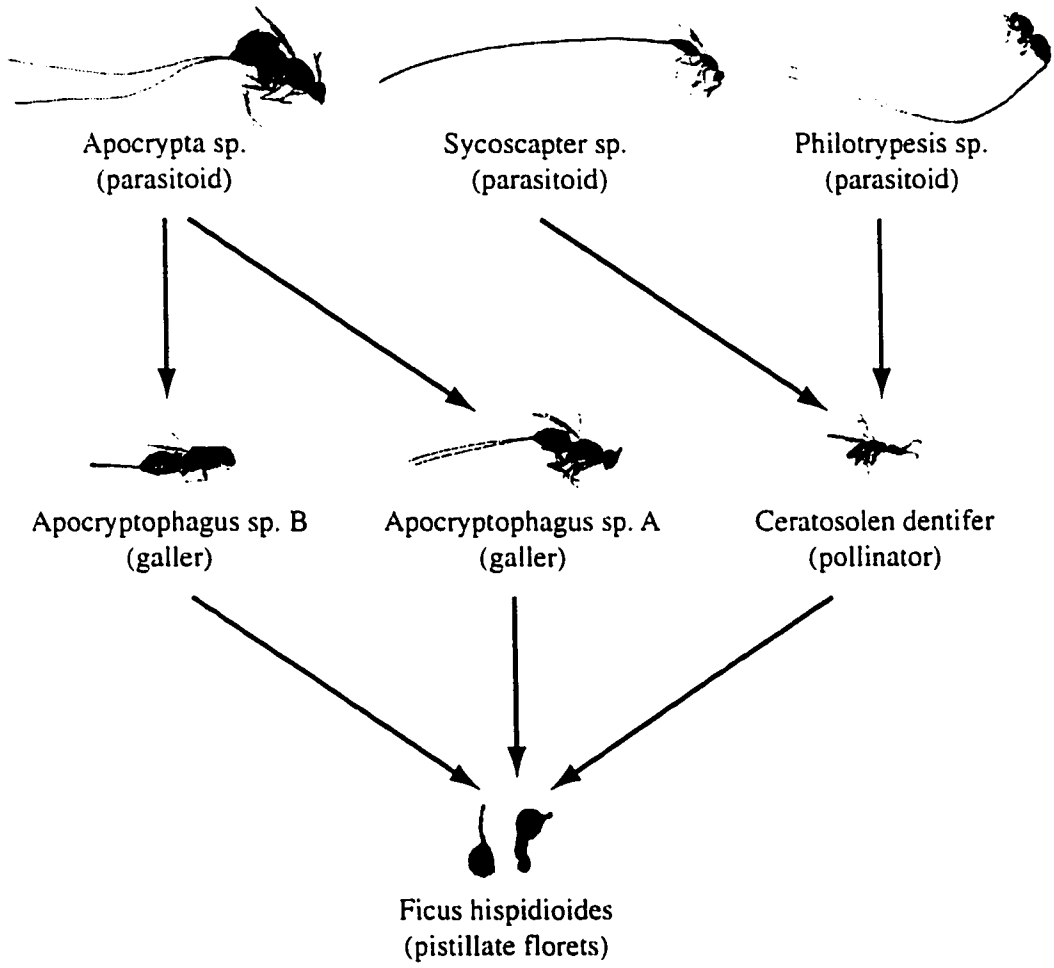

Apocrypta sp.
(parasitoid)
Sycoscapter sp.
(parasitoid)
Philotrypesis sp.
(parasitoid)
Apocryptophagus sp. B
(galler)
Apocryptophagus sp. A
(galler)
Ceratosolen dentifer
(pollinator)
Ficus hispidioides
(pistillate florets)

Figure 4

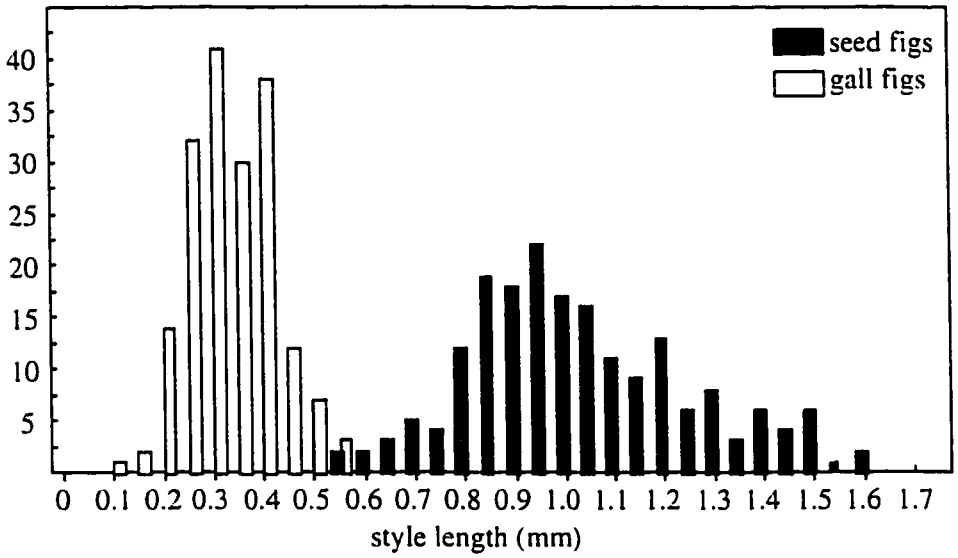

seed figs
gall figs
40
35
30
25
20
15
10
5
0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0 1.1 1.2 1.3 1.4 1.5 1.6 1.7
style length (mm)

Figure 5

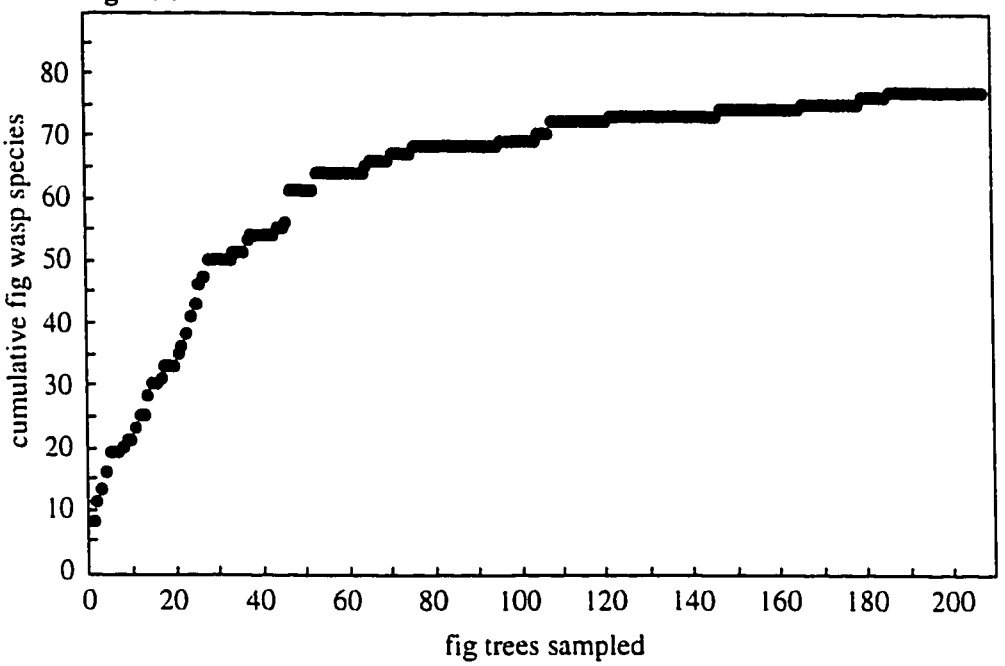

cumulative fig wasp species
0
10
20
30
40
50
60
70
80
0
20
40
60
80
100
120
140
160
180
200
fig trees sampled

Figure 6A

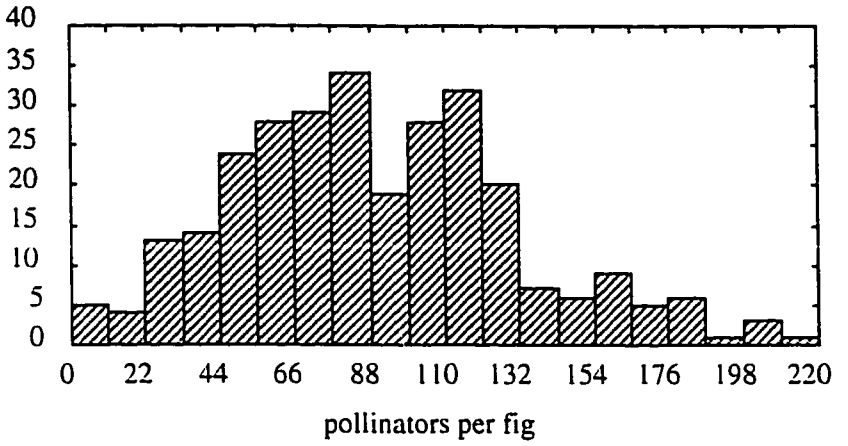


Figure 6B

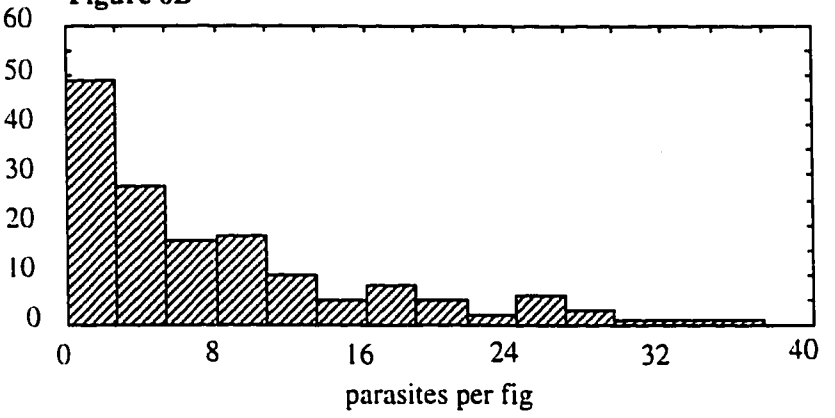


Figure 6C

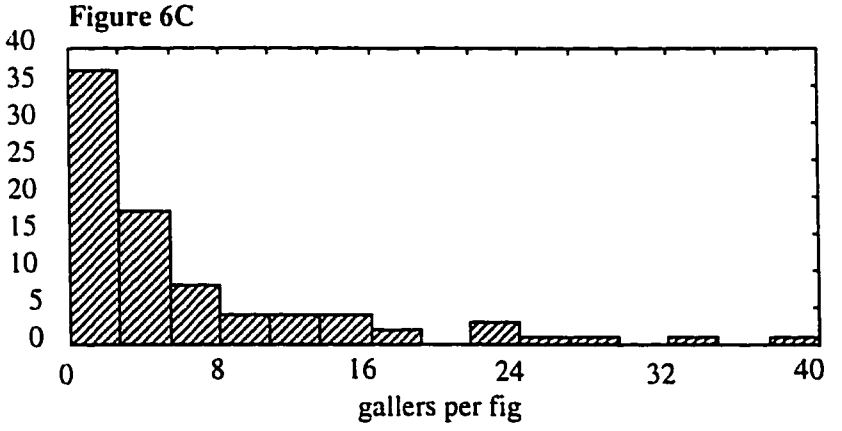


Figure 7A

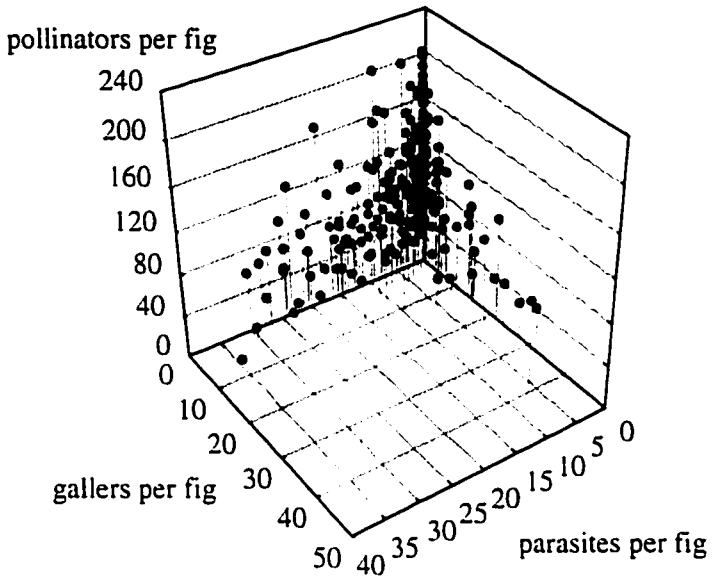
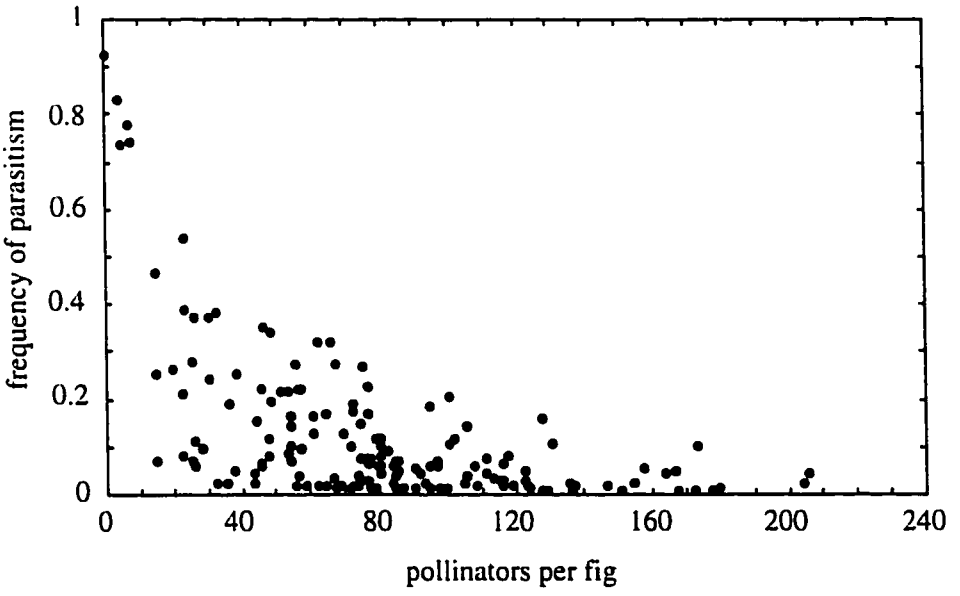


Figure 7B



CHAPTER 5

Fig phylogeny and associations with insect herbivores in New Guinea

"In the old days, a botanist might collect insects; now it seems that students are too specialized." Corner (1977), p. 381

Introduction

Recent developments in phylogenetic analysis have opened new approaches to the study of interactions between plant and insects. In the case of the obligate mutualism between figs and their pollinators, phylogeny suggests the coevolution of interacting lineages (Chapter 3). Although obligate mutualisms between plants and pollinators provide compelling evidence for coevolution (e.g. Ramirez 1974, Wiebes 1979, Aker and Udovic 1981, Corner 1985, Bronstein 1992, Pellmyr and Thompson 1992, Pellmyr and Huth 1994, Pellmyr et al. 1996), most plant species support diverse insect communities including a range of herbivore guilds (Moran and Southwood 1982). How common is coevolution for insect herbivores and their host plants in general? In seeking overall patterns in the evolution of plant-insect interactions, focusing on extreme specialists such as fig wasps can be misleading (Fox 1988).

The extent to which historical associations between insects and plants are reflected in contemporary interactions has attracted the interest of evolutionary biologists for at least half a century (Dethier 1954, Mitter and Books 1983, Futuyma and Keese 1992). Herbivore specificity and the conservatism of associations over evolutionary time will determine the extent to which historical patterns can be inferred from current interactions. Jaenike (1990) suggested that most insect herbivores are oligophagous and therefore less specialized than fig wasps. Herbivore associations show geographical and temporal variability and host shifts occur in each of these dimensions (Thompson 1994a). Host switching complicates the reconstruction of the ancestral associations of herbivores from contemporary interactions. Although the relationship between historical and contemporary factors in shaping interactions is complex, some ecological patterns can be

explored in a phylogenetic framework using comparative methods (Brooks and McLennan 1991, Harvey and Pagel 1991, Eggleton and Vane-Wright. 1994).

Chapter 3 showed a pattern of congruence between fig and pollinator phylogenies that supported the hypothesis of cospeciation. Evidence for parallel diversification has also been shown for beetles (Farrell and Mitter 1990) and to some extent for yucca moths (Brown et al. 1994a). However, closely related insects rarely feed on closely related plants (Jermy 1984). It is more common for closely related herbivores to specialize on more distantly related host plants. Oligophagous herbivores are sometimes associated with a restricted group of hosts (e.g. a plant genus or family) while polyphagous species often feed on more distantly related hosts (e.g. several plant families). The most general model accounting for patterns in plant-insect interactions is sequential evolution, in which host shifts are common and new herbivore associations evolve without having major impacts on plant diversification (Zwolfer 1982, Jermy 1984).

Ronquist and Nylin (1990) accounted for phylogenetic patterns in species associations by three processes: colonization, extinction and successive specialization. Successive specialization is similar to cospeciation in that ancestral associations are divided into two or more descendant associations but even specialists are not expected to speciate with their hosts unless host conservatism is correlated with fitness over the long term (Futuyma 1983). Successive specialization could produce patterns of herbivore association that are potentially informative with regard to host phylogenetic relationships but this possibility has not received much attention in the literature (Abrahamson et al. 1998).

Herbivores differing in dietary specialization and mode of feeding may differ in the extent to which their feeding preferences are correlated with host plant phylogeny. For example, we expect the associations of specialized herbivores to be more closely correlated with host phylogeny than the associations of generalists. Examining the relationship between feeding mode and insect-plant coevolution is hindered by the lack of information on phylogenetic relationships and on insect feeding patterns. Herbivore species belonging to a particular lineage often differ markedly in their range of host preferences and dietary specialization (Miller 1992). However, herbivores can be assigned to functional guilds and predictions can be made about the extent to which patterns of associations within guilds correlate with host phylogeny. Endophagous guilds, for example, may contain more specialized herbivores than ectophagous guilds because of their capacity to overcome specialized plant defenses (Cornell 1989). Similarly, sap sucking species feeding on xylem fluid tend to be polyphagous (Press and Whittaker 1993) while phloem and mesophyll feeders are typically more host specific (Cobbin 1988, Wilson et al. 1994).

The relationship between herbivore specificity and host phylogeny was explored using records of herbivores feeding on Ficus in New Guinea lowland forests. The host preferences of fig wasps, leaf chewing insects, and sap sucking insects were inferred from massive sampling on locally abundant Ficus in Madang, Papua New Guinea and comparative methods were used to examine correlations between feeding patterns within insect guilds and host phylogeny. The rationale for sampling and for delimiting functional guilds within the Ficus-feeding community was described by Basset et al. (1997). Relationships between insect guilds and host phylogeny were compared using

two different approaches. First, the host associations of herbivores were mapped on the fig phylogeny (Chapter 1) to reconstruct patterns in the evolution of host use. Second, host species were grouped according to the similarity of herbivore associations within guilds to examine the relationship between faunal similarity and host phylogeny.

Methods

Study area

The study area was situated in the Madang district of Papua New Guinea, stretching from the coast to the slopes of the Adelbert mountains, between the Gogol and Sempi rivers. This approximate rectangular area of 17 x 31 km encompasses about 434 km^2 of relatively disturbed lowland rainforest and 21 km^2 of coastal habitat (0-400 m above sea level). Plants and insects were sampled in primary and secondary lowland forests near Baitabag (145°047' E, 5°08' S, ca. 100 m), Ohu (145°41' E, 5°14' S, ca. 200 m) and Mis (145°47' E, 5°11' S, ca. 50 m) villages, and to some extent around Baiteta, Erima, Gamoe, Nainai, Ninfon, Pau and Reinduk villages. Coastal sampling occurred near Riwo (145°48'E, 5°09' S, 0 m), Babau, Jais Aben, Mazidaben, Mililat and Nagada. Islands in the Madang Lagoon including Bektukuzan, Duadtinan, Malapau, Panudau, Sek, Sinub, Tab and Wongad were also sampled.

Fig trees and fig wasps

Fifteen Ficus species were selected for this study (Table 1). The rationale for choosing these host species on the basis of local abundance and ease of field identification was outlined by Basset et al. (1997). Phylogenetic relationships of the 15 host species were

obtained from the combined analysis of nuclear ribosomal DNA sequences and morphology in Chapter 1. Among eight most parsimonious trees recovered in the combined analyses, there was conflict regarding the placement of two out of the 15 species. Ficus septica and F. botryocarpa appeared either as sister species or as shown in Figure 1. Apart from this conflict, the phylogeny for the 15 species was robust. All other clades in Figure 1 were supported by bootstrap values >50%, except for F. conocephalifolia as sister to ser. Copiosae and the relationships within the clade including F. phaeosyce, F. trachypison and F. tinctoria. Comparison of phylogenies for the 15 Ficus species and their obligate pollinators (Chapter 2) found 9 out of 14 nodes in agreement (Figure 1B). The observation that none of the conflicting nodes were contradicted by bootstrap values >50% in both analyses was suggestive of fig/pollinator cospeciation generally (Chapter 3). In addition, the non-pollinating fig wasp assemblage of the 15 host species was described in Chapter 4. The distribution of fig wasp genera across the host species is summarized in Table 1. Pollinating and non-pollinating wasps associated with the fig inflorescence (Agaonidae s.l.) were grouped as a guild for comparison with leaf chewing and sap sucking guilds.

Leaf chewing insects

Orthoptera, Phasmatodea, Cerambycidae, Chrysomelidae, Curculionidae and Lepidoptera were sampled by hand collecting and beating the foliage of Ficus trees. Trees <10 m in height were sampled from the ground or climbed. Larger trees were accessed using single rope technique. In 1994-1996, insects were collected during day and night by village assistants and parataxonomists at each of the four main sites (Baitabag, Ohu, Mis

and Riwo). Collecting effort was measured as the time spent looking at the foliage of each tree species. Sampling was similar for each tree species, averaging 24.7 hours and 455 tree-inspections per fig species and totaling 370 hours and 6831 tree-inspections. It is estimated that >1000 trees were sampled from a range of size classes.

Live leaf chewing insects collected in the field were stored in plastic vials at room temperature and provided with fresh foliage until they fed or died. Caterpillars were raised to adults whenever possible. Procedures for rearing, mounting and sorting into morphospecies (hereafter "species") by parataxonomists were described by Novotny et al. (1997). Voucher specimens of insects and plants are deposited at the Bishop Museum. Only specimens that were observed feeding were considered in the analyses in order to exclude transient species. Overall, 13,191 individuals representing 349 species from 25 families of leaf chewing insects were collected from the 15 Ficus species. Chrysomelidae, Choreutidae, Crambidae (Pyraustinae) and to a lesser extent, Cerambycidae dominated the samples. Subfamilies Lamiinae (Cerambycidae), Galerucinae (Chrysomelidae), Eumolpinae (Chrysomelidae), Brenthinae (Choreutidae), Choreutinae (Choreutidae) and Pyraustinae (Crambidae) were most abundant. These subfamilies represented 49.7% of the total species and 84.2% of the total number of individuals collected on all fig species.

Leaf chewing insects were divided into guilds according to three modes of feeding: (1) larval feeders, (2) adult feeders, and (3) lifetime feeders. Larvae feeding on leaves included all Lepidoptera, mostly in the families Crambidae and Choreutidae. Adult feeders included Cerambycidae, mostly in the subfamily Lamiinae, with larvae feeding on dead wood and adults feeding on Ficus leaves. Lifetime feeders were defined

as species with larvae possibly feeding on Ficus wood or roots and with adults feeding on Ficus leaves. Lifetime feeders included Orthoptera, Phasmatodea, Curculionidae and most Chrysomelidae, mainly in the subfamilies Eumolpinae and Galerucinae. Note that only adult feeding records were obtained for lifetime feeders. However, many of these taxa are known wood borers or root feeders and it is suspected that they feed on Ficus as larvae.

Sap sucking insects

Auchenorrhyncha (Homoptera) were sampled from 1995 to 1996 following the same protocol for leaf chewing insects. Collecting effort, again similar for each tree species, averaged 18.6 hours and 353 tree inspections per fig species. More than 1000 trees were sampled for a total of 280 hours and 5139 tree-inspections. Unlike leaf chewing insects, Auchenorrhyncha were not subjected to feeding trials as there was no comparable test that could be carried out in the laboratory. Instead, sap sucking host associations were based on the number of specimens collected from each host species. Assignment to species was based on the examination of male genitalia whenever possible.

In total, 39,975 individuals representing 390 species of Auchenorrhyncha from 19 families were collected. Cicadellidae, Aphrophoridae, and Derbidae represented more than 70% of both total species and individuals. Species feeding on Ficus were assigned to guilds based on published evidence on feeding modes in higher auchenorrhynchan taxa (Novotny and Basset 1998). Sap sucking species were placed in three distinct feeding guilds: (1) xylem feeders, (2) phloem feeders, and (3) mesophyll feeders.

Xylem feeding is probably universal in Cercopoidea (Wiegert 1964, Horsfield 1978) and in Cicadoidea (Cheung and Marshall 1973, White and Strehl 1978). In Cicadelloidea, only members of Cicadellini sensu Hamilton (1983) and Cicadellinae sensu Young (1968) have been shown to feed on xylem fluid. The position of Mileewanini (Cicadellidae) is controversial, as they are placed either under Typhlocybinae (Young 1968) or under Cicadellinae (Hamilton 1983). Mileewanini were treated as xylem feeders based on field observations. Evidence of xylem feeding in taxa other than cercopoids, cicadoids and cicadellines is either equivocal or refers to facultative rather than obligatory xylem feeding (Press and Whittaker 1993). Phloem feeding is probably universal in Fulgoroidea and in Cicadelloidea except Cicadellini and Typhlocybinae, but strong evidence is lacking in several tribes of Cicadellidae and in some families of Fulgoroidea. This study did not involve Stenorrhyncha, which belong in the phloem-feeding guild. Mesophyll feeding is found in the majority of Typhlocybinae (Backus 1988). The tribe Empoascini may contain phloem feeding species or taxa feeding on phloem and mesophyll, but the data are controversial (Kabrick and Backus 1990, Tavella and Arzone 1993). All typhlocybines were therefore treated as mesophyll feeders in this study.

Coding of host associations

Each herbivore species was treated as a character with a discrete distribution across the 15 Ficus host species. Herbivore feeding records, grouped according to guild, were used in analyses of homoplasy and phylogenetic incongruence. Fig wasps were assumed to be monophagous, and therefore, autapomorphic for each fig species. However, the

associations of pollinator genera were potentially informative in phylogenetic analysis. Host associations of the non-pollinating fig wasp genera were also potentially informative, although phylogenetic relationships were not available for most taxa (cf Ulenberg 1985). The pattern of host association in each genus was coded as an independent, binary character (Table 1).

Host records for herbivore species were tallied from field collections and supplemented by laboratory feeding experiments in the case of leaf chewing insects (Appendix 8). The feeding patterns of herbivore species were transformed into independent characters for phylogenetic analysis. Only feeding patterns supported by a minimum number of records were considered. The rationale for setting thresholds was to eliminate erroneous records. This is especially important for field collections that often include transient species not feeding on the plants from which they are collected (Basset et al. 1996a). As a threshold is increased, fewer transient species are included but there is also an increased possibility that true feeding records are omitted. Choosing a threshold value represents a compromise between maximizing data volume and reducing the impact of erroneous records on the analysis. Feeding records were transformed under two different assumptions to examine the sensitivity of results to threshold settings.

The first assumption included all herbivores recorded more than once on at least one host. Singletons were excluded from the analysis because they could not be separated from rare transient species. Each herbivore species meeting this assumption was coded as a binary character with states: (0) not feeding or (1) feeding. Herbivores were assumed to feed on each host having two or more records. The second assumption included herbivores with at least three and six records on a host for leaf chewing and sap

sucking guilds, respectively. Separate thresholds for the two main guilds were based on a minimum estimate of feeding records per host under the null hypothesis that herbivores were evenly distributed across hosts. Threshold values were calculated by dividing the total abundance of each guild by the total number of species per guild times the number of host species. Each herbivore species meeting these more restrictive assumptions was coded as a binary character. For example, a sap sucking species with four records on Ficus bernaysii and six records on F. botryocarpa was coded (0) and (1) for the respective hosts.

Comparative analysis

Patterns of homoplasy and statistical measures of conflict between host phylogeny and herbivore feeding records were explored using several approaches. When herbivore associations are reconstructed on a host phylogeny, it is possible to compare collective patterns of association among guilds. MacClade (Maddison and Maddison 1992, Maddison 1994) was used to reconstruct ancestral host associations of herbivores on the fig phylogeny (Figure 1). Homoplasy in systematics is interpreted as a false hypothesis of homology (Sanderson and Donoghue 1989), but when ancestral herbivore associations are reconstructed on a host phylogeny, homoplasy suggests that herbivore colonization and extinction has occurred in multiple host lineages independently. Factors such as host chemistry, abundance or seasonality could influence patterns of homoplasy. Homoplasy of herbivore associations within guilds was compared using the consistency index (CI) defined as the minimum number of evolutionary changes in the absence of homoplasy divided by the observed number of changes on the host phylogeny. In the case of

herbivore associations coded as binary characters, the minimum number of changes on the host phylogeny is equal to the number of potentially informative herbivores. Uninformative herbivores were excluded from all analyses because they inflate the consistency index (Sanderson and Donoghue 1989).

Herbivores with conservative host associations will exhibit less homoplasy (i.e. higher CI) than herbivores that colonized or went extinct on multiple host lineages (i.e. lower CI). Monte Carlo simulations addressed whether homoplasy in herbivore associations was lower than expected by chance. Permutation has a broad range of applications in comparing homoplasy (Archie 1996) and two specific tests were implemented for herbivore associations. One hundred data sets were generated with PAUP* (Swofford 1998) by permuting the observed associations of each herbivore across all hosts while holding constant the observed number of host records. The permuted data sets provided a null distribution of CI assuming that herbivores were associated with hosts at random. Each herbivore guild was tested as to whether the observed CI was as low as expected by chance alone. This procedure is similar to the homoplasy excess ratio (Archie 1989) in comparing observed homoplasy to the mean homoplasy of permuted data except that an independent tree topology (i.e. host phylogeny) was used instead of minimum length trees for each data set (i.e. guild). In addition, topology-dependent permutation (T-PTP) tests compared the shortest trees for each data set to the length of the host phylogeny (Faith 1991). Although the T-PTP test is not powerful as a test of monophyly (Hueslenbeck et al. 1996), it can also address whether the length difference between two trees is more than expected by chance. As implemented here, the test examined whether host relationships inferred from herbivore

associations were less similar to host phylogeny than expected by chance. All parsimony searches were conducted on unordered and equally weighted characters in PAUP* (Swofford 1998) under the heuristic setting with 100 random addition sequences replicates.

In addition, Templeton tests compared alternative hypotheses of host relationships inferred from insects associated with figs, leaves and sap (Larson 1994). Wilcoxon signed-ranks tests compared the minimum length trees inferred from herbivore associations to the shortest trees resulting from rival searches constrained by host phylogenetic relationships. Constraint trees for rival searches included: (1) the bootstrap consensus of combined molecular and morphological data, (2) two basal clades including sect. Sycidum s.s. and subg. Sycomorus s.l., (3) subsect. Sycocarpus and (4) two tip clades including sect. Neomorphe and ser. Copiosae. Again, parsimony searches were conducted on unordered and equally weighted characters in PAUP* (Swofford 1998) under the heuristic setting with 100 random addition sequences replicates. Cluster analyses were also performed using the unweighted pair group method (UPGMA) to arrange host species according to faunal similarity. Phenograms were used to compare relationships between herbivore associations and host phylogeny.

Results

Ancestral fig wasp associations reconstructed on a host phylogeny are shown in Figure 2. Eight out of the 14 fig wasp genera were potentially informative with regard to host relationships, meaning that 8 genera were associated with more than one but less than all host species (Table 1). All Ceratosolen pollinators were associated with monophyletic

subg. Sycomorus s.l. (Figure 2A), suggesting that the ancestor of this clade and all its descendants were Ceratosolen-pollinated. Gall-inducing Apocryptophagus species were associated with all members of subg. Sycomorus except F. pungens and F. dammaropsis (Figure 2B), suggesting two scenarios for ancient colonization. Apocryptophagus either colonized the ancestral Sycomorus clade and was lost in the F. pungens lineage or colonized sect. Neomorphe and subsect. Sycocarpus lineages independently. Apocrypta, a parasitoid of Apocryptophagus (Ulenberg 1985), appeared to have attacked sect. Neomorphe and F. hispidioides plus F. bernaysii lineages independently. Parasitic Philotrypesis was less restricted than Apocrypta (Figure 2D). Philotrypesis appeared to have colonized the common ancestor of all host species and may have been lost in three or four lineages. In addition, pollinating Kradibia was inferred to have been associated with the common ancestor of sect. Sycidium s.s. (Figure 2E). Gall-inducing Grandiana was also restricted to members of this clade but the ancestral relationships with hosts were equivocal with regard to a single colonization and two extinctions or three independent colonization events (Figure 2F).

When herbivore associations based on more than one feeding record were considered, 293 out of 450 herbivore species were potentially informative (Table 2). Informative herbivores were divided into leaf chewing and sap sucking guilds with 115 and 178 species, respectively. In the case of leaf chewers with at least three feeding records per host and sap suckers with at least six records, only 184 out of 291 species were potentially informative. Informative herbivores under this more restrictive assumption included 84 leaf chewing species and 100 sap sucking species. Numbers of

informative herbivores in each of the six guilds under the two coding assumptions are also listed in Table 2.

The associations of fig wasps exhibited less homoplasy with regard to the host phylogeny than expected by chance (CI_{obs} = 0.40 vs. CI_{perm} = 0.24; P = 0.01). Permutation tests suggested that fig wasp associations contained phylogenetic signal comparable to that of Ficus molecular and morphological data (Table 2). The point is best illustrated by comparing the observed CI value to the distribution of CI vales for permuted data (Figure 3). In the case of fig nuclear ribosomal DNA sequences (Figure 3A) and fig wasp associations (Figure 3B), the observed CI was significantly greater than the average CI of 100 permuted data sets.

By contrast, the associations of herbivores with greater than one feeding record were more homoplasious. The CI for herbivores in general did not differ significantly from the expectation of random associations with hosts (Figure 3C). Leaf chewing insects departed from the expectation of random associations (Figure 3D) but a significant reduction in homoplasy was apparent only in the larval feeding guild (Figure 3E). Homoplasy in the associations of sap sucking insects was similar to chance expectations (Table 2). Under the more restrictive assumption of three and six host records for leaf chewers and sap suckers, respectively, the overall associations of herbivores were less homoplasious than expected by chance. As in the case of the less restrictive assumption, the deviation from random associations could be attributed, in large part, to the larval feeding guild of leaf chewing insects. Homoplasy in the lifetime feeding guild was also significantly lower than expected by chance (Figure 3F). Homoplasy in sap sucking insects was similar to the expectation of random associations

(Figure 3G) although there was a marginally significant deviation from randomness in the mesophyll-feeding guild (Figure 3H). Topology-dependent permutation tests (Table 2), on the other hand, did not detect any differences among herbivore guilds. These tests indicated that the differences between host trees and shortest trees inferred from the observed herbivore associations were not significantly greater than differences between host trees and trees inferred from permuted herbivore associations. Only fig wasps rejected the null hypothesis that nonrandom associations could account for differences between the shortest trees.

Results of Templeton tests for incongruence are summarized in Table 3. Templeton tests examined conflicts between the shortest trees inferred from herbivore associations and the shortest trees compatible with a range of rival constraint trees. Only the associations of fig wasps showed no significant conflict with the 50% bootstrap consensus tree based on combined analyses of fig molecular and morphological data. All herbivore guilds rejected the fig bootstrap consensus tree but some guilds produced different results in local tests of incongruence. Under the less restrictive assumption, all guilds significantly rejected two basal clades in the host phylogeny (sect. Sycidium s.s. and subg. Sycomorus s.l.) except for larval leaf chewers. Under the more restrictive assumption, the associations of larval feeders, adult feeders and mesophyll feeders did not reject the existence of these two clades. Results were similar under both coding assumption with regard to subsect. Sycocarpus. Herbivores collectively rejected the Sycocarpus clade, although phloem feeders were the only guild with associations that showed significant conflict. Under the less restrictive coding scheme, tip clades including sect. Neomorphe and ser. Copiosae were not rejected by any single guild

except for sap suckers collectively. Under more restrictive coding, however, larval feeders and phloem feeders also showed significant conflict with the tip clades.

There was little correspondence between the faunal similarity and phylogenetic relationships of host species (cf Figure 1 and Figures 4-5). Clustering host species with respect to similarity of herbivore associations did not recover many phylogenetic relationships and all herbivore phenograms were statistically incongruent with host phylogeny (Templeton tests; $P < 0.0001$). Fig wasp associations (Figure 4A) recovered subg. Sycomorus s.l. and sect. Sycidium except for F. tinctoria, which clustered with F. microcarpa. Also, the similarity of F. botryocarpa and F. septica was in agreement with one of the two most parsimonious host trees. Ficus bernaysii, F. variegata and F. nodosa had highly similar fig wasp faunas but the association of fig wasp genera did not provide enough information to resolve sect. Neomorphe. Faunal similarity under the more restrictive coding of herbivore associations is also shown in Figures 4. Overall, only a single host clade shared a similar herbivore fauna (ser. Copiosae; Figure 4B). Although comparison of UPGMA distances indicated that F. microcarpa and F. tinctoria shared a highly similar fauna, no other clusters were so distinct. In the case of leaf chewer associations (Figure 4C), similar faunas were shared by less closely related hosts. In the case of sap sucking associations, members of ser. Copiosae had similar faunas while the shared fauna of F. microcarpa and F. tinctoria was most distinct (Figure 4D).

Faunal similarity for the six guilds under the more restrictive coding of herbivore associations is shown in Figure 5. The overall patterns were consistent with the results of homoplasy analysis (Table 2). In particular, the associations of larval feeders, lifetime feeders and mesophyll feeders were in closer agreement with host phylogeny than the

associations of adult feeders, phloem feeders or xylem feeders. No host clades were detected in herbivore phenograms for the latter three guilds. On the other hand, host species in sect. Neomorphe shared similar larval feeding and mesophyll-feeding faunas. Lifetime feeders recovered two host clades, including subsect. Sycocarpus and ser. Copiosae (Figure 5C). In addition, there were patterns in faunal similarity that could not be explained by host phylogeny. In particular, the phloem and xylem feeding faunas of F. microcarpa and F. tinctoria were highly distinct, especially for xylem feeders.

Discussion

Insect guilds differed in the extent to which host associations were correlated with Ficus phylogenetic relationships. In particular, the associations of specialized parasitic wasps were better predictors of host phylogeny than the associations of oligophagous and polyphagous herbivores. Earlier studies have compared patterns of insect associations with regard to plant phylogeny but few have compared feeding guilds in a sympatric assemblage of closely related host species. For example, the associations of gall-inducing insects (Cynipidae: Hymenoptera) were congruent with the phylogenetic relationships of oak species (Quercus, Fagaceae; Abrahamson et al. 1998) while the associations of leaf chewing Lepidoptera were not strongly indicative of host relationships (Futuyma and Gould 1979). Differences in the conservatism of host use could be explained by the evolution of specialization as a consequence of different feeding modes. For example, internal feeding on floral structures within the syconium in the case of fig insects could require greater specialization in relation to host chemistry

and morphology than external feeding on leaves or sap in the case of other herbivorous guilds.

Guilds containing more specialists were better predictors of host phylogeny than guilds composed mostly of generalists. As an overall estimate of specialization, the average number of host species per herbivore species was calculated for the oligophagous species in each guild. On average, leaf chewers had half as many hosts as sap suckers and leaf chewers showed less homoplasy on the host phylogeny than sap suckers. In addition, the associations of leaf chewers departed significantly from chance expectations while sap sucking associations did not. Lower homoplasy in the associations of leaf chewers was, for the most part, due to larval feeding moths (Lepidoptera). Only larval feeders strongly rejected chance as an explanation for the pattern of association in the leaf chewing guild. Larval feeders (mean 2.1 hosts per herbivore) were also less polyphagous than either adult feeders or lifetime feeders (3.4 and 3.9 hosts per herbivore, respectively).

The correlation between homoplasy in host associations and herbivore specialization was also upheld in comparisons of sap sucking guilds. Under the most restrictive coding of host records, mesophyll feeders (3.9 hosts per herbivore) were less polyphagous than phloem feeders (6.1 hosts per herbivore) and xylem feeders (9.1 hosts per herbivore). The overall trend in specialization, from mesophyll to phloem to xylem feeding, is consistent with the distribution of host plant defenses. Xylem fluid is low in secondary metabolites compared to mesophyll cells (Raven 1983), a condition possibly favoring colonization of distantly related hosts by xylem feeders. In general, xylem feeders are extremely polyphagous (Novotny and Wilson 1997) and their feeding

preferences appear to be more closely correlated with solute concentrations in xylem fluid than with host secondary chemistry or phylogenetic relationships (Brodbeck et al. 1990, Thompson 1994b). Homoplasy in the associations of xylem and phloem feeders with respect to Ficus phylogeny did not differ from chance expectations, whereas the relatively more specialized mesophyll feeders showed a marginal reduction in homoplasy.

Under different assumptions regarding host records, there was much less phylogenetic signal in the host associations of leaf chewers and sap suckers compared to fig wasps. In contrast to fig wasps, the overall associations of herbivores did not correlate with the two major lineages of dioecious figs in New Guinea (Figure 1; Table 3). Many herbivores are not restricted to Ficus (Novotny et al. 1999) and the presence of polyphagous generalists within guilds would obscure patterns of historical association shown by more specialized herbivores. However, there was limited evidence for similar herbivore faunas on closely related hosts in several guilds. Lepidoptera feeding on leaves as larvae comprised the most specialized guild and a few species even showed no homoplasy in their associations with hosts. For example, a leaf-rolling moth (FCRAM015; Crambidae) and a species of Brenthia (FTORT015; Choreutidae) were restricted to F. variegata and F. nodosa in sect. Neomorphe under each coding of host records. Most Lepidoptera showed homoplasy in their associations, such as Euploea leucosticos Gmelin (FNYMP001; Nymphalidae: Danainae), which fed on F. bernaysii and all species in sect. Sycidium s.s. except for F. trachypison. Euploea are also known to sequester latex. However, highly polyphagous moths such as Adoxophyes sp. (FTORT045; Tortricidae) and Homona spp. (Tortricidae) also feed on hosts besides Ficus

and probably confounded the attempt to recover plant phylogeny from the associations of larval feeders (Figure 5A). A restricted analysis of known Ficus specialists might yield a different result.

In the case of lifetime feeders (Figure 5C), faunal similarity based on the more restrictive coding of host records recovered two clades; subsect. Sycocarpus and ser. Copiosae. For example, a species of Atysa (FCHRY004; Chrysolmelidae) was associated with the host clade including F. bernaysii, F. hispidioides and F. septica. Also, Rhinoscapha cf tricolor Faust (FCURC003; Curculionidae) was restricted to ser. Copiosae. In the case of mesophyll feeders (Figure 5F), faunal similarity recovered sect. Neomorphe, reflected in the associations of a single species of Typhlocybinae (TYP071; Cicadidae). It must be repeated, however, that the overwhelming majority of herbivore associations were more homoplasious in regard to host phylogeny than expected by chance. It is also noteworthy that cluster analysis (Figures 4-5) revealed hierarchical structure in the associations of herbivores arising from sources other than phylogenetic history.

Hierarchical structure in the associations of herbivore guilds may also result from ecological factors (Basset et al. 1996b). For example, a similar overall fauna was associated with F. microcarpa and F. tinctoria (Figure 4B); the only species grouping supported by both leaf chewing and sap sucking guilds (Figures 4C-4D). These host species are both hemi-epiphytic stranglers but F. microcarpa is a member of a large monoecious clade while F. tinctoria is nested within a major dioecious lineage (Chapter 1). Such highly similar faunas on distantly related hosts could reflect repeated colonization of hosts with convergent defensive chemistry or morphology, notably latex

and cystoliths in Ficus. However, the most striking similarity between F. microcarpa and F. tinctoria is their abundance in coastal habitats. Although both species occur in lowland forests, they are most abundant in rheophytic habitats. The physiology of salt tolerance in rheophytic hosts may have an especially strong impact on xylem and phloem feeders. Indeed, the faunal similarity of F. microcarpa and F. tinctoria is most extreme in xylem and phloem feeding guilds (Figures 5D and 5E). This finding points to the role of ecological factors in structuring herbivore communities (Basset et al. 1996b).

Few studies of ecological communities have followed a phylogenetic approach (Losos 1996) and phylogenetic studies of plant-insect interactions have rarely extended to the community level (Farrell and Mitter 1993). Most phylogenetic studies of plant-insect interactions have focused on monophyletic groups of herbivores showing convergent host associations (e.g. Miller 1987, Becerra 1997, Mardulyn et al. 1997, Kelley and Farrell 1998). However, guilds rather than monophyletic groups should be examined in studies of community structure because the interacting species are not necessarily close relatives (Losos 1996). Why include the temperate relatives of Curculionidae in a community study of beetles associated with Ficus? For instance, African Ficus were not included as potential hosts for herbivores in this study even though African sect. Sycomorus is more closely related to sect. Neomorphe than other sympatric species of Ficus in New Guinea forests. Herbivore species were treated as independent entities in examining insect communities on Ficus, in part, because phylogenetic relationships were not known. However, a potential refinement of this approach with better knowledge of insect phylogeny would divide guilds into monophyletic groups and examine them as independent replicates of a given feeding mode (Smiley 1982). For example, xylem

feeders could be divided into two or three clades and analyzed separately with regard to host phylogeny (Hamilton 1983, Emelyanov 1987, Sorensen et al. 1995).

In general, the results suggest that phylogenetic relationships in Ficus are not of great consequence to herbivores and comparable studies are needed to establish the generality of this result in other plant lineages. The escalation of defenses against herbivory through the production of latex in Ficus would tend to favor a specialized herbivore fauna (Farrell et al. 1991) but most herbivores appear to have colonized multiple lineages within Ficus. Significant incongruence between the faunal similarity and phylogenetic relationships of host species indicates that specialization and host conservatism are not pervasive among Ficus herbivores. Although some guilds are more conservative than other guilds (e.g. larval feeders compared to adult feeders), the majority of associations in each guild indicate that colonization is the predominant mode for the evolution of herbivore associations. A general survey of the literature on phytophagous insects yielded a similar conclusion (Miller and Wenzel 1995). When colonization of new hosts is common, it is difficult to reconstruct ancestral associations and even more difficult to compare alternative models for the evolution of associations (Ronquist and Nylin 1990). However, ecological factors and host traits such as plant secondary chemistry should be considered in accounting for patterns of colonization. For example, abundant host shifts in chrysomelid beetles (Blepharida: Coleoptera) feeding on Bursera species (Burseraceae) were recently shown to correspond to similarities in host defensive chemistry (Becerra 1997). Similar conclusions were reached with papilionid butterflies (Miller 1987, Miller 1992). With additional information on ecological and

chemical traits in Ficus, it will be possible to examine these factors as determinants of herbivore community structure in the context of host phylogeny (Losos 1996).

This chapter has outlined how alternative scenarios for the evolution of herbivorous insect associations (e.g. Ehrlich and Raven 1964, Brooks 1979, Jermy 1984, Farrell and Mitter 1993, Jermy 1993) can be compared through phylogenetic analyses of ecological data (Wanntorp et al. 1990). The focus of most recent approaches to this problem has been on specialized plant-insect interactions that are likely candidates for parallel diversification. For example, phylogenies for specialized leaf beetles (Phyllobrotica and Tetraopes) were shown to be congruent with their hosts' phylogenies (Farrell and Mitter 1990, Farrell and Mitter 1998). Cospeciation studies provide insights on the evolution of specialized interactions but the majority of insect herbivores are less specialized and the exclusive study of specialists leads to a biased view of evolving interactions in general (Miller and Wenzel 1995). Recent phylogenetic studies of butterflies in relation to their host plants (Janz and Nylin 1998) also indicate that host shifting is more common than cospeciation in herbivores that are restricted to a particular host lineage (e.g. a plant family or genus). In the case of Ficus, host associations were reconstructed for a range of herbivore guilds and cospeciating clades. Most herbivores are oligophagous and host switching is common. Although some fig specialists including fig wasps and moths show conservative patterns of host use, the associations of most insect herbivores are not easily explained by the phylogeny of Ficus.

Table 1: Host associations of 14 fig wasp genera coded as binary characters for 15 Ficus species at Madang, Papua New Guinea. Fig wasp genera are labled: (1) Ceratosolen, (2) Eupristina, (3) Liporrhopalum, (4) Kradibia, (5) Apocrypta, (6) Philotrypesis, (7) Sycoscapter, (8) Apocryptophagus, (9) Epichrysomalla, (10) Grandiana, (11) Neosycophila, (12) Odontofroggatia, (13) Orymus, and (14) Walkerella.

		pollinators				parasitoids			gallers						
	Ficus host	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	bernaysii	1	0	0	0	1	0	1	1	0	0	0	0	0	0
2	botryocarpa	1	0	0	0	0	1	1	1	0	0	0	0	0	0
3	conoceph.	0	0	0	1	0	1	1	0	0	0	0	0	0	0
4	copiosa	0	0	0	1	0	0	1	0	0	1	0	0	0	0
5	dammaropsis	1	0	0	0	0	0	1	0	0	0	0	0	0	0
6	hispidioides	1	0	0	0	1	1	1	1	0	0	0	0	0	0
7	microcarpa	0	1	0	0	0	1	1	0	1	0	1	1	1	1
8	nodosa	1	0	0	0	1	0	1	1	0	0	0	0	0	0
9	phaeosyce	0	0	0	1	0	1	1	0	0	1	0	0	0	0
10	pungens	1	0	0	0	0	1	1	0	0	0	0	0	0	0
11	septica	1	0	0	0	0	1	1	1	0	0	0	0	0	0
12	tinctoria	0	0	1	0	0	1	1	0	1	0	1	0	0	0
13	trachypison	0	0	0	1	0	1	1	0	1	1	0	0	0	0
14	variegata	1	0	0	0	1	0	1	1	0	0	0	0	0	0
15	wassa	0	0	0	1	0	1	1	0	1	1	1	0	0	0

Table 2: Homoplasy of herbivore associations with respect to host phylogeny (Figure 1). Consistency index (CI) was calculated for fig nrDNA, fig morphology, fig wasps (Table 1) and herbivores (Appendix 8) under two different coding assumptions. The consistency index for the observed data (CI_{obs}) was compared to the mean of 100 permuted datasets (CI_{perm}) to test whether CI_{obs} could have resulted by chance (P). Topology-dependent permutation tests (T-PTP) compared the lengths of shortest trees for each data set (L_{min}) to the host phylogeny (L_{phyl}).

	characters		CI		P	length		T-PTP
	total	inf	obs	perm		min	phyl	
fig nrDNA	722	56	0.66	0.30	0.01	94	101	0.01
fig morphology	64	42	0.57	0.42	0.02	127	135	0.01
fig wasps	14	8	0.40	0.24	0.01	12	20	0.04
herbivores (>1)	450	293	0.31	0.31	n.s.	802	946	n.s.
leaf chewers	200	115	0.32	0.30	0.01	313	356	n.s.
larval feeders	82	43	0.35	0.32	0.01	101	122	n.s.
adult feeders	33	18	0.32	0.32	n.s.	38	56	n.s.
lifetime feeders	85	54	0.30	0.29	n.s.	151	178	n.s.
sap suckers	250	178	0.30	0.31	n.s.	462	590	n.s.
xylem feeders	34	30	0.35	0.36	n.s.	59	85	n.s.
phloem feeders	172	117	0.29	0.30	n.s.	296	399	n.s.
mesophyll feeders	44	31	0.29	0.29	n.s.	81	106	n.s.
herbivores (>2)	291	184	0.32	0.31	0.01	475	571	n.s.
leaf chewers	143	84	0.34	0.32	0.01	212	244	n.s.
larval feeders	63	32	0.37	0.34	0.01	69	86	n.s.
adult feeders	16	11	0.29	0.30	n.s.	24	38	n.s.
lifetime feeders	64	41	0.34	0.32	0.04	101	120	n.s.
sap suckers	148	100	0.31	0.30	n.s.	244	327	n.s.
xylem feeders	24	19	0.30	0.29	n.s.	38	64	n.s.
phloem feeders	97	62	0.30	0.30	n.s.	142	207	n.s.
mesophyll feeders	27	19	0.34	0.32	0.05	41	56	n.s.

Table 3: Templeton test results for incongruence between herbivore associations and host phylogeny. Minimum length trees (L_{min}; Table 2) inferred from fig wasp and herbivore datasets (Table 1; Appendix 8) were compared to shortest trees from searches constrained by host phylogeny. Constraints included a strict consensus tree (Figure 1), two major clades, subsect. Sycocarpus and two tip clades (see Methods). Significance at the 0.05 level of Wilcoxon's sum of signed ranks tests was calculated following Larson (1994).

rival tree:	host consensus		major clades		Sycocarpus		tip clades	
data set	L	P	L	P	L	P	L	P
fig wasps	18	n.s.	13	n.a.	13	n.a.	13	n.a.
herb. (>1)	904	<0.0001	875	<0.0001	819	0.03	808	n.s.
leaf chewers	348	0.0001	333	<0.005	321	n.s.	322	n.s.
larval	118	<0.005	109	n.s.	106	n.s.	109	n.s.
adult	55	<0.005	44	<0.05	40	n.s.	43	n.s.
lifetime	171	<0.001	162	<0.05	153	n.s.	156	n.s.
sap suckers	555	<0.0001	531	<0.0001	484	n.s.	473	0.01
xylem	85	0.0001	77	<0.005	63	n.s.	64	n.s.
phloem	370	<0.0001	343	<0.0001	312	0.03	306	n.s.
mesophyll	99	<0.005	93	<0.025	85	n.s.	87	n.s.
herb. (>2)	554	<0.0001	532	<0.0001	485	0.03	486	n.s.
leaf chewers	240	0.001	228	0.001	219	n.s.	218	n.s.
larval	84	<0.005	76	n.s.	72	n.s.	75	<0.05
adult	37	<0.005	29	n.s.	28	n.s.	28	n.s.
lifetime	117	<0.005	111	<0.005	104	n.s.	104	n.s.
sap suckers	308	<0.0001	287	<0.0001	253	n.s.	257	<0.01
xylem	57	<0.005	52	<0.005	41	n.s.	41	n.s.
phloem	195	<0.0001	170	<0.0001	150	<0.05	161	<0.005
mesophyll	53	0.02	50	n.s.	47	n.s.	44	n.s.

Figure 1: Phylogeny of 15 Ficus species from Madang, Papua New Guinea. (**A**) One of eight most parsimonious trees resulting from a combined analysis of Ficus morphology and nuclear ribosomal DNA sequences (Chaper 1). Some taxonomic groups are indicated in brackets. Contrary to Corner (1965), Ficus pungens is excluded from sect. Sycidium and the circumscription of subg. Sycomorus follows Ramirez (1977). Clades supported by bootstrap values <50% are marked by closed circles. (**B**) Comparison of fig and pollinator phylogenies provides evidence of cospeciation. The pollinator phylogeny was inferred from a combined analysis of morphology and mitochondrial DNA sequences (Chapter 2). None of the topological conflicts between fig and pollinator relationships (e.g. in sect. Sycidum) were supported by bootstrap values greater than 50% in the host phylogeny.

Figure 2: Associations of fig wasps mapped on a host phylogeny. Closed bars indicate the inferred ancestral associations of genera including (**A**) pollinating Ceratosolen, (**B**) gall-making Apocryptophagus, (**C**) parasitic Apocrypta, (**D**) parasitic Philotrypesis, (**E**) pollinating Kradibia and (**F**) gall-making Grandiana . The associations of several genera are homoplasious. For example, Apocrypta invaded two host lineages independently but it is unclear whether Apocryptophagus colonized two times or was lost in the F. pungens lineage (equivocal reconstructions are indicated by dashed lines).

Figure 3: Null distributions of the consistency index (CI) obtained through permutation. Homoplasy in fig nrDNA (**A**) was compared to the host associations of (**B**) fig wasps, (**C**) herbivores collectively, (**D**) leaf chewing insects, (**E**) larval feeders, (**F**) lifetime feeders,

(**G**) sap sucking insects and (**H**) mesophyll feeders. Monte Carlo simulations tested whether the observed level of homoplasy in each data set was significantly different than expected by chance. Significance is indicated at the 0.05 level.

Figure 4: Cluster analysis of host associations (UPGMA) for (**A**) fig wasps, (**B**) all herbivores collectively, (**C**) leaf chewing insects and (**D**) sap sucking insects. In the case of herbivores, only feeding records based at least three and six individuals were included for leaf chewers and sap suckers, respectively. Scale bars are proportional to 0.05 changes.

Figure 5: Cluster analysis of host associations (UPGMA) for six herbivore guilds, including (**A**) larval feeders, (**B**) adult feeders, (**C**) lifetime feeders, (**D**) xylem feeders, (**E**) phloem feeders, and (**F**) mesophyll feeders. Only feeding records based on more than two and five individuals were included for leaf chewers and sap suckers, respectively. Scale bars are proportional to 0.05 changes.

Figure 1A: host phylogeny

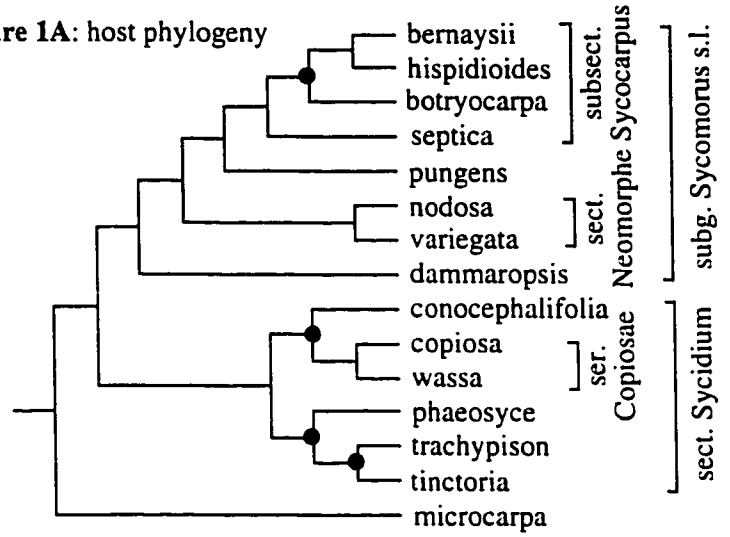


Figure 1B: fig and pollinator phylogenies

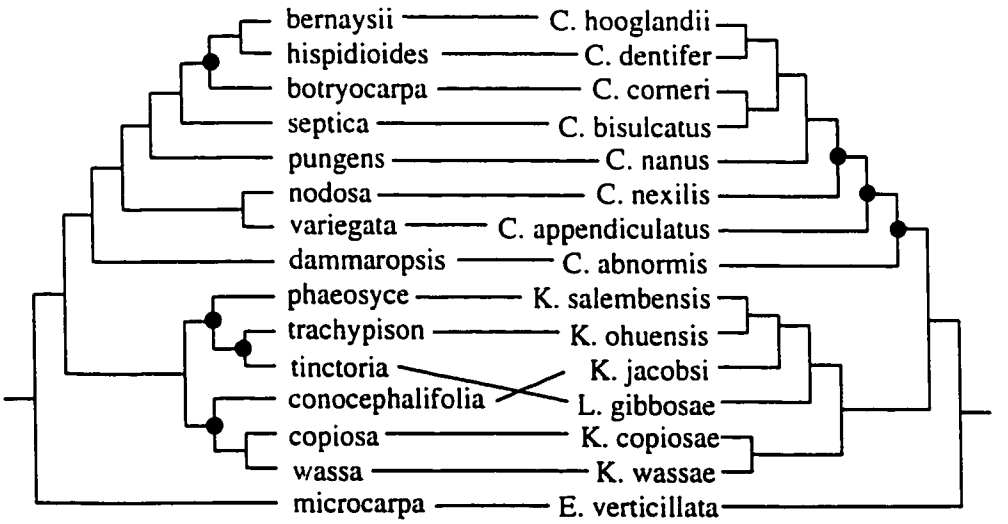


Figure 2A: Ceratosolen

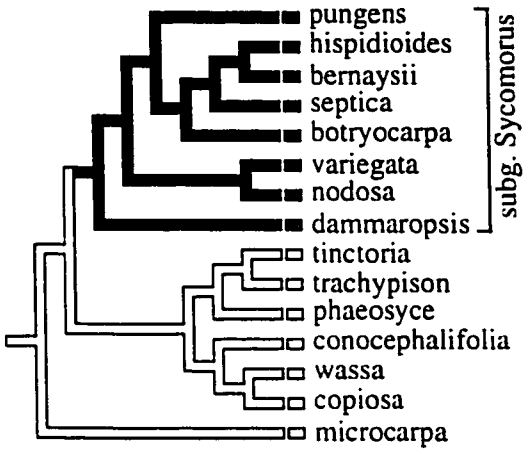


Figure 2B: Apocryptophagus

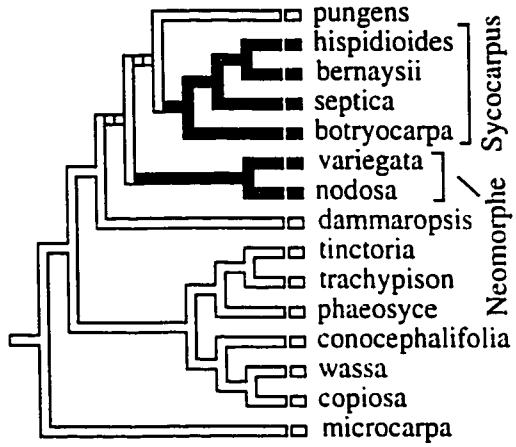


Figure 2C: Apocrypta

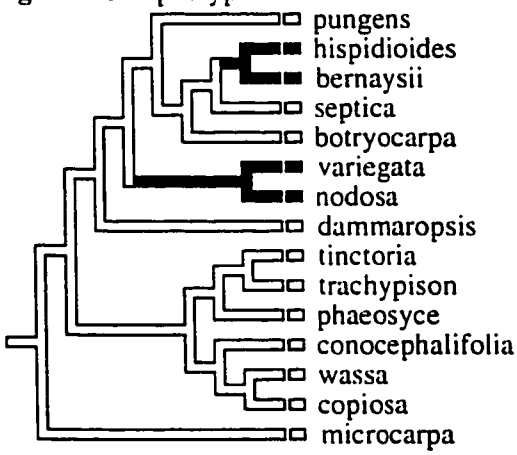


Figure 2D: Philotrypesis

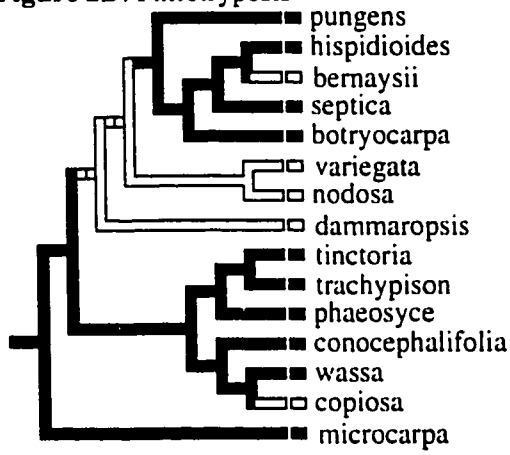


Figure 2E: Kradibia

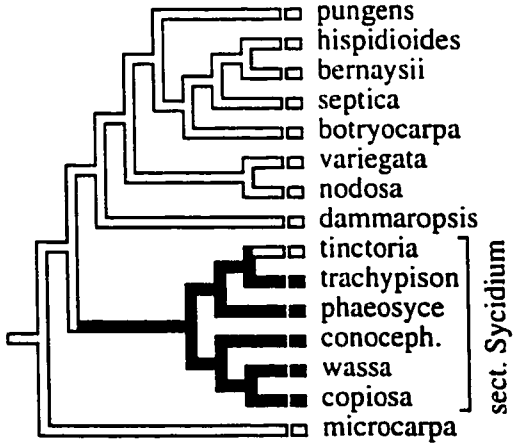


Figure 2F: Grandiana

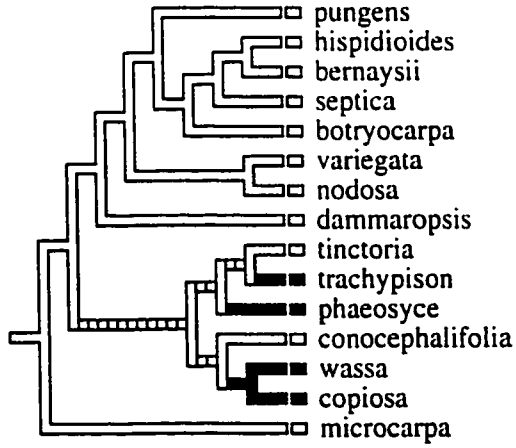


Figure 3A: fig nrDNA

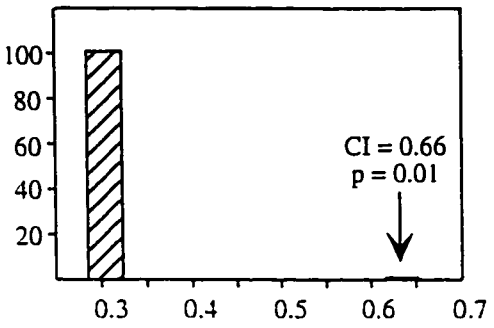


Figure 3B: fig wasps

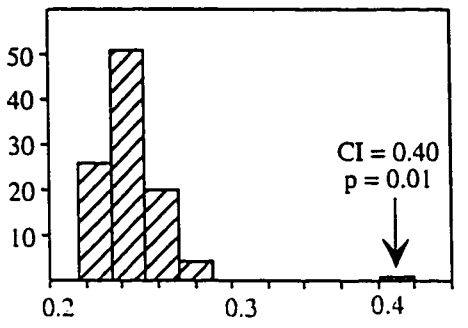


Figure 3C: herbivores

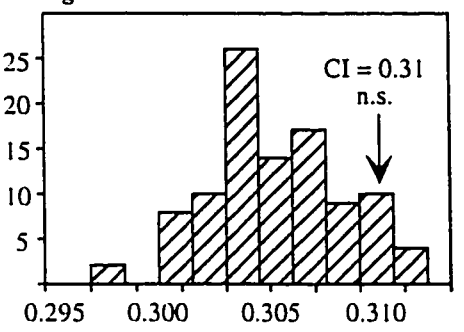


Figure 3D: leaf chewers

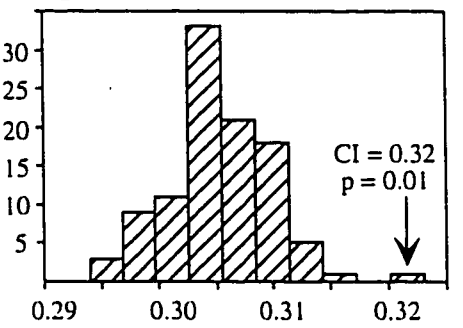


Figure 3E: larval feeders

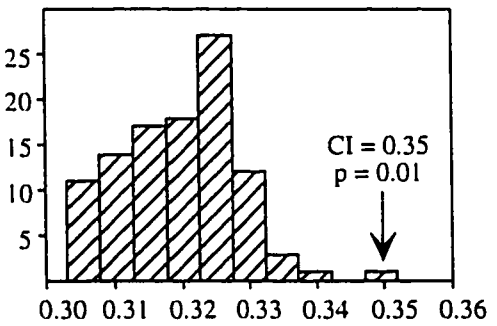


Figure 3F: lifetime feeders

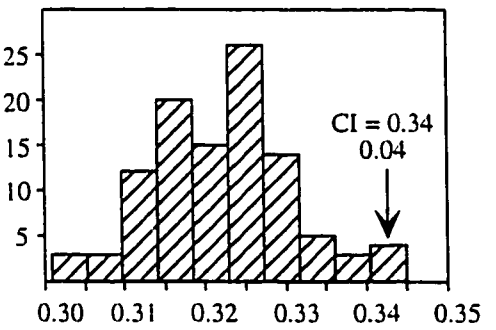


Figure 3G: sap suckers

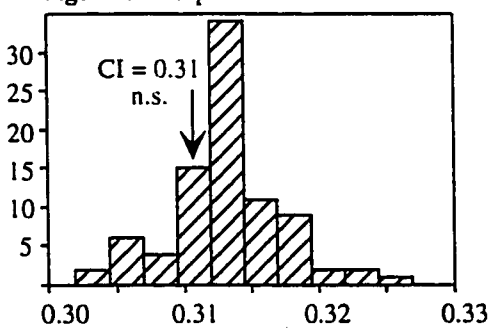


Figure 3H: mesophyll feeders

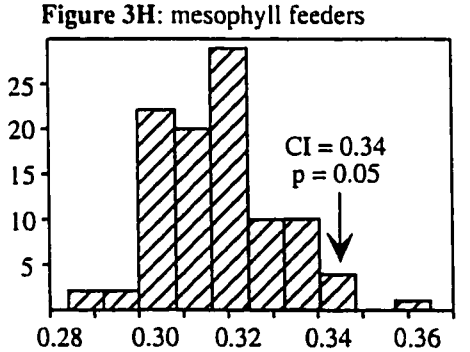


Figure 4A: figwasps

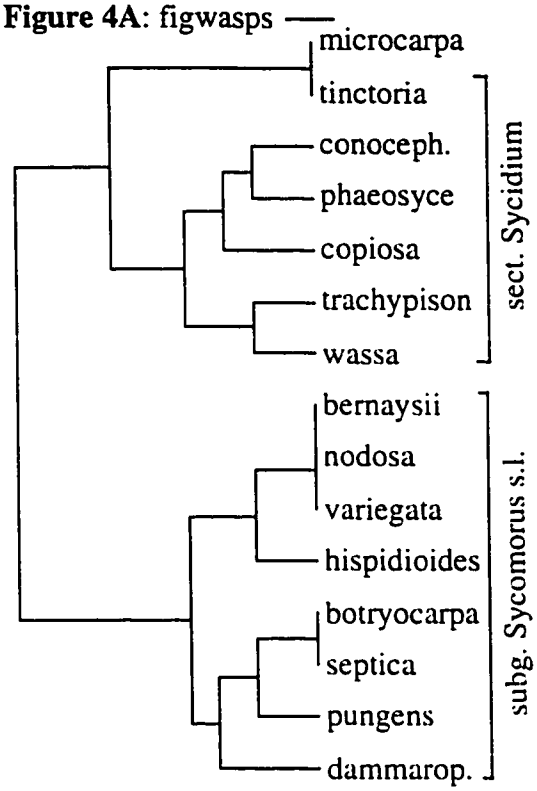


Figure 4B: herbivores

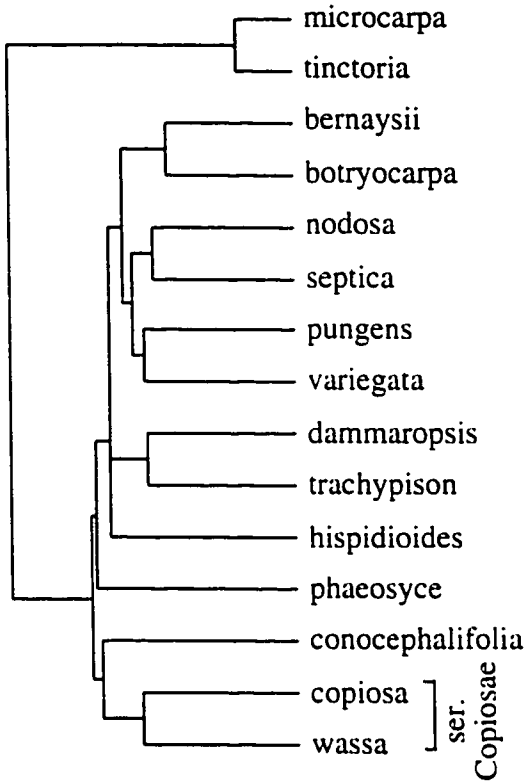


Figure 4C: leaf chewers

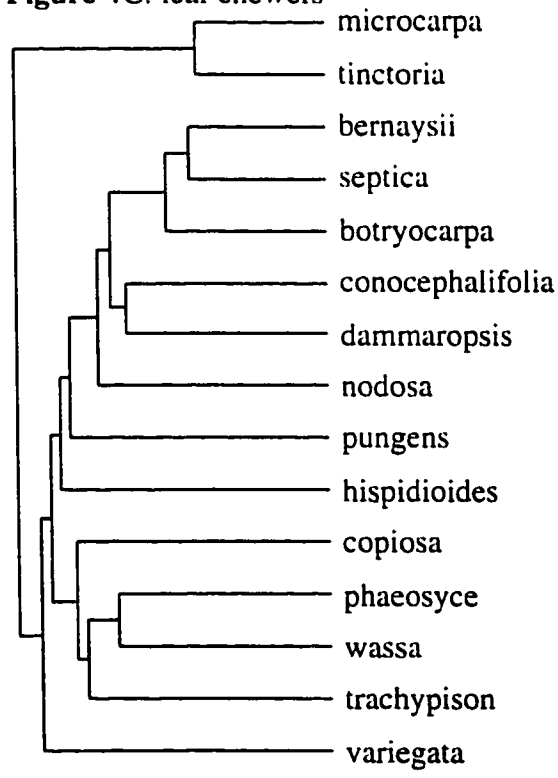
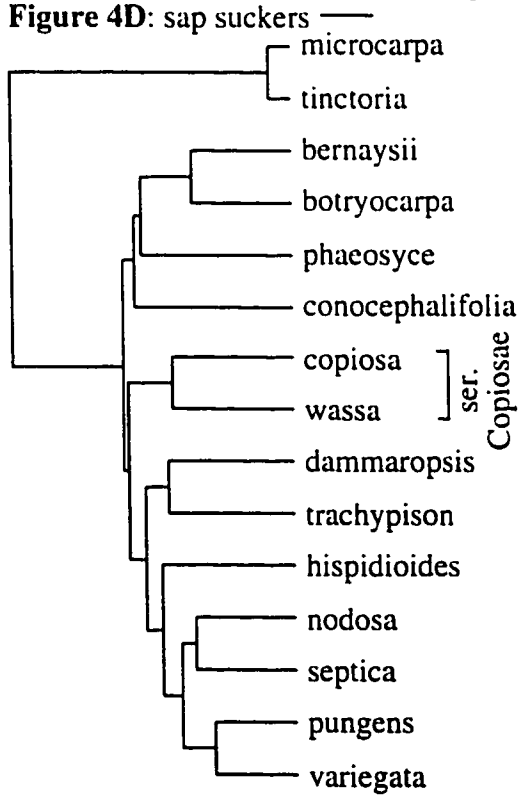


Figure 4D: sap suckers



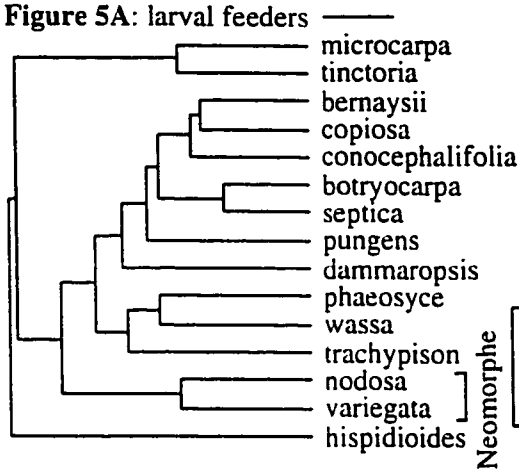


Figure 5A: larval feeders

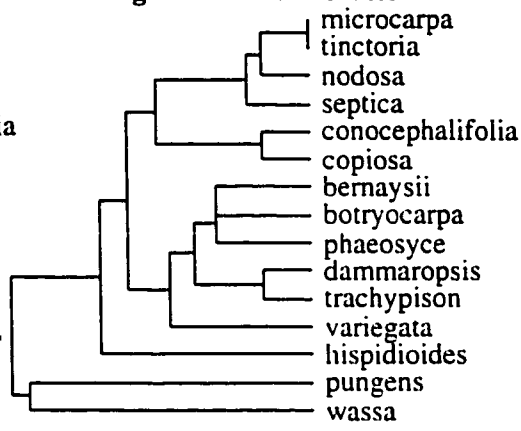


Figure 5B: adult feeders

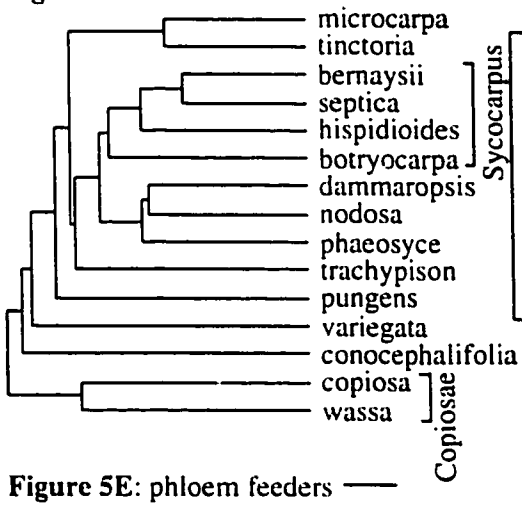


Figure 5C: lifetime feeders

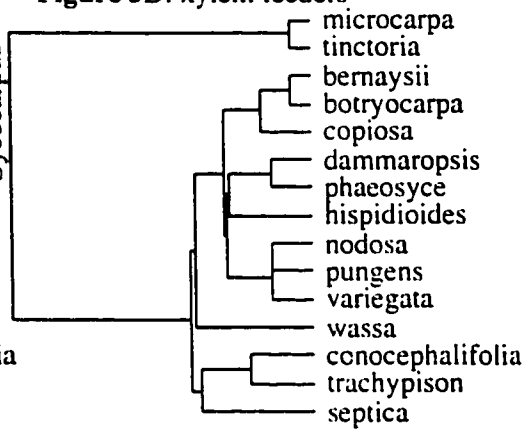


Figure 5D: xylem feeders

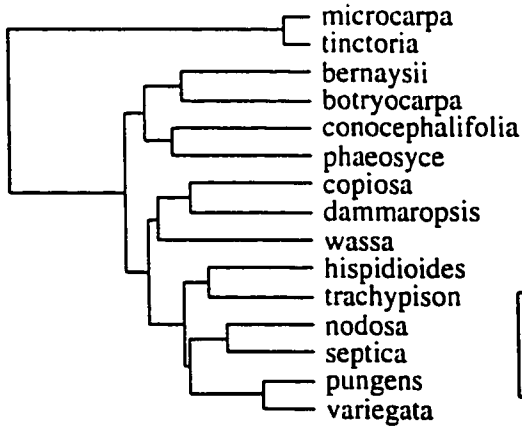


Figure 5E: phloem feeders

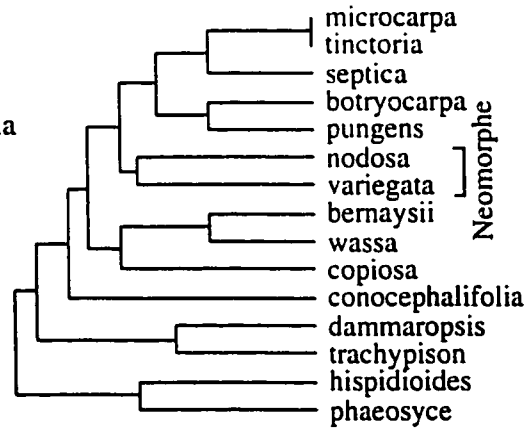


Figure 5F: mesophyll feeders

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APPENDICES

Appendix 1: Specimens included in the phylogenetic analysis of Ficus. Collectors, voucher numbers and localities are listed for sources of molecular and morphological data. Abbreviations following voucher numbers refer to (c)ultivated, (g)all and (s)eed figs. GenBank accession numbers correspond to ITS sequences. Vouchers are deposited at the Harvard University Herbaria (A). Genbank accession numbers are AF165374-AF165419.

no.	Ficus	collector	voucher	location
1	adenosperma Miq.	Weiblen	GW553	Madang, PNG
		Weiblen	GW674 (g)	Tabubil, PNG
		Weiblen	GW808 (s)	Crater Mountain, PNG
2	albipila (Miq.) King	Weiblen	GW1070	Bogor, Java, Indonesia
		Henty	NGF 13636	Morobe, PNG
3	auriculata Lour.	Ho	726 (c)	Hainan, China
		Liang	65169	Hainan, China
		MacClure	641961	Hainan, China
4	baeuerleni King	Isua & Weiblen	B121	Madang, PNG
		Isua & Weiblen	B120 (g)	Madang, PNG
5	bernaysii King	Weiblen	GW541 (c)	Madang, PNG
		Isua & Weiblen	B55 (g)	Madang, PNG
		Weiblen	GW526 (s)	Madang, PNG
		Koil & Weiblen	D9 (s)	Madang, PNG
6	botryocarpa Miq.	Koil & Weiblen	D3 (c)	Madang, PNG
		Isua & Weiblen	B138 (s)	Madang, PNG
		Weiblen	GW468 (g)	Madang, PNG
7	botryoides Baker	Kerdelhue	GW841	Madagascar
		Humbert	2330	Madagascar
		Humbert	5785	Madagascar
8	conocephalifolia Ridley	Koil & Weiblen	D7 (c)	Madang, PNG
		Koil & Weiblen	G049 (g)	Madang, PNG
		Weiblen	GW532	Madang, PNG
		Takeuchi	8776	PNG
9	copiosa Steud.	Koil & Weiblen	G057	Madang, PNG
		Koil & Weiblen	D8 (c)	Madang, PNG
		Koil & Weiblen	G052 (g)	Madang, PNG
10	dammaropsis Diels	Isua & Weiblen	B34	Madang, PNG
		Koil & Weiblen	G050 (g)	Madang, PNG
		Weiblen	GW849 (s)	Hawaii ex PNG
		Brass	8892	PNG
		Brass	12964	PNG
11	destruens F.v.M.	Weiblen	GW943	Queensland, Australia
12	edelfeltii King	Weiblen	GW821	Madang, PNG
		Isua & Weiblen	B195	Madang, PNG
13	grossularioides Burm. f.	Weiblen	GW858 (s)	Kalimantan, Indonesia
		Weiblen	GW851 (g)	Kalimantan, Indonesia
14	hesperidiiformis King	Weiblen	GW825	Madang, PNG
		Weiblen	GW624	Madang, PNG
15	hispidioides S. Moore	Koil & Weiblen	G053 (g)	Madang, PNG
		Weiblen	GW533 (g)	Madang, PNG
		Isua & Weiblen	B15 (s)	Madang, PNG

Appendix 1 (continued): Specimens included in the phylogenetic analysis of Ficus. Collectors, voucher numbers and localities are listed for sources of molecular and morphological data. Abbreviations following voucher numbers refer to (c)ultivated, (g)all and (s)eed figs. GenBank accession numbers correspond to ITS sequences. Vouchers are deposited at the Harvard University Herbaria (A). Genbank accession numbers are AF165374-AF165419.

no.	Ficus	collector	voucher	location
16	Hombroniana Corner	Weiblen	GW953	Madang, PNG
17	Insipida Willd.	Weiblen	V08	Venezuela
		DeWolf	1998	Brazil
		DeWolf	1965	Venezuela
		Lundell	3945	Honduras
		Dywer	10734	Brazil
18	itoana Diels	Weiblen	GW622 (g)	Madang, PNG
		Weiblen	GW406	New Britain, PNG
		Weiblen	GW421	New Britain, PNG
19	maxima P. Miller	Weiblen	BRA02	Brazil
		Irwin	18063	Brazil
		DeWolf	1991	Venezuela
		DeWolf	1993	Venezuela
		DeWolf	2077	Venezuela
		Steyermark	107390	Venezuela
20	microcarpa L.	Weiblen	GW535	Madang, PNG
		Dal & Weiblen	G111	Madang, PNG
21	microdictya Diels	Weiblen	GW954	Madang, PNG
		Weiblen	GW590	Madang, PNG
22	nodosa Teysm. et Binn.	Weiblen	GW603 (c)	Madang, PNG
		Weiblen	GW403	New Britain, PNG
		Isua & Weiblen	B42.0	Madang, PNG
23	ochrochlora Ridl.	Weiblen	GW735 (g)	Crater Mountain, PNG
		Weiblen	GW752 (s)	Crater Mountain, PNG
24	odoardi King	Weiblen	GW708 (s)	Crater Mountain, PNG
		Isua & Weiblen	B205 (g)	Madang, PNG
		Brass	31757	PNG
		Brass	23639	PNG
		Brass	8993	PNG
25	padana Burm. f.	Weiblen	GW1066	Java, Indonesia
		McDonald	3308	Java, Indonesia
		Bangham	960	Sumatra, Indonesia
26	pellucido-punctata Griff.	Weiblen	GW868	Kalimantan, Indonesia
		Weiblen	GW880	Kalimantan, Indonesia
27	pertusa L.	Weiblen	V09	Venezuela
		NYBG	58584	Venezuela
28	phaeosyce Laut.	Isua & Weiblen	B142 (c)	Madang, PNG
29	prasinicarpa Elm.	Weiblen	GW827	Solomon Islands
		Dal & Weiblen	G070	Madang, PNG
30	punctata Thunb.	Laman	TL1022 (g)	Kalimantan, Indonesia

Appendix 1 (continued): Specimens included in the phylogenetic analysis of Ficus. Collectors, voucher numbers and localities are listed for sources of molecular and morphological data. Abbreviations following voucher numbers refer to (c)ultivated, (g)all and (s)eed figs. GenBank accession numbers AF165374-AF165419 correspond to ITS sequences. Vouchers are deposited at the Harvard University Herbaria (A).

no.	Ficus	collector	voucher	location
31	pungens Rein. ex Bl.	Weiblen	GW539 (c)	Madang, PNG
		Weiblen	GW467 (g)	New Britain, PNG
		Koil & Weiblen	G047 (g)	Madang, PNG
32	racemosa L.	Spencer & Flick	GW940	Queensland, Australia
		Panton	GW683	Darwin, Australia
		Craven 2313	2313	Darwin, Australia
		Fernandes	41	India
33	robusta Corner	Weiblen	GW952	Madang, PNG
		Corner	NGF 13651	PNG
34	ruginerva Corner	Weiblen	GW854 (s)	Kalimantan, Indonesia
35	semivestita Corner	Weiblen	GW700 (g)	Madang, PNG
		Isua & Weiblen	B79.1 (g)	Madang, PNG
		Isua & Weiblen	B185 (s)	Madang, PNG
36	septica Burm f.	Weiblen	GW836 (c)	Solomon Islands
		Novotny	GW686 (s)	Madang, PNG
		Koil & Weiblen	G041 (g)	Madang, PNG
		Koil & Weiblen	G109 (g)	Madang, PNG
		Koil & Weiblen	G110 (g)	Madang, PNG
37	superba Miq.	Weiblen	GW851	Bogor, Java, Indonesia
38	sur Forssk.	Kerdelhue	GW840	Tanzania
		Linder	70	Liberia
		Linder	495	Liberia
39	theophrastoides Seem.	Weiblen	GW826 (g)	Solomon Islands
		Kajewski	1971	Solomon Islands
		?	NGF 13386	Solomon Islands
40	tinctoria Forst. f.	Dal & Weiblen	G067 (g)	Madang, PNG
		Weiblen	GW822 (s)	Madang, PNG
		Weiblen	GW611 (s)	Madang, PNG
		Weiblen	GW537	Madang, PNG
41	trachypison K. Schum.	Weiblen	GW950	Madang, PNG
		Weiblen	GW506	New Britain, PNG
		Koil & Weiblen	G046	Madang, PNG
42	variegata Bl.	Weiblen	GW682 (c)	Madang, PNG
		Weiblen	GW602	Madang, PNG
		Isua & Weiblen	B24 (g)	Madang, PNG
		Koil & Weiblen	D10 (s)	Madang, PNG
43	virens Ait.	Weiblen	GW555	Madang, PNG
44	virgata Reinw.	Weiblen	GW704	Madang, PNG
		Isua & Weiblen	B168 (g)	Madang, PNG
45	wassa Roxb.	Koil & Weiblen	G051	Madang, PNG
		Koil & Weiblen	G048 (g)	Madang, PNG
		Isua & Weiblen	B22 (g)	Madang, PNG
		Isua & Weiblen	B155 (s)	Madang, PNG
46	xylosycia Diels	Weiblen	G066	Madang, PNG
		Weiblen	G059	Madang, PNG

Appendix 2: ITS alignment for 46 Ficus species numbered as in Appendix 1. Indels are numbered according to the 5' end. Abbrev: (.) same as first row; (-) indel; (x) excluded.

	•1	•10	•20	•30	•40
1	T T T C C G T A G G T G A A C C T G C G G A A G G A T C A T T G T C G A A A C C T G
2	. . . . . G . . . . .
3	. . . . . G . . . . .
4	. . . . .
5	. . . . . A . . . . . G . . . . .
6	. . . . . G . . . . .
7	. . . . . G . . . . . G T . . . .
8	. . . . .
9	. . . . . T . . . .
10	. . . . . T . . . .
11	. . . . .
12	. . . . . A . . . . .
13	. . . . .
14	. . . . .
15	. . . . . A . . . . . G . . . . .
16	. . . . .
17	. . . . .
18	. . . . . A . . . . . T . .
19	. . . . .
20	. . . . . G . . . . .
21	. . . . . A . . . . .
22	. . . . . G . . . . .
23	. . . . . T . . . .
24	. . . . .
25	. . . . . T . . . . .
26	. . . . . G . . . . .
27	. . . . .
28	. . . . . C . . . . .
29	. . . . .
30	. . . . .
31	. . . . . A . . . . . G . . . . .
32	. . . . . G . . . . . G T . . . .
33	. . . . . G . . . . .
34	. . . . . G . . . . .
35	. . . . . T . . . . .
36	. . . . . A . . . . . G . . . . .
37	. . . . .
38	. . . . . G . . . . . G . . . . . G T . . .
39	. . . . .
40	. . . . .
41	. . . . .
42	. . . . . G . . . . .
43	. . . . .
44	. . . . .
45	. . . . .
46	. . . . .

Appendix 2: ITS alignment for 46 Ficus species numbered as in Appendix 1. Indels are numbered according to the 5' end. Abbrev: (.) same as first row; (-) indel; (x) excluded.

```
                    •50                 •60                 x x x x             •80
1   CCCAGCAGAAAGACCCGCGAACACGTT- - - - ACAACACCCGA
2   . . . . . G. . . . . - - - - . . . . . T. . .
3   . . . . . - - - - . . . . . .
4   . . . . . G. . . . . - - - - . . . . . .
5   . . . . . - - - - . . . . . C.
6   . . . . . - - - - . . . . . .
7   . . . . . - - - - . . . . G. . . . .
8   . . . . . G. . . . . - - - - . . . . . .
9   . . . . . G. . . . . G- - - - . . . . . .
10  . . . . T. G. . . . . T. . . . . - - - - . . . . T. . . . .
11  . . . . . G. . . . G. . . . . - - GTG. . . . . T. . .
12  . . . . . A. . . . . - - - - . . . . . T. . .
13  . . . . . G. . . . . - - - - . . . . . .
14  . . . . . G. . . . G. . . . . ACAC. AC. . . . T. . .
15  . . . . . - - - - . . . . . C.
16  . . . . . - - - - G. . . . . T. . .
17  . . . . . - - - - G. G. . . . T. . .
18  . . . . . - - - - . . . . . .
19  . . . . . - - - - G. G. . . . T. . .
20  . . . . . G. . . . G. . . . . - - - - . . . . . T. . .
21  . . . . C. . . . . - - - - . . . . . .
22  . . . . . - - - - . . . . . .
23  . . . . . - - - - . . . . . .
24  . . . . . G. . . . . - - - - . . . . . .
25  . . . . . G. . . . . - - - - . . . . . .
26  . . . . . G. . . . G. . . . . - - - - . . . . . T. . .
27  . . . . . G. . . . G. . . . . - - - - . . . . . T. . .
28  . . . . . G. . . . . - - - - . . . . . .
29  . . . G. . . . . G. . . . . - - - - . . . . . .
30  . . . . . G. . . . . - - - - . . . . . .
31  . . . G. . . . . - - - - - . G. . . . . .
32  . . . . . - - - - . . . . . .
33  . . . . . - - - - . . . . . .
34  . . . . . G. . . . . - - - - . . . . . .
35  . . . . . - - - - . . . . . .
36  . . . . . - - AC. . . . . .
37  . . . . . G. . . . . - - - - . . . . . .
38  . . . . . - - - - . . . . G. . . . .
39  . . . . . - - - - T. . . . . C.
40  . . . . . G. . . . . - - - - . . . . . .
41  . . . . . G. . . . . - - - - . . . . . .
42  . . . . . - - - - . . . . . .
43  . . . G. . . . . G. . . . . - - - - . . . . . .
44  . . . . . G. . . . . - - - - . . . . . .
45  . . . . . G. . . . . - - - - . . . . . .
46  . . . . . G. . . . G. . . . . ATAT. . T. . . . T. . G
```

Appendix 2: ITS alignment for 46 Ficus species numbered as in Appendix 1. Indels are numbered according to the 5' end. Abbrev: (.) same as first row; (-) indel; (x) excluded.

```
                 • x                • x x              •110                •120
1   GGGGGG-CGAGGGGCGTTAGCACGCCCCGGACCCTCCTTTTTC
2   ......-..........CCGA.T..................CGC.
3   ......-..........CG.A.....................CG..
4   ......-T.........---A.....................CGC.
5   T.....-..........ACG.A.....................CG..
6   T.....-..........CG.A.....................CG..
7   ......-...T.....CG.T.....................CG..
8   ......-.......GAC..A.....T...............CGC.
9   ......-..........CG.A.....................CGC.
10  ......-..........CAC.A.....GA...T..T..C.C.
11  .....G.A.........CG.A.....................CGC.
12  ......-..........CG.A.G...................CGC.
13  ......-..........---A.....................CGC.
14  .....G.A.........CG.AA..T.................CGC.
15  T.....-..........ACG.A.....................CG..
16  ......-..........CG.A.G...................CGC.
17  ..AA..CA.........CG.........TC.............CGC.
18  ......-..........CG.A.....................C...
19  ..AA..CA.........CG.........TC.............CGC.
20  .....GA-------------------------A...CG..
21  ......-..........CG.A.....................C...
22  ......-..........CG.A.....................CG..
23  ......-..CC.....................................C.C.
24  ......-T.........---A.....................CGC.
25  ......-..........---A.....................CGC.
26  .....G.A.........CG.A.....................CGC.
27  .....G.A.........CG.A.....T...............CGC.
28  ......-..........CG.A.....T...............CGC.
29  ......-AA........CG..................A.....CGC.
30  ......-T.....G.G-.A.....................CGC.
31  ......-.....T.CG.A.....T.................G..
32  ......-G..T.....CG.A.................G..A.CG..
33  ......-..........CG.A.....................CG..
34  ......-T.....G.G-.A.....................CGC.
35  ......-..........CG.....................C.C.
36  T.....-..........CG.A.....................CG..
37  ......-AA........CG..................A.....CGC.
38  ......-..........CG.A.....................CG..
39  ......-..........CG.A.G...A...............C...
40  ......-..........CG.....T.................CGC.
41  ......-..........CG.A.....................CGC.
42  ......-..........CG.A.....................CG..
43  ......-GA........CG..................A.....CGC.
44  ......-...C.....CG.....T.................CGC.
45  ......-..........CG.A.....................CGC.
46  .....C.A.........CG.A.....................CGC.
```

Appendix 2: ITS alignment for 46 Ficus species numbered as in Appendix 1. Indels are numbered according to the 5' end. Abbrev: (.) same as first row; (-) indel; (x) excluded.

```
                 •130          •140          •150          x •160
1   GGGTGCGTGTGGCCCCGTCCTTCGCCCCCGG- CACCAAACG A
2   . . . . . A . . T . C . . G . . . . . - . . . . . .
3   . . . . . C . . C . . . . . - . . . . . .
4   . . . . . G . . C . . . . . A - . . . A . . . . .
5   . . . . . C . . C . . . . A . . . . - . . . . . .
6   . . . . . C . . C . . . . . . . . - . . . . . .
7   . . . . . C . . . . . . . . . . . - . . . . . .
8   . . . A . . . . . C . . . . . . . . . G . . . . . .
9   . . . . . C . . C . . . . . . . . G . . . . . .
10  . . A . T . . . . . G A . C . T . . . . . G . . - . . . . . .
11  . . . . . G . . C . . . . . . . . - . . . . . .
12  . . . . . C . . C . . . . . . . . - . . . . . .
13  . . . . . C . . A C . . . . . . . . - . . A . . . . .
14  . . . . . G . . C . . . . . . . . - . . . . . .
15  . . . . . C . . C . . . . A . . . . - . . . . . .
16  . . . . . C . . C . . . . . . . . - . . . . . .
17  . . . . . C . C . . . . . C . . G . T . . . . . - . . . . . .
18  . . . . . A . C . . C . . . . . . . - . . . . . .
19  . . . . . C . C . . . . . C . . G . T . . . . . - . . . . . .
20  . . . . . C . A C . . . . . . . . - . . . . . .
21  . . . . . A . C . . C . . . . . . . - . . . . . .
22  . . . . . C . . C . . . . . . . . - . . . . G . . . .
23  . . . . . - . . . . . .
24  . . . . . G . . C . . . . . . . . A - . . . A . . . . .
25  . . . . . C . . C C . . . . . . . - . . . A . . . . .
26  . . . . . C . . . . . C . . C . . . . . - . . . . . .
27  . . . . . C . C . G . . . C . . C . . . . . - . . . . . .
28  T . . . . A . . . . . G . C . . . . . G . . . . . .
29  . . . . . C . . C . . . . . - . . . . G . . . .
30  . . . . . G . . C . . . . . - . . . A . . . . .
31  . . . . . C . . C . . . . . - . . . . . .
32  . . . . T . . . . . C . . . . . - . . . . . .
33  . . . . . C . . C . . . . . - . . . . G . . . .
34  . . . . . G . . C . . . . . - . . . A . . . . .
35  . . . . . C . . . T . . . . . - . . . . G . . . .
36  . . . . . C . . C . . . . . - . . . . . .
37  . . . . . C . . C . . . . . - . . . . . .
38  . . . . . C . . . . . - . . . . . .
39  . . . . . C . . C . . . . . - . . . . . .
40  . . . . . C . . C . . . . . G . . . . . .
41  . . . . . C . . . . . G . . . . . .
42  . . . . . C . . C . . . . . - . . . . . .
43  . . . . . C . . C . . . . . - . . . . . .
44  . . . . . C . . . . . G . . . . . .
45  . . . . . A . C . . C . . . . . G . . . . . .
46  . . . . . G . . C . . . . . - . . . . . .
```

Appendix 2: ITS alignment for 46 Ficus species numbered as in Appendix 1. Indels are numbered according to the 5' end. Abbrev: (.) same as first row; (-) indel; (x) excluded.

	•170	•180	•190	x x	•200	•
1	ACCCCGGCGCGGAATGCGTCAAGGAAA			--	GACAACGAGACGA	
2				--		
3				--		
4				--		
5				A-		
6				A-		
7				--	G	
8				--	G	
9				--		
10		G	T	--		
11	G			--		
12				--		
13		G		--		
14	G			GA		
15				A-		
16				--		
17				--		
18				--		
19				--		
20	G			--		
21				--		
22				--		
23				--		
24				--		
25				--		
26	G			--		
27	G			A-	A	
28				--		
29				--		
30				--		
31				--		
32				--		
33				--		
34				--		
35			T	--		G
36				AA		
37				--		
38				--	G	
39				--		
40				--		
41				--		
42				--		
43				--		
44				--		
45				--		
46	G			--		

Appendix 2: ITS alignment for 46 Ficus species numbered as in Appendix 1. Indels are numbered according to the 5' end. Abbrev: (.) same as first row; (-) indel; (x) excluded.

```
                                •220          x x     •230                •240                •250
1   TCCCCGCCATCGAGG--CCCCGGAAACGGTGACTCTGCCTCG
2   ...............--.............................-..........
3   ...............--.............................
4   ...............--.........G...................
5   ...............--.....................C.......
6   ...............--.............................
7   ...............--.............................
8   ...............--.............................
9   ....-..........--.....-.......................
10  .......T..A....---...GC......A....GA.....
11  .......G..G....--..............................C..
12  ...............--.............................
13  ...A...........--.........G.........A.........
14  .......G..G....--.........G.....................C..
15  ...............--.....................C.......
16  ...............--.............................
17  ....T..........GG........G.........G.....
18  ...............--.............................
19  ....T..........GG........G.........G.....
20  ....T..G..G....--.............................C..
21  ...............--.............................
22  ...............--.............................
23  ...............--.............................
24  ...............--.........G...................
25  ....A..........--.........G.........A.....
26  ....T..G..G....--.............................
27  ..........G....--.............................
28  ...............--.............................
29  ...............--.....T.......................
30  ...............--.........G...................
31  ...............--..........G..................
32  ...............--.............................
33  ...............--.............................
34  ...............--.........G...................
35  .T.....A.......--.....................T.....
36  ...............--.....................T.....
37  ...............--.....T.......................
38  ...............--.............................
39  ...............--.............................
40  ....A..........--.............................
41  .......G.......--.....................A.....
42  ...............--.............................
43  ...............--.....T.......................
44  ...............--.............................
45  ...............--.............................
46  .......G..G....--.............................C..
```

Appendix 2: ITS alignment for 46 Ficus species numbered as in Appendix 1. Indels are numbered according to the 5' end. Abbrev: (.) same as first row; (-) indel; (x) excluded.

```
                    •260                •270      x        x    x x x x      •290
1   GTGGTCGCCTCGGGATTGGTAC-GAGT-AT----GAAGAACG
2   ........T..........TTT..........-----.....
3   ..........................-.....-.....----A.....
4   .C.....T.....C...G.....-...ATATA.....
5   ..........................-.....-.....----A.....
6   ..........................-.....-.....----A.....
7   ..................C.A.....-.....-.....----A.....
8   ........T.....C...TTT.....-.....-----.....
9   ........T.....C...TTT.....-.....-----.....
10  ..T.....................-.....-G.....----A.....
11  .C.....T.....C...TT-.....-.....-----AG.....
12  ........T..........-.....-.....-----A.....
13  ....T..T.....C..CG.-.....-.....-----A.....
14  .C.....T.....C...TT-.....-...ATGAA.....
15  ..........................-.....-.....----A.....
16  ........T..........-.....-.....----A.....
17  ....T..T..........CT-.....-G.....----AG.A....
18  ..........................-.....-G.....----A.....
19  ....T..T..........CT-.....-G.....----AG.A....
20  ....T..T.....C...TT-.....--C----.....
21  ..........................-.....-G.....----A.....
22  ....T.....................-.....G.....----A.....
23  ..................C.....-.....-.....----.....
24  .C.....T.....C...G.....-...ATATA.....
25  ........T.....C..CG.-.....-.....----A.....
26  ....T..T.....C...TT-.....--C----.....
27  ....T..TG.....C...TTT.....---AAAAG.....
28  ........T.....C...TTT.....--AT--.....
29  ........T..........TTT.....-.....----A.....
30  .C.....T.....C.....-.....-.....----A.....
31  ..........................-.....-C----A.....T.
32  ..........................-.....-.....----A.....
33  ......C.....................-.....G.....----A.....
34  .C.....T.....C.....-.....--C----A.....
35  .....T.....................-.....-AT--A.....
36  ..........................-.....-.....----A.....
37  ........T..........TTT.....-.....----A.....GT
38  ..................C.A.....-.....-.....----A.....
39  .......G.....-.....-.....-.....----A.....
40  ........TA.....C....TT.....-.....----T.....
41  .C.....T.....C...TTT.....-.....-----.....
42  .C.....T..G....C.....-.....-.....----.....
43  ........T..........TTT.....-.....----A.....
44  ........TG.....C....TT.....-.....-----.....
45  ........T.....C...TTT.....-.....----.....
46  .C.....T.....C...TT-.....-G.ATAAA.....
```

Appendix 2: ITS alignment for 46 Ficus species numbered as in Appendix 1. Indels are numbered according to the 5' end. Abbrev: (.) same as first row; (-) indel; (x) excluded.

```
                 •300                 •310                 •320                 •330
1   ACTCTCGGCAACGGATCTCTCGG-CTCTCGCATCGATGAAGA
2   ..................A.....-T.T.T....T.......
3   .-.A..............A.T...-T.T.G.....G.....
4   ..................A.....-..................
5   ..................A.....-..................
6   ..................A.....-..................
7   ..................A.....-..................
8   .-................A.T...-T.T.-..............
9   ..................A.....-..................
10  ..................A.....-..................
11  ..................A.....-..................
12  ..................A.....-..................
13  ..................A.....-..................
14  ..................A.....-..................
15  ..................A.....-..................
16  ..................A.....-..................
17  ..................G.T.-..................
18  ..................A.....-..............A...
19  ...A..............G.T.-..................
20  ..................A.....-..................
21  ..................A.....-..................
22  ..................A.....-..................
23  ..................x.....-..................
24  ..................A.....-..................
25  ..................A.....-..................
26  ..................A.....-..................
27  ..................A.....-..................
28  ..................A.....-..................
29  ..................A.....-..................
30  ..................A.....-..................
31  ..................A....T.-..................
32  ..................x.....-..................
33  .GAAA.............A.....-..................
34  ..................A.....-..................
35  ..................A.....-..................
36  ..................A.....-..................
37  .TG.AT...C..T....A.....-..................
38  .G................A.....-..T................
39  .........N........A.....-..............T...
40  ..................A.....-..................
41  .A................-A.T.T..GT.T..............
42  ..................A.....-..................
43  ..................A.....-..................
44  ..................A.A...-..................
45  ..................A.....-..................
46  ......T...........A.....-..................
```

Appendix 2: ITS alignment for 46 Ficus species numbered as in Appendix 1. Indels are numbered according to the 5' end. Abbrev: (.) same as first row; (-) indel; (x) excluded.

```
                •340                 •350                 •360                 •370
1   ACGCAGCGAAATGCGATACTTGGTGTGAATTGCAGAATCCCG
2   ........G.....................................
3   ...T..............A...G.......................
4   ...T.....................................
5   ...T.....................................
6   ...T.....................................
7   ...T.....................................
8   ...T.....................................
9   ...T.....................................
10  ..........................................A....
11  ...T.....................................
12  ...T.....................................
13  ...T.....................................
14  ...T.....................................
15  ...T.....................................
16  ...T.....................................
17  ..TTG....TC.....A.....................
18  ...T.....................................
19  ..TT.....TGT....A.....................
20  ...T.....................................
21  ...T.....................................
22  ...T.....................................
23  ........................................
24  ...T.....................................
25  ...T.....................................
26  ...T.....................................
27  ...T.....................................
28  ...T.....................................
29  ...T.....................................
30  ...T.....................................
31  ...T.....................................
32  ...T.....................................
33  ...T.....................................
34  ...T.....................................
35  ...T.....................................A..
36  ...T.....................................
37  ...T.....................C.....C.....
38  ...T.....................................
39  ...TC.G..................................
40  ...T.....................................
41  ...T.....................................
42  ...T.....................................
43  ...T.....................................
44  ...T.....................................
45  ...T.....................................
46  ...T.....................................-....
```

Appendix 2: ITS alignment for 46 Ficus species numbered as in Appendix 1. Indels are numbered according to the 5' end. Abbrev: (.) same as first row; (-) indel; (x) excluded.

```
          •380    x       •390            •400            •410            •
1         TGAAC-ATCGAGTCTTTGAACGCAAGTTGCGCCCGAAGCC--
2         .....C.....................................................--
3         .....C.....................................................--
4         .....C.....................................................--
5         .....C.....................................................--
6         .....C.....................................................--
7         .....C.....................................................--
8         .....C.....................................................CC
9         .....C.....................................................--
10        .....-.....................................................--
11        .....C.....................................................--
12        .....C.....................................................--
13        .....C.....................................................--
14        .....C.....................................................--
15        .....C.....................................................--
16        .....C.....................................................--
17        .....C.....................................................--
18        .....C.....................................................--
19        .....C.....................................................--
20        .....C.....................G.....--
21        .....C.....................................................--
22        .....C.....................................................--
23        .....-.....................................................--
24        .....C.....................................................--
25        .....C.....................................................--
26        .....C.....................G.....--
27        .....C.....................................................--
28        .....C.....................................................--
29        .....C.....................................................--
30        .....C.....................................................--
31        .....C.....................................................--
32        .....C.....................................................--
33        .....C.....................................................--
34        .....C.....................................................--
35        .....C.....................................................--
36        .....C.....................................................--
37        .....C.....................................................--
38        .....C.....................................................--
39        .T...C.....................................................--
40        .....C.....................C.....--
41        .....C.....................................................--
42        .....C.....................................................--
43        .....C.....................................................--
44        .....C.....................C.....--
45        .....C.....................................................--
46        .....-.....................................................--
```

Appendix 2: ITS alignment for 46 Ficus species numbered as in Appendix 1. Indels are numbered according to the 5' end. Abbrev: (.) same as first row; (-) indel; (x) excluded.

	•430	•440	•450	•460
1	ATCAGGTCGAGGGCACGTCTGCCTGGGCGTCACACGCGGTTG			
2				C
3				C
4				C
5				TC
6				TC
7				C
8				C
9				C
10				C
11	A			C
12				C
13				C
14				C
15	A			TC
16				C
17	G			AC C
18				CC C
19	G			AC C
20	G			C
21				CC C
22				C
23				
24				C
25				C
26				C
27				C
28				C
29				C
30				C
31				C
32				C
33				C
34				C
35				C
36				TC
37				C
38				C
39				C
40				T
41	CC			C
42	C			C
43				C
44				C
45				C
46				C

Appendix 2: ITS alignment for 46 <u>Ficus</u> species numbered as in Appendix 1. Indels are numbered according to the 5' end. Abbrev: (.) same as first row; (-) indel; (x) excluded.

```
                x x x x x x x x x x x x x x x x x x x x           •490          x   •500
1     C C C C C C C - - - - - - - - - - - - - - - C C A A C G A A A C C C C C - T C C C G C
2     . . . . . . . - - - - - - - - - - - - - - - - - - - - - - - - C . . . . . . . . . . .
3     G . . . . . . - - - - - - - - - - - - - - - . C . . . . . . . . . . . C . G . . . .
4     . T . . . . . - - - - - - - - - - - - - - - . C C . A . . G . . . . . - . . . . . .
5     . . . . . . . - - - - - - - - - - - - - - - . . G . . . . G . . . . . - . A . T . .
6     . . . . . . . - - - - - - - - - - - - - - - . . G . . . . G . . . . . - . A . T . .
7     G . . . . . . - - - - - - - - - - - - - - - . C . . . . . G . . . . C C A . . . .
8     . T . . . . . - - - - - - - - - - - - - - - . C C A A . . C . . . . . - . . . . . .
9     . T . . . . . - - - - - - - - - - - - - - - . C C A A . . C . . . . . C . . . . . .
10    . . . . . . . C C C C C C C C C T - - - - . . . . . . . . G . . . . . - . . . . . .
11    . . . . . . . - - - - - - - - - - - - - - - . C C A . G . C . . . T T - . . . . . .
12    . . . . . . . - - - - - - - - - - - - - - - . . C . . . . C . . . . T - . . . . . .
13    . T . . . . . C C C C - - - - - - - - - - - . C C . A . . C . . . . G - . . . . . T
14    . . . . . . . - - - - - - - - - - - - - - - . C C . . G . C . . . . T - . . . . . .
15    . . . . . . . - - - - - - - - - - - - - - - . . G . . . . G . . . . . - . A . T . .
16    . . . . . . . - - - - - - - - - - - - - - - . G C . . . . C . . . . T - . . . . . .
17    . . . . . . . - - - - - - - - - - - - - - - . C C . . . . T . . . . T - . . . . . .
18    . . . . . . . C C C C C C C - - - - - - - - - - - - - - - - - - - - - - . . . T . . T
19    . . . . . . . - - - - - - - - - - - - - - - . C C . . . . T . . . . T - . . . . . .
20    . . . . . . . - - - - - - - - - - - - - - - . C C A A . . C . . . T T - . . . . . .
21    . G . . . . . C C C C C C C C C C - - - - - - - - - - - - - - - - - - - - - - - - - -
22    G . . . . . . - - - - - - - - - - - - - - - . C . . . . . G . . . . . C . A . . . G
23    . . . . . . . C C C A C C - - - - - - - - . . . . . . . . G . . . . . - . . . . . .
24    . T . . . . . - - - - - - - - - - - - - - - . C C . A . . G . . . . . - . . . . . .
25    . T . . . . . C C C C C C C C - - - - - - - . G C A A . . C . . . . G - . . . . . T
26    . . . . . . . - - - - - - - - - - - - - - - . C C G A . . C . . . T T - . . . . . .
27    . . . . . . . A C C - - - - - - - - - - - T . C C G A G . C . . . T . - . . . . . .
28    . T . . . . . - - - - - - - - - - - - - - - . C C A A . . C . . . . . - . . . . . .
29    . T . . . . . - - - - - - - - - - - - - - - . C . . . . . C . . . . . - . . . . . .
30    . T . . . . . C - - - - - - - - - - - - - A . T C . A . . C . . . . . - . . . . . .
31    . . . . . . . C C C C C C C C A C A C C C . . C G . . . . G . . A . A - . A . . . .
32    G . . . . . . - - - - - - - - - - - - - - - T C T . C C C C . . . . . C . A . . . .
33    G . . . . . . - - - - - - - - - - - - - - - . C . . . . . G . . . . . C . A . . . .
34    . T . . . . . C - - - - - - - - - - - - - A . T C . A . . C . . . . . C . . . . . .
35    . . . . A . . C C C C C C - - - - - - - - A . . . . . . . G . . . . . - . . . . . .
36    . . . . . . . - - - - - - - - - - - - - - - . G G . . . . G . . . . . - . A . T . .
37    . T . . . . . - - - - - - - - - - - - - - - . C C . . . . C . . . . . - . . . . . .
38    G . . . . . . - - - - - - - - - - - - - - - . C . . . . . G . . . . . C . A . . . .
39    . . . . T . . C C C - - - - - - - - - - . . . . . . . . . G . . . . . - . . . . . .
40    . T . . . . . C C T C C C - - - - - - - - . . . . A A . . C . . . T . - . . . . . .
41    . T . . . . . - - - - - - - - - - - - - - - . . C A A . . C . . . . . - . . . . . .
42    G . . . . . . C - - - - - - - - - - - - - - . C . A A . G G . . . . . C . G . . . .
43    . T . . . . . - - - - - - - - - - - - - - - . C . . . . . C . . . . . - . . . . . .
44    . T . . . . . T C C C C A - - - - - - - - A A . . A A . . C . . . T . - . . . . . .
45    . . . . . . . - - - - - - - - - - - - - - - . C C A A . . C . . . . . - . . . . . .
46    . . . . . . . C C C - - - - - - - - - - - - . C C G . T . C . . . T T - . . . . . .
```

Appendix 2: ITS alignment for 46 Ficus species numbered as in Appendix 1. Indels are numbered according to the 5' end. Abbrev: (.) same as first row; (-) indel; (x) excluded.

```
                  •510 x          •520            •530          x x x x x x x x
1   CCCTATCC-GGGCGAAGGGGG-ACCGTGGGTGGGGGGGG--C
2   .....C.....A.....A.....G...G.............
3   T....G.....G.....G.....G.....G.....G.....
4   T...GG.G-....A.GT.....-.....G.....-.....
5   T....G.....G.....-.....G.....G.....-.....
6   T..A.G.....G.....-.....G.....G.....-.....
7   T....G.....G.....-.....C.CG.....-.....
8   T...GG.G-....G.....-.....G.....-.....
9   T...GG.G-....G.....-.....G.....-.....
10  T.A..C.....G.....-.....G..T....G-.
11  T....G.....GGT.....-....C...G.....-.....
12  T....G.....G.....-.....G.....-.....
13  T...GG.G-....G.....-...A.....-.....
14  T....G.....G-C.....-....C...G.....-.....
15  T....G.....G.....-.....G.....-.....
16  T....G.....TG.....-.....G.....-.....
17  T...GG.....GC.....-.....G.....-.....
18  T.T..A.....G..G.....-...C....G.....-.....
19  T...GG.....GC.....-.....G.....-.....
20  T....G..G.....GC.....-.....G.....-.....
21  ----------AA.ATG.....-.....G.....GG.
22  T....A.....G.....-.....G.....-.....
23  T....A.G-...G..G.....-...S...G.....-.....
24  T...GG.G-....A.GT.....-.....G.....-.....
25  T...GG.G-....G.....-....A....G.....-.....
26  T....G..G.....GC.....-....C...G.....-.....
27  T....G.....GT....G.....G.....-.....
28  T...GG.G-....G.....-.....G.....-.....
29  T...GG.....G.....-.....G.....-.....
30  T...GG.G-....A.G.....-.....G.....-.....
31  T....G.....G.....-.....G..A....G-.
32  T....G.....G.....-.....G.....G-.
33  T....G.....G.....-.....G.....-.....
34  T...GG.G-....A.G.....-.....G.....-.....
35  T....G.....G.....-.....G.C.....-.....
36  T....G.....G.....-....T..G.....-.....
37  T....G.....G.....-.....G.....-.....
38  T....G.....G.....-....T..G.....-.....
39  T.....-.....G.....-.....G.....-.....
40  T...GG.G-.C....G.....-.....G.....-.....
41  T...GG.G-....G.....-.....G.....-.....
42  .....G.....G.....-.....G.....-.....G
43  T...GG.....G.....-.....G.....-.....
44  T...GG.G-....G.....-.....G.....-.....
45  T...GG.G-....G.....-.....G.....-.....
46  T.T..G.....GGT.....-....C...G...T.....-.
```

Appendix 2: ITS alignment for 46 Ficus species numbered as in Appendix 1. Indels are numbered according to the 5' end. Abbrev: (.) same as first row; (-) indel; (x) excluded.

```
                 •550        •560        x x         •580
1   GGACAATGACCTCCCGTGCGCGCGCTTCGACCCGCGGTTGGT
2   ....G.....T.....C.G.....--..................
3   ..........................G.................
4   ...A..............GT...---...T..............
5   ..................T...H.....................
6   .....................................
7   .....................................
8   ...A.....................--.....A..........
9   ...-A....................--.....A..........
10  ....G....................--.....A..........
11  ....G....................--.....A..........
12  ..................C.....--.....A..........
13  ...A.....................ATT--.....A..........
14  ...TG....................---C.....A..........
15  ..........................T..................
16  ..................C.....--.....A..........
17  ..........................--.....A..........
18  .....................................
19  ..........................--.....A..........
20  ...GG....................--.....A..........
21  ..........................T..................
22  .....................................
23  ..........................A..................
24  ...A.....................GT...---...T..........
25  ...A.....................ATT--.....A..........
26  ....G....................--.....A..........
27  ..........................--.....A..........
28  ...A.....................--.....A..........
29  ....G....................--.....A.....C....
30  ...A.....................GTT--.....A..........
31  ..........................R..................
32  .....................................
33  ....G....................--.....A..........
34  ...A.....................GTT--.....A..........
35  .....................................
36  .....................................
37  ....G....................--.....A..........
38  .....................................
39  .....................................
40  ...A.....................--.....A..........
41  ...A.....................--.....A..........
42  .....A...................--.....A.....A....
43  ....G....................--.....A..........
44  ...A.....................--.....A..........
45  ...A.C...................---C....A..........
46  ....GT...................--.....A..........
```

Appendix 2: ITS alignment for 46 Ficus species numbered as in Appendix 1. Indels are numbered according to the 5' end. Abbrev: (.) same as first row; (-) indel; (x) excluded.

```
      •590        •600        •610        •620  x     •
1     TC-AAAAAACGAGTCCCCTGTCAC--GTCGTCTT-GGCAA-C
2     C.-.....T.....----------.....-.....-.
3     C.-.....T.....----------.....G.-.....-.
4     C.-.....T.....----------.....T..T...-.
5     C.-.....T.....----------.....-.....-.
6     C.-.....T.....----------.....-.....-.
7     C.-.....GT....----------.....-.....-.
8     C.-.....G.....----------.....-.....-.
9     C.-.....G.....----------.....-.....-.
10    C.-.....T.....----------.....-.....-.
11    C.-.....T.....----------.....-....G-.
12    C.-.....T.....----------.....-.....-.
13    C.-.....T.....----------.....-.....-.
14    C.-.....T.....C.....----------.....-.....-.
15    C.-.....T.....----------.....-.....-.
16    C.-.....T.....----------.....-.....-.
17    C.-.....T.....----------.....C.....-.....-.
18    C.-.....T.....----------.....-.....-.
19    C.-.....T.....----------.....C.....-.....-.
20    C.-.....T.....----------.....-.....-.
21    C.A.....T.....----------.....-.....-.
22    C.-.....----------.....-.....-.
23    ..-.....T.....----------.....-.....-.
24    C.-.....T.....----------.....T..T...-.
25    C.-.....T.....----------.....-.....-.
26    C.-.....T.....----------.....C.....-.....-.
27    C.-.....T.....----------.....C.....-.....-.
28    C.-.....G.....----------.....-.....-.
29    C.-.....CG....C.....----------.....-.....-.
30    C.-.....T.....----------.....-..T...-.
31    C.-.....T.T...----------.....AC....-.....-.
32    C.-.....T.....----------.....-....G-.
33    C.-.....----------.....C.....-.....-.
34    C.-.....T.....----------.....-..T...-.
35    C.-.....T.....----------.....-.....-.
36    C.-.....T.....----------.....-.....-.
37    C.-.....TG....T..C.....----------.....-.....-.
38    C.-.....T.....----------.....-.....-.
39    C.-.....T.....----------.....-.....-.
40    C.-.....G.....----------.....-.....-.
41    C.-.....T.....----------.....-.....A.
42    C.-.....T...T....C.....----------.....-.....-.
43    C.-.....CG....C.....----------.....-.....-.
44    C.-.....G.....----------.....-.....-.
45    C.-.....G.....----------.....-.....-.
46    C.-.....T.....----------.....-.....-.
```

Appendix 2: ITS alignment for 46 Ficus species numbered as in Appendix 1. Indels are numbered according to the 5' end. Abbrev: (.) same as first row; (-) indel; (x) excluded.

```
                    •640                •650                •660                •670
1   AGGTAGTCGATCATTCGGTGCCACCGCCACGTGCGTCGGGCA
2   ..................G.................A..
3   ..........A.......................
4   ..................G...........C...A..
5   ..................................
6   ..................T...............
7   ..................G...............
8   ..................................A..
9   ..................................A..
10  ..................................
11  ..................G...T.........T...
12  ..................................A..
13  ..................................A..
14  ....G...............G...T.C.--------
15  ..................................
16  ..................................A..
17  ........G...........A.............A..
18  ..................................
19  ........G...........A.............A..
20  ..........G.........G...GT.C.....T...
21  ..................................
22  ..................................
23  ..................................
24  ..................G...........C...A..
25  ..................................A..
26  ..................G...GT-C.....T...
27  ..........C.......G.G...T.......T...
28  ..................................A..
29  ..................................A..
30  ..................G...........C...A..
31  G.........G.........T..............
32  ..................................
33  ..................................
34  ..................G...........C...A..
35  ..................................
36  ..................................
37  ..................................
38  ..................................
39  ..................................
40  .......T..........................A..
41  ..................................A..
42  ..........A...A...................A...
43  ..................................A..
44  ..................................A..
45  ..................................A..
46  ........G...........G...T.........TA..
```

Appendix 2: ITS alignment for 46 Ficus species numbered as in Appendix 1. Indels are numbered according to the 5' end. Abbrev: (.) same as first row; (-) indel; (x) excluded.

```
                  •680                •690                x     •700                •710
1   CGCATCGGGACTCCGATAGACCCC-AATGCGCCCGTCACGGG
2   ..................C.....CGT.................
3   ...................................-...C.....T..
4   ...G..............C.....-..G.T.....T...
5   ..................C.....-..................
6   ..................C.C...-..................
7   ...................................-...C.....
8   T.................C.....C.....G....
9   T.................C.....C.....G....
10  ...................................-..................
11  ...................................-.GT...............
12  ...................................-..................
13  ..................C.....-...C.....
14  --..GG.............G.....-..G.....
15  ..................C.....-..................
16  ...................................-..................
17  ......T.....GCG.....-..................
18  ..................G.....-..................
19  ......C.....GCG.....-..................
20  ..T...............C.....-..G.....G....
21  ...................................-..................
22  ...............G.....-...C.....
23  ...................................-...C.....
24  ...G..............C.....-..G.TG.....T...
25  ..................C.....-...C.....
26  ..................C.....-..G.....
27  ...................................-.GGC..............
28  T...............T..C.....C.....G....
29  ...................................-.....TG....
30  ...G..............C.....-..G.T.....T...
31  ...........................A...-.....G.G.....
32  ...................................-...C.....
33  ...................................-...C.....
34  ...G..............C.....-..G.T.....T...
35  ...................................-...C.....
36  ..................C.....-..................
37  ...................................-.....TG....
38  A.................................-...C.....
39  ...................................-..................
40  ..........T.....C.....C.....G....
41  .T...............C.....C.....G...T
42  .......C.....C.C.....-.....A..A.....
43  ...................................-.....TG....
44  ..........T.....C.....C.....G....
45  T.................C.....C.....G....
46  ...G..............................-.GG..............
```

Appendix 2: ITS alignment for 46 Ficus species numbered as in Appendix 1. Indels are numbered according to the 5' end. Abbrev: (.) same as first row; (-) indel; (x) excluded.

```
                  • x                •730               •740               •750
1   TGCCTCACAACGCGACCCCAGGTCAGGCGGGGCTACCCGCTG
2   .......-...........................................
3   .......-...........................................
4   .C.....-...........................................
5   .......-...........................................
6   .......-...........................................
7   .......-...........................................
8   .......-...........................................
9   .......-...........................................
10  ......................T..G......T..................
11  .......-.....................................C.....
12  .......-...........................................
13  .......-.....T.....................................
14  .......-....A......................................
15  .......-...........................................
16  .......-...........................................
17  .......-...........................................
18  ........T..........................................
19  .......-...........................................
20  .......-...........................................
21  .......-...........................................
22  .......-...........................................
23  C......-...........................................
24  .C.....-...........................................
25  .......-.....T.....................................
26  .......-...........................................
27  .......-...........................................
28  .......-.....................T.....................
29  .......-...........................................
30  .......-...........................................
31  .......-...........................................
32  .......-...........................................
33  .......-...........................................
34  .......-...........................................
35  C......-...........................................
36  C......-...........................................
37  ...G...-.....T.....................................
38  .......-...........................................
39  .......-.....................T.....................
40  .......-...........................................
41  .......-...........................................A
42  ........A..........................................
43  .......-...........................................
44  .......-...........................................
45  .......-...........................................
46  .......-....A......................................
```

Appendix 2: ITS alignment for 46 Ficus species numbered as in Appendix 1. Indels are numbered according to the 5' end. Abbrev: (.) same as first row; (-) indel; (x) excluded.

		70	91	101	158	196	226	275	280	364	498	512	570	622	697	721
1	AGTTT	0	0	0	0	0	0	0	0	1	0	0	1	0	0	1
2		.	.	.	.	.	.	1	.	0	.	.	0	.	1	0
3		.	.	.	.	.	.	.	.	0	1	.	.	.	.	.
4		.	.	1	.	.	.	.	.	0	.	.	0	1	.	0
5		.	.	.	.	1	.	.	.	0	.	.	.	.	.	.
6		.	.	.	.	1	.	.	.	0	.	.	.	.	.	.
7		.	.	.	.	.	.	.	.	0	1	.	.	.	.	.
8		.	.	.	1	.	.	1	.	0	.	.	0	.	1	0
9		.	.	.	1	.	.	1	.	0	1	.	0	.	1	0
10		.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
11		1	1	.	.	.	.	.	.	0	.	.	0	.	.	0
12		.	.	.	.	.	.	.	.	0	.	.	0	.	.	0
13		.	.	1	.	.	.	.	.	0	.	.	0	.	.	0
14		1	1	.	.	1	.	.	.	0	.	.	0	.	.	0
15		.	.	.	.	1	.	.	.	0	.	.	.	.	.	.
16		.	.	.	.	.	.	.	.	0	.	.	0	.	.	0
17	A	.	1	.	.	.	1	.	.	0	.	.	0	.	.	0
18		.	.	.	.	.	.	.	.	0	-	.	.	.	.	.
19	A	.	1	.	.	.	1	.	.	0	.	.	0	.	.	0
20		.	1	-	.	.	.	.	.	0	.	1	.	.	.	0
21		.	.	.	.	.	.	.	.	0	-	-	.	.	.	.
22		.	.	.	.	.	.	.	1	0	1	.	.	.	.	.
23		.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
24		.	.	1	.	.	.	.	.	0	.	.	0	1	.	0
25		.	.	1	.	.	.	.	.	0	.	.	0	.	.	0
26		.	1	.	.	.	.	.	.	0	.	1	.	.	.	0
27		.	1	.	.	1	.	1	.	0	.	.	0	.	.	0
28		.	.	.	1	.	.	1	.	0	.	.	0	.	1	0
29		.	.	.	.	.	.	1	.	0	.	.	0	.	.	0
30		.	.	1	.	.	.	.	.	0	.	.	0	.	.	0
31		.	.	.	.	.	.	.	.	0	.	.	.	.	.	.
32		.	.	.	.	.	.	.	.	0	1	.	.	.	.	.
33		.	.	.	.	.	.	.	1	0	1	.	.	1	.	.
34		.	.	1	.	.	.	.	.	0	1	.	0	.	.	0
35		.	.	.	.	.	.	.	.	0	.	.	.	.	.	.
36		1	.	.	.	1	.	.	.	0	.	.	.	.	.	.
37		.	.	.	.	.	.	1	.	0	.	.	0	.	.	0
38		.	.	.	.	.	.	.	.	0	1	.	.	.	.	.
39		.	.	.	.	.	.	.	.	0	.	.	.	.	.	.
40		.	.	.	1	.	.	1	.	0	.	.	0	.	1	0
41		.	.	.	1	.	.	1	.	0	.	.	0	.	1	0
42		.	.	.	.	.	.	.	.	0	1	.	.	.	.	.
43		.	.	.	.	.	.	1	.	0	.	.	0	.	.	0
44		.	.	.	1	.	.	1	.	0	.	.	0	.	1	0
45		.	.	.	1	.	.	1	.	0	.	.	0	.	1	0
46		1	1	.	.	.	.	.	.	.	.	.	0	.	.	0

Appendix 3: Ficus morphological characters. Figures are in Chapter 1.

1. Breeding system [0] monoecious or [1] gynodioecious (Figures 1.20-1.22). Morphologically gynodioecious species are functionally dioecious due to complex interactions between the heterostylous florets and pollinating seed predators.
2. Syconia [0] solitary or paired in the axils of expanded leaves (axillary) or [1] additionally or entirely produced on leafless branchlets (cauliflorous). As is common in Moraceae, paired inflorescences are located in the axils of the two lateral prophylls associated with the leaf. Geocarpic syconia occurring on leafless branchlets at ground level were coded as [1].
3. Syconia on leafless branchlets with [0] short internodes or [1] with elongated internodes. Only applicable to cauliflorous taxa. Members of sects. Ficus, Kalosyce, Rhizocladus and Sycidium may produce facsiculate syconia on short branchlets lacking measurable internodes. These specialized branchlets become thickened and tubercular after several reproductive episodes. The reproductive branchlets of certain members of sects. Neomorphe, Sycomorus, and Sycocarpus, including the geocarpic species, have internodes up to 1 m in length.
4. Syconia with [0] two or [1] three basal bracts. Bracts subtending the syconium or displaced along the peduncle represent a modified leaf and two associated prophylls. The condition of having two bracts suggests the loss of the leaf or connation of the prophylls.
5. Basal bracts [0] caducous or [1] persistent in ripe syconia (D phase in the phenology of Galil and Eisikowich (1968)).

6. Basal bracts [0] glabrous (or glabrescent; without persistent hairs), [1] pubescent (with persistent hairs but not rough like sandpaper) or [2] scabrid (rough like sandpaper due to raised cystoliths).
7. Syconia [0] sessile (without a stalk as in Figure 1.12) or [1] pedunculate (with a stalk as in Figures 1.13-2.15). The peduncle is an elongated inflorescence axis.
8. Syconium basal bracts [0] at the bottom (Figure 1.13), [1] between the bottom and the top (Figure 1.14) or [2] at the top of the peduncle (Figure 1.15). Only applicable to pedunculate taxa.
9. Peduncles [0] glabrous, [1] pubescent, [2] scabrid or [3] muricate (with epidermal hooks 0.5-2 mm in length). Only applicable to pedunculate taxa.
10. Syconia [0] without lateral bracts or transverse ridges, [1] with lateral bracts (Figure 1.17) or [2] with transverse ridges on the receptacle. In some taxa, apical bracts are not confined to the ostiole but are also scattered along the receptacle. Multibracteate syconia appear to have evolved independently in sects. Sycocarpus and Sycidium. Displaced bracts range from one to many. The syconia of F. dammaropsis in sect. Sycocarpus, for example, are completely enclosed by numerous lateral bracts. The syconia of some members of subsect. Sycocarpus are marked by transverse ridges on the outer epidermis which become especially prominent in dried specimens.
11. Syconium outer epidermis [0] glabrous, [1] pubescent, [2] scabrid, [3] muricate or [4] pustulate (covered with lenticels).
12. Syconia [0] without or [1] with sclereids in the receptacle. Layers of sclerotic cells are visible in sections of the receptacle upon staining with phloroglucinol.

13. External ostiolar bracts [0] two or three or [1] more than three (Figure 1.18), or [2] sunken and not visible on fig exterior. The number of bracts visible at the apex of the syconium varies according to their degree of overlap and orientation. Two or three closely overlapping bracts form a flat disc or umbonate apex in most members of subg. Urostigma. More than three apical bracts were counted in subg. Ficus, Sycomorus and Pharmacosycea, except for members of sect. Rhizocladus where they are sunken and not visible.
14. Internal ostiolar bracts [0] overlapping (in cross-section of figs; Figure 1.19) or [1] inflexed and not overlapping. Ostiolar bracts are involved in facilitating the entry of pollinators but preventing their escape from the syconium. All syconia have at least some overlapping internal bracts except for species in series. Perforatae of sect. Conosycea (e.g. F. pellucido-punctata).
15. Syconium lumen [0] dry or [1] fluid-filled during the interfloral phase. Following pollination, syconia fill with fluid and remain so during seed maturation in sects. Adenosperma, Neomorphe, Sycocarpus and Sycomorus.
16. Syconium inner epidermis [0] without or [1] with glandular hairs.
17. Pistillate florets [0] all without pedicels (sessile) or [1] varying within syconia from sessile to pedicellate. Syconia in most species have sessile and pedicellate florets whose length varies inversely to that of style length, resulting in a uniform stigmatic surface or synstigma during the pollination phase. All pistillate florets in sect. Adenosperma are sessile.
18. Pedicels of pistillate florets [0] glabrous or [1] setose. Only applicable to pedicellate taxa.

19. Pistillate perianth with tepals [0] free (Figure 1.23), [1] fused at the base (Figure 1.24), [2] fused completely along their length (Figure 1.25) or [3] without tepals in seed figs (Figure 1.26). The pistillate perianth in some species of sect. Sycocarpus is saccate due to the complete fusion of the tepals, which are only fused along part of their length in sects. Neomorphe, Sycomorus and Oreosycea.
20. Pistillate perianth with tepals [0] glabrous (Figure 1.27) or [1] pubescent on the abaxial surface (Figure 1.28). Not applicable to seed figs lacking tepals.
21. Pistillate perianth margins [0] entire, [1] ciliate (hairy; Figure 1.29) or [2] dentate (toothed; Figure 1.30). The completely fused perianth may split secondarily due to the swelling of the galled ovaries. Not applicable to seed figs lacking tepals.
22. Pistillate perianth [0] white (without pigment) or [1] red. Not applicable to seed figs lacking tepals.
23. Style [0] subterminal to lateral or [1] gynobasic.
24. Style [0] glabrous in all florets (as in Figure 1.31) or [1] setose in long-styled florets (hairy as in Figure 1.32). Only applicable to heterostylous (functionally dioecious) taxa.
25. Style [0] not divided or [1] divided at the apex (Figure 1.33).
26. Stigma [0] clavate or [1] funneliform (funnel-shaped) in gall figs (as in Figure 1.34). Short-styled florets in gall figs may be specially adapted to ovipositing fig wasps. Only applicable to heterostylous (functionally dioecious) taxa.
27. Ovary [0] white (without pigment), [1] with a red spot near the base of the style or [2] red throughout. Ovaries with a red spot on the stylar side occur in sects. Americana, Conosycea, and Malvanthera.

28. Ovary [0] superior in all pistillate florets or [1] inferior in seed-producing florets (embedded in the receptacle). Ovaries of seed-producing florets in sect. Malvanthera may be embedded in the receptacle and surrounded by a layer of lignin.

29. Achene [0] not flattened or auriculiform (Figure 1.35) or [1] auriculiform to flattened; more than twice as long as wide (Figure 1.36). Applicable only to viable seeds at maturity.

30. Achene [0] smooth or [1] tuberculate (Figure 1.37). Achenes in sect. Ficus subsect. Eriosycea are tuberculate, being rough due to microscopic projections caused by the variable length of elongate prismatic cells in the endocarp.

31. Achene [0] with a single ridge arising from the hilum (Figure 1.35-37) or [1] with a forked, double ridge arising from the hilum (Figure 1.38). The prominence of the ridge when single varies, but forked ridges in sect. Adenosperma and subsect. Eriosycea are always distinctly raised.

32. Staminate florets [0] dispersed, scattered among the pistillate florets (Figure 1.20) or [1] ostiolar (clustered around the ostiole as in Figure 1.22).

33. Staminate florets [0] without pistillodes, [1] with pistillodes (Figure 1.41) or [2] with functional gall ovaries (Figure 1.42). Staminate florets containing a pistillode occur in sects. Sycomorus and Neomorphe. Apparently hermaphroditic florets occur in the ostiolar position in subsect. Paleomorphe of sect. Sycidium, and although the ovary has an ovule, it does not produce an achene since the ovary is galled. F. copiosa and F. wassa in subsect. Sycidium often have a pistillode and rarely a functional gall ovary.

34. Syconia [0] without or [1] with staminodia. Not applicable to monoecious species. Some members of sects. Neomorphe and Sycocarpus have abortive staminate florets in the ostiolar position in seed figs.

35. Stamens per floret [0] one (Figure 1.39), [1] two (Figure 1.40) or [2] varying from one to three within syconia. Florets are all unistaminate within syconia throughout subg. Urostigma and all bistaminate throughout sects. Ficus and Rhizocladus. However, the number of stamens per floret varies within syconia in subg. Sycomorus, sect. Neomorphe, and some members of sect. Sycidium and sect. Sycocarpus.

36. Staminate florets [0] sessile or [1] pedicellate.

37. Staminate perianth with tepals [0] free (not fused), [1] fused at the base or [2] fused completely along their length and splitting at anthesis. The staminate perianth is partly fused in sects. Kalosyce and Neomorphe, Sycomorus and is completely fused in sect. Sycocarpus. The perianth in both staminate and pistillate florets is fused in sects. Neomorphe, Sycomorus, and Sycocarpus but not in sect. Kalosyce.

38. Staminate perianth with tepals [0] glabrous or [1] pubescent on the abaxial surface. Epidermal hairs on the perianth in staminate and pistillate florets are correlated in sect. Sycidium subsect. Paleomorphe but not in subsect. Sycidium.

39. Filaments [0] without epidermal hairs at the base or [1] with epidermal hairs at the base (Figure 1.43).

40. Anthers [0] not mucronate or [1] mucronate (filament or connective projecting beyond the pollen sacs as in Figure 1.44).

41. Anthers [0] bilocular or [1] unilocular. The kidney-shaped locule in sect. Malvanthera dehisces by a crescentic slit and contains two thecae suggesting the fusion of two locules into one. These taxa were scored [1].

42. Growth habit [0] hemi-epiphytic or strangling, [1] climbing, or [2] free-standing. Hemi-epiphytes or stranglers in subg. Urostigma and sect. Sycidium subsect. Paleomorphe produce contractile aerial roots which may fuse, the plant then becoming free-standing. Climbers in sects. Kalosyce and Rhizocladus produce aerial roots but the plant is never free-standing. Trees and shrubs in subg. Ficus, Pharmacosycea and Sycomorus were scored as [2].

43. Buttresses in mature trees [0] less than 0.5 m or [1] more than 1 m in height. Not applicable to epiphytes, hemiepiphytes, climbers, shrubs or immature trees. Buttresses increase in size with age and therefore the character was scored from mature specimens only.

44. Growth [0] continuous or [1] discontinuous. Deciduousness or discontinuous growth in sect. Neomorphe and subg. Sycomorus results from the synchronized abscission of leaves followed by a pulse of new growth. Discontinuous growth is often associated with seasonally dry environments (Spencer et al. 1996).

45. Branches [0] orthotropic or [1] plagiotropic by apposition. "Terminalia" branching or plagiotropy by apposition occurs in sect. Adenosperma and sect. Oreosycea.

46. Twigs [0] without hollow or spongy pith or [1] with hollow or spongy pith. In some species, expanded and spongy pith between the nodes may separate, resulting in hollow twigs (Figure 1.1).

47. [deleted]

48. Twigs [0] glabrous or glabrescent, [1] pubescent, with persistent hairs, but not rough like sandpaper or [3] scabrid due to raised cystoliths. Pubescence on twigs is often not correlated with pubescence on other structures, such as syconium peduncles.
49. Twigs [0] without waxy glands below the node, [1] with a waxy gland below the node (Figure 1.2) or [2] with two glands below the node. The single subnodal gland in sect. Sycocarpus secretes cuticular wax. F. nodosa and F. robusta (sect. Neomorphe) have paired subnodal glands located between the leaf and the lateral prophyllar buds.
50. Stipules [0] clasping the bud or [1] reflexed and not clasping the bud at the apex. The latter condition is known only from F. copiosa and F. wassa.
51. Stipules [0] glabrous or [1] pubescent on the abaxial surface.
52. Stipules [0] caducous (falling) or [1] persistent. Specimens with stipules remaining attached at least six nodes below the apical bud were scored as [1].
53. Latex [0] white or [1] yellow.
54. Ptyxis (leaf bud in cross-section) [0] convolute (rolled in bud) or [1] plicate (folded in bud). Leaves in subg. Pharmacosycea and Urostigma are convolute whereas leaves in subg. Ficus and Sycomorus are plicate.
55. Phyllotaxis [0] spiral or [1] distichous. Leaves on juvenile and mature plants are distichous in sects. Rhizocladus and Kalosyce. Leaves are opposite in some members of sect. Sycocarpus but juveniles have spiral phyllotaxis and were scored [0].
56. Petioles [0] glabrous, [1] pubescent, [2] scabrid or [3] muricate.

57. Leaves (laminae) [0] cuneate (wedge-shaped) to rounded or [1] cordate (heart-shaped) at the base.

58. Leaves [0] symmetric or [1] asymmetric. Leaves that are unequal in area on either side of the midrib are considered to be asymmetric.

59. Leaf glands [0] none, [1] solitary at the base of the midrib (Figure 1.3), [2] solitary in the axil of a basal vein (Figure 1.4), [3] paired in the axils of basal veins (Figure 1.5), [4] in the axils of basal and secondary veins or [5] only in the axils of lateral veins (Figure 1.6). A single gland at the base of the midrib is common in subg. Urostigma. Glands in the axils of basal or secondary veins are common in other taxa and they may be paired or solitary, as in some members of sect. Sycidium. Leaf glands appear to secrete cuticular wax that is collected by insects.

60. Tertiary veins (as in Figure 1.10) [0] parallel to secondary veins, [1] reticulate (net-like, not parallel or perpendicular to secondary veins), or [2] scalariform (ladder-like; perpendicular to secondary veins). Secondary and tertiary veins are numerous and closely parallel in sect. Malvanthera and were scored [0]. Tertiary veins are reticulate in most subg. Urostigma (except sect. Malvanthera), subg. Pharmacosycea, and in some members of subg. Ficus. Scalariform tertiary veins are common in subg. Ficus.

61. Leaf margin [0] entire (not toothed) or [1] serrate to dentate (toothed). Leaves with ascending or looping secondary veins tend to have entire margins. Serrate or dentate leaves tend to have secondary veins terminating in the teeth.

62. Leaf epidermis [0] glabrous, [1] pubescent , [2] scabrid or [3] muricate.

63. Cystoliths [0] none, [1] abaxial (only on the lower surface), [2] paraxial (on the upper and lower surfaces) or [3] adaxial (only on the upper surface). Cystoliths are secretory cells containing calcium oxalate visible at 20x magnification on the upper and lower epidermis of the leaves. Apart from sect. Ficus subsect. Eriosycea, all species have cystoliths.

64. Stomata [0] not aggregated or [1] aggregated in sunken and foevate areoles (hairy depressions). Stomatal pits are visible at 20x magnification in sect. Kalosyce and may be associated with the climbing habit, although they are absent in climbing sect. Rhizocladus.

Appendix 4: Ficus morphological matrix. Species and characters are numbered as in Appendices 1 and 3, respectively. Abbrev: (.) state same as first row; (-) not applicable.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	1	1	0	1	1	0	1	1&2	0	1	0&1	1	1	0
2	0	0	-	.	0	.	.	2	1	0	.	.	.	.
3	.	.	.	.	0	.	.	2	.	0	1	0	.	.
4	.	0	-	.	0	1	.	1	1	0	1	0	2	.
5	.	.	.	.	.	.	.	1	.	2	4	0	.	.
6	.	.	1	.	.	.	.	2	.	2	4	0	.	.
7	0	.	1	.	.	1	.	2	.	0	1	0	.	.
8	.	.	.	.	.	1	.	2	1	.	1	0	.	.
9	.	.	.	.	.	2	.	1	3	.	3	0	.	.
10	.	.	.	.	.	.	.	.	.	.	0	0	.	.
11	0	0	-	.	0	1	.	2	1	0	0	.	0	.
12	0	0	-	.	.	1	.	2	1	0	.	.	.	.
13	.	0	-	.	.	1	0	-	-	0	0	0	.	.
14	0	0	-	.	0	.	.	2	.	0	0	.	0	.
15	.	.	.	.	.	.	.	2	.	2	4	0	.	.
16	0	0	-	.	.	1	.	0&1	.	0	0	.	.	.
17	0	0	-	.	0	.	.	.	.	0	0	.	.	.
18	.	.	.	.	0	.	.	.	.	0	0	0	.	.
19	0	0	-	.	.	.	.	1	.	0	0	.	.	.
20	0	0	-	.	0	1	0	-	-	0	0	0	0	.
21	0	.	.	.	0	.	.	1	.	0	0	0	.	.
22	.	.	1	.	0	.	.	2	.	0	0	0	.	.
23	.	.	.	.	.	.	.	.	0&1	.	.	.	.	.
24	.	.	.	.	0	1	.	1	1	0	1	0	2	.
25	.	0	-	.	.	1	.	0	1	0	1	0	.	.
26	0	0	-	.	.	.	0	-	-	0	0	0	0	1
27	0	0	-	0	.	.	.	2	.	0	0	0	0	.
28	.	.	.	.	.	1	.	2	1	0	1	0	.	.
29	0	0	-	.	0	.	.	2	.	0	0	0	0	.
30	.	.	.	.	0	1	.	1	1	0	1	0	.	.
31	.	.	1	.	.	.	.	2	.	0	0	0	.	.
32	0	.	1	.	.	1	.	.	.	0	1	0	.	.
33	.	.	.	.	0	1	.	2	.	0	1	0	.	.
34	.	.	.	.	.	.	.	1	.	0	0	0	.	.
35	.	0	-	.	.	1	.	2	1	.	1	0	.	.
36	.	.	.	.	.	.	.	2	.	2	4	.	.	.
37	0	0	-	.	0	.	.	2	.	0	0	0	0	.
38	0	.	1	.	.	1	.	2	.	0	1	0	.	.
39	.	.	.	0&1	.	.	.	2	.	0	0	0	.	.
40	.	.	.	.	.	.	.	0	2	0	2	.	.	.
41	.	.	.	.	.	2	.	.	2	.	2	0	.	.
42	.	.	.	.	0	.	.	.	.	0	0	0	.	.
43	0	0	-	.	.	0&1	.	2	.	0	0	0	0	.
44	.	.	.	.	.	.	.	0	2	0	2	.	.	.
45	.	.	.	.	.	2	.	1	3	.	3	0	.	.
46	0	0	-	.	0	.	.	2	.	0	0	.	0	.

Appendix 4 (continued): Ficus character matrix.

	15	16	17	18	19	20	21	22	23	24	25	26	27	28
1	1	1	0	-	0	0	0	1	1	0	0	1	1	0
2	0	.	1	1	.	.	.	.	0	-	1	-	0	.
3	.	0	1	0	1	.	1	.	0	1	.	.	0	.
4	0	.	1	0	.	.	.	.	0	.	.	0	0	.
5	.	.	1	0	2	.	-	.	0	1	.	.	.	.
6	.	.	1	0	2	.	-	.	0	.	.	.	.	.
7	.	0	1	0	0&1	.	1	.	0	-	.	-	.	.
8	0	0	1	0	.	.	1	.	0	.	.	0	0	.
9	0	0	1	0	.	.	1	0	0	0&1	.	0	0	.
10	.	0	1	0	2	.	-	.	.	.	.	.	0	.
11	0	0	1	0	.	.	.	.	0	-	.	-	.	0&1
12	0	0	1	0	1	.	.	.	0	-	1	-	0	.
13	0	.	1	0	.	.	.	.	0	.	.	0	0	.
14	0	0	1	0	.	.	.	.	0	-	1	-	.	1
15	.	0	1	0	2	.	-	.	0	1	.	.	.	.
16	0	0	1	0	1	.	.	.	0	-	1	-	0	.
17	0	0	1	0	.	.	.	.	0	-	1	-	0	.
18	.	0	1	0	2	.	-	.	0	.	.	.	0	.
19	0	0	1	0	.	.	.	.	0	-	1	-	0	.
20	0	0&1	1	0	.	.	.	0	0	-	.	-	.	.
21	.	0	1	0	2	.	-	.	0	-	.	-	0	.
22	.	0	1	0	1	.	1	.	0	.	.	.	0	.
23	.	.	.	-	0&1	.	.	.	.	.	.	.	.	.
24	0	.	1	0	.	.	.	.	0	.	.	0	0	.
25	0	.	1	0	.	.	.	.	0	.	.	0	0	.
26	0	0	1	0	.	.	.	0	0	-	.	-	.	.
27	0	0	1	0	.	.	.	.	0	-	.	-	.	.
28	0	.	1	0	.	.	.	0	0	.	.	0	0	.
29	0	.	1	0	1	.	.	.	0	-	.	-	2	.
30	0	.	1	0	.	.	.	.	0	.	1	0	0	.
31	.	.	1	0	.	.	.	.	0	1	.	.	.	.
32	.	0	1	0	1	.	1	.	0	-	.	-	.	.
33	.	.	1	0	0&1	.	1	.	0	.	.	.	.	.
34	0	.	1	0	.	.	.	.	0	.	1	0	0	.
35	.	.	1	0	0&1	.	.	.	.	.	.	.	.	.
36	.	.	1	0	2	.	-	.	0	1	.	.	.	.
37	0	0	1	0	.	.	1	.	0	-	.	-	0	.
38	.	0	1	0	1	.	1	.	0	-	.	-	.	.
39	.	0	1	0	2	.	-	.	.	.	.	.	0	.
40	0	0	1	1	.	1	.	0	0	.	.	0	0	.
41	0	.	1	0	.	1	1	0	0	0&1	.	0	0	.
42	.	0	1	0	1	.	0&1	.	0	.	.	.	.	.
43	0	.	1	0	.	.	.	.	0	-	.	-	2	.
44	0	0	1	1	.	1	.	0	0	.	.	0	0	.
45	0	0	1	0	.	1	1	0	0	.	.	0	0	.
46	0	0	.	-	.	.	.	.	0	-	1	-	2	1

Appendix 4 (continued): Ficus morphological matrix.

	29	30	31	32	33	34	35	36	37	38	39	40	41	42
1	1	0	1	1	0	0	0	0	1	0	0	0	1	2
2	0	.	0	0&1	.	-	2	0&1	0	.	.	.	.	.
3	0	.	0	.	.	1	2	0&1	.	.	.	?	.	.
4	.	.	0	.	.	.	1	.	0	.	.	1	.	1
5	0	.	0	.	.	.	.	.	2	.	.	.	.	.
6	0	.	0	.	.	.	.	1	2	.	.	.	.	.
7	0	.	0	.	?	-	2	.	.	.	.	.	.	.
8	0	.	0	.	.	.	.	1	0	.	.	.	.	.
9	0	.	0	.	1	.	2	.	0	.	.	1	.	.
10	0&1	.	0	.	.	.	1	.	2	.	.	.	.	.
11	0	.	0	0	.	-	.	1	0	.	.	.	0	0
12	0	.	0	0	1	-	.	0&1	.	.	.	1	.	.
13	0	1	.	.	.	.	1	.	0	.	1	1	.	.
14	0	.	0	0	.	-	.	1	0	.	.	.	0	0
15	0	.	0	.	.	.	.	1	2	.	.	.	.	.
16	0	.	0	0&1	.	-	.	0&1	.	.	.	?	.	.
17	0	.	0	0&1	.	-	1	1	0	.	.	.	.	.
18	0	.	0	.	.	.	2	.	2	.	.	1	.	.
19	0	.	0	.	.	-	1	1	0	.	.	.	.	.
20	0	.	0	0	.	-	.	0&1	0	.	.	1	.	0
21	0	.	0	.	.	-	2	.	2	.	.	1	.	.
22	0	.	0	.	1	1	2	1	2	.	.	1	.	.
23	.	.	.	.	1	.	.	.	.	.	1	.	.	.
24	.	.	0	.	.	.	1	.	0	.	.	1	.	1
25	0	1	.	.	.	.	1	.	0	.	1	1	.	.
26	0	.	0	0	.	-	.	0&1	0	.	.	1	.	0
27	0	.	0	0	.	-	.	.	0	.	.	1	.	0
28	0	.	0	.	.	.	.	.	0	.	.	.	.	.
29	0	.	0	.	.	-	.	.	0	.	.	.	.	0
30	.	.	0	0	.	.	.	1	.	.	.	.	.	1
31	0	.	0	.	.	.	.	.	0	.	.	.	.	.
32	0	.	0	.	1	-	2	0&1	.	.	.	.	.	.
33	0	.	0	.	1	1	2	0&1	2	.	.	?	.	.
34	.	.	0	0	.	.	.	1	.	.	.	.	.	1
35	.	.	?	.	1	.	2	0&1	2	.	.	1	.	.
36	0	.	0	.	.	.	.	1	2	.	.	.	.	.
37	0	.	0	.	.	-	.	.	0	.	.	.	.	0
38	0	.	0	.	1	-	2	.	.	.	.	.	.	.
39	.	.	0	.	.	.	.	0&1	2	.	.	.	.	.
40	0	.	0	.	2	.	.	0&1	0	1	.	1	.	0
41	0	1	0	.	0&1	.	.	.	0	.	.	.	.	.
42	0	.	0	.	1	1	2	.	2	.	.	1	.	.
43	0	.	0	.	.	-	.	.	0	.	.	.	.	0
44	0	.	0	.	2	.	.	0&1	0	1	.	1	.	0
45	0	.	0	.	1	.	2	.	0	1	.	1	.	.
46	0	.	0	0	.	-	.	0&1	0	.	.	.	0	0

Appendix 4 (continued): Ficus morphological matrix.

	43	44	45	46	47	48	49	50	51	52	53	54	55	56
1	0	0	1	1	-	0&1	0	0	0&1	0	0	1	0	0&1
2	1	1	0	.	-	.	.	.	1	.	.	0	.	1
3	.	?	0	.	-	0	.	.	.	.	.	.	.	.
4	-	.	0	.	-	1	.	.	1	.	.	.	1	1
5	.	.	0	.	-	1	1	.	1	1	.	.	.	1
6	.	.	0	.	-	1	1	.	1	.	1	.	.	.
7	?	?	0	.	-	.	.	.	.	.	.	.	.	0
8	.	.	0	.	-	1	.	.	1	1	1	.	.	1
9	.	.	0	.	-	3	.	1	0	.	.	.	.	3
10	.	.	0	.	-	0	.	.	0	1	.	.	.	0
11	-	.	0	0	-	1	.	.	1	.	.	0	.	1
12	1	.	.	.	-	0	.	.	1	.	.	0	.	1
13	.	.	0	.	-	1	.	.	1	.	.	.	.	1
14	-	.	0	0	-	0	.	.	1	.	.	0	.	0
15	.	.	0	.	-	1	1	.	1	.	1	.	.	1
16	1	.	.	.	-	0	.	.	0	.	.	0	.	0
17	1	.	0	.	-	0	.	.	0	.	.	0	.	0
18	.	.	0	.	-	0	.	.	0	.	.	.	.	0
19	1	.	0	.	-	0	.	.	0	.	.	0	.	0
20	-	.	0	0	-	0	.	.	0	.	.	0	.	0
21	.	.	0	.	-	0	.	.	0	.	.	.	.	0
22	1	1	0	.	-	0	2	.	0	.	.	.	.	0
23	.	.	.	.	-	1	.	.	1	.	.	.	.	.
24	-	.	0	.	-	1	.	.	1	.	1	.	1	1
25	.	.	0	.	-	1	.	.	1	.	.	.	.	1
26	-	.	0	0	-	0	.	.	0	.	.	0	.	0
27	-	.	0	0	-	0	.	.	0	.	.	0	.	0
28	.	.	0	.	-	1	.	.	1	.	.	.	.	1
29	-	1	0	0	-	0	.	.	0	.	.	0	.	0
30	-	.	0	.	-	1	.	.	1	1	.	.	1	1
31	.	.	0	.	-	1	1	.	0	1	.	.	.	1
32	1	1	0	.	-	0	.	.	1	.	.	.	.	1
33	1	?	0	.	-	1	2	.	1	.	.	.	.	1
34	-	.	0	.	-	0	.	.	0	.	.	.	1	0
35	1	.	.	.	-	1	.	.	1	.	.	.	.	1
36	.	.	0	.	-	0	1	.	0	.	1	.	.	0
37	-	0&1	0	0	-	0	.	.	1	.	.	0	.	0
38	1	1	0	.	-	.	.	.	1	.	.	.	.	0
39	.	.	0	.	-	0	.	.	0	1	.	.	.	0
40	-	.	0	.	-	2	.	.	0	.	.	.	1	2
41	.	1	0	.	-	2	.	.	1	.	.	.	1	2
42	1	1	0	.	-	0	.	.	0	.	.	.	.	0
43	-	1	0	0	-	0	.	.	0	.	.	0	.	0
44	-	.	0	.	-	0	.	.	0	.	.	.	1	1
45	.	.	0	.	-	3	.	1	0	.	.	.	.	3
46	-	.	0	0	-	0	.	.	0	.	.	0	.	0

Appendix 4 (continued): Ficus morphological matrix.

	57	58	59	60	61	62	63	64
1	0&1	0	3	1	0	0&1	2	0
2	1	.	0	.	.	.	.	.
3	1	.	.	2	1	0	1	.
4	.	.	4	2	.	1	.	.
5	.	1	5	2	1	1	1	.
6	0	1	5	2	1	1	.	.
7	0	.	.	.	0&1	0	1	.
8	1	.	.	2	1	1	.	.
9	1	.	.	2	1	3	.	.
10	1	.	.	2	.	0	1	.
11	0	.	1	.	.	.	.	.
12	.	.	.	.	.	0	1	.
13	.	.	4	2	1	1	0	.
14	0	.	1	0	.	0	.	.
15	1	1	5	2	1	1	1	.
16	0	.	.	.	.	0	1	.
17	.	.	.	.	.	0	.	
18	0	.	.	.	.	0	1	.
19	0	.	.	.	.	0	.	.
20	0	0&1	1	.	.	0	.	.
21	0	.	.	.	.	0	1	.
22	1	.	.	2	1	0	1	.
23	0	.	.	2	.	1	.	.
24	.	0&1	4	2	.	1	.	.
25	1	.	4	2	1	1	0	.
26	0	.	1	.	.	0	.	.
27	0	.	1	.	.	0	.	.
28	1	1	.	2	1	2	.	.
29	0	.	1	.	.	0	1	.
30	1	1	.	.	.	0	.	1
31	1	.	.	2	1	1	1	.
32	.	.	.	.	1	0	1	.
33	1	.	.	2	1	1	1	.
34	1	1	.	.	.	0	.	1
35	1	.	.	.	.	1	.	.
36	0	1	5	.	.	0	1	.
37	.	.	1	.	.	0	1	.
38	.	.	.	.	1	0	1	.
39	1	.	.	.	.	0	.	.
40	0	1	2	.	.	0	.	.
41	1	1	4	2	1	2	.	.
42	.	.	.	2	1	0	1	.
43	0	.	1	.	.	0	1	.
44	0	1	2	.	.	0	.	.
45	1	.	.	2	1	3	.	.
46	0	.	1	0	.	0	.	.

Appendix 5: Morphological characters for a phylogenetic analysis of pollinating fig wasps.

1. Female head (0) less than twice as long as wide or (1) twice or more as long as wide. When pollinators attempt to gain access to the interior of the syconium through the ostiole, the head, antennae and mandibles are first to interact with the ostiolar bracts. vanNoort and Compton (1996) argued that the evolution of female head shape in response to arrangements of ostiolar bracts could obscure phylogenetic relationships. The ratio of median length to width across the compound eyes is close to unity (X = 1.06; SD = 0.37) and ranges from 0.8 to 3.0. Wiebes (1982b) remarked that elongate heads (with ratios greater than 1.2) in Dolichoris, Pleistodontes and Tetrapus are pleisiomorphic. However, elongate heads are derived Ceratosolen and only some Pleistodontes have ratios >2.0.
2. Female ocelli (0) three or (1) two. A median ocellus and two lateral ocelli are situated in a groove at the posterior of the head in most species. The median ocellus is absent in Waterstoniella brevigena.
3. Female epistomal margin or clypeus (0) rounded, (1) with a pointed median, (2) bilobate, or (3) trilobate. The clypeus is trilobate in most species although the lateral and median points vary in length. The clypeus is rounded or pointed in Pleistodontes and Eupristina or bilobate in Kradibia copiosae.
4. Female facial groove (0) closed or (1) open. A medial groove fits the antennal scapes in species other than Pleistodontes. As a pollinator pushes through the ostiole, the antennae are folded into the facial groove and pointed backward, eventually breaking beyond the third segment.
5. Female antennae with (0) nine segments (1) ten segments or (2) eleven or more segments. Eleven segments are common although the apical two or three segments may be fused into a club.

6. Female scape (0) less than twice as long as wide or (1) more than twice as long as wide. Pleistodontes, Tetrapus and Waterstoniella brevigena have elongate antennal scapes ranging 2.5-4.0 times as long as wide.
7. Female pedicel (0) as long as wide or (1) elongate. Pleistodontes have elongate antennal scapes ranging 2.5-4.0 times as long as wide.
8. Female pedicel (0) with less than ten or (1) with more than ten recurved axial spines. Axial spines on the pedicel interact with the ostiolar bracts during entry to the syconium. Numerous axial spines are present in most species but spines are scarce in Pleistodontes and absent Tetrapus.
9. Female third antennal segment (0) without a pointed apex, (1) with a pointed apical process or (2) with a pointed apical appendage. The third antennal segment is distinguished from the other funicular segments in most species by a pointed apical process, which may be secondarily divided and appended to the segment.
10. Female funicular segments (0) less than three times as long as wide or (1) more than three times as long as wide. Except in Lipporhopalum, the funicular segments are less than twice as long as wide. Lipporhopalum has funicular segments ranging from three to five times as long as wide.
11. Female funicular segments (0) with sensilla linearia or (1) sensilla chaetica. Sensilla may enable pollinators in detecting fig volatiles, which act as chemosensory stimuli and maintain pollinator specificity (Hossaert-McKey et al. 1994, Ware and Compton 1994b). Sensilla linearia are embedded longitudinally in the cuticle and do not extend beyond the apex of the segment in which they are embedded. Sensilla chaetica project beyond the apex of the segment or radiate from it laterally.
12. Female sensilla (0) in one row, (1) in two rows or (2) in three rows. There is considerable variation in the number and arrangement of sensilla on the funicular segments of the antennae. Antennae with a fused club may have up to nine rows of sensilla on the apical segment, representing the fusion of three segments. To examine

the distribution of sensilla in homologous segments, rows were counted on the eighth segment only.

13. Female maxilla (0) with a palpus (1) with subapical setae or (2) atrophied. The "baciliform process" of Wiebes (1994) refers to a reduced maxillary palpus. The palpus may be reduced to a single hair on the maxilla or the maxilla may be indistinguishable from an atrophied maxillolabial complex.
14. Female labium (0) with two or more subapical setae, (1) with one subapical seta, or (2) without setae. Labial palps are reduced to setae in all species and in some species the labium is atrophied and fused to the maxilla (see character 13).
15. Female mandibular appendage (0) horizontal in orientation and appended to the mandible or (1) subvertical in orientation and fused to the mandible. The subvertical orientation of the mandibular appendage in Pleistodontes and Tetrapus may be involved in passage though the ostiole (Grandi 1925). The appendage is fused to the mandible in all genera except Pleistodontes and Tetrapus (Ramirez 1991).
16. Female mandible with (0) one or (1) two apical teeth.
17. Female mandible with (0) one or (1) two glands. Ramirez (1991) showed that the distribution of mandibular glands is phylogenetically informative although more intensive sampling indicates that the number of glands is not fixed at the generic level. For example, D. hombronianae has two glands while D. inornata and D. vasculosae have a single gland.
18. Number of ridges on female mandible (0) four or less, (1) five, (2) six, (3) seven or (4) eight or more.
19. Number of ventral lamellae on female mandibular appendage (0) four, (1) five, (2) six, (3) seven, (4) eight, (5) nine, (6) ten, (7) eleven or (8) twelve or more.
20. Ventral lamellae (0) not produced into teeth or (1) produced into teeth. In some species, the narrowed and upturned lamellae resemble teeth.

21. Mesosternal pollen pockets (0) absent, (1) present or (2) present but evidently closed. Ramirez (1978) argued that pollen pockets evolved independently in several pollinator lineages. Wiebes (1982b) argued that the absence of pockets in some Pleistodontes was pleisomorphic. Wiebesia have pollen pockets but they appear to be closed. In this genus, the mechanism of pollen transport may involve gaps between the posterior abdominal tergites.
22. Mesoscutum (0) entire or (1) with a longitudinal groove along the median. A grooved mesoscutum is present in Wiebesia but not in the type species, W. partita.
23. Front coxae (0) smooth or (1) with combs or (2) with corbiculae. Ramirez (1978) examined the distribution of coxal combs and corbiculae in relation to pollen collection and transport. Combs consist of even rows of hairs on the axial surface of the coxae. Corbiculae consisting of mesal cavities on the fore coxae covered by a row of hairs are present in Eupristina, Dolichoris, Platyscapa and some Pleistodontes.
24. Sternal corbiculae (0) absent or (1) present. Ramirez (1978) reports the presence of sternal corbiculae in all genera except for Blastophaga, Wiebesia and Pleistodontes.
25. Fore tibia with (0) two, (1) three, (2) four, or (3) more than four dorso-apical teeth. Axial spines or ventral spurs may also be present on the fore tibia.
26. Mid leg with (0) five or (1) four tarsal segments.
27. Ventral spines on the first tarsomere (0) absent or (1) present. Conical spines are abundant on the first tarsal segment in Tetrapus.
28. Hind coxae (0) without or (1) with a groove for the reception of the tibia.
29. Antaxial tooth in the hind tibia (0) bicuspidate or (1) tricuspidate. Teeth in the antaxial position on the hind tibia are bicuspidate or tricuspidate.
30. Axial tooth in the hind tibia (0) simple or (1) bicuspidate. Teeth in the axial position on the hind tibia may be simple or bicuspidate.
31. Forewing venation (0) complete or (1) obsolete beyond the submarginal vein. Forewing venation is often reduced in the Chalcidoidea relative to other Hymenoptera

(Boucek 1988). Complete venation in the Agaonine forewing includes submarginal, marginal, stigmal and postmarginal veins. When complete, the postmarginal vein is 1-2 times as long as the stigmal vein. When obsolete, the postmarginal vein is absent or shorted than the stigmal vein.

32. Forewing with (0) two, (1) three, (2) four, (3) five or more pustules scattered along the stigmal vein or (4) without pustules. Pustules may also be scattered along the submarginal vein.

33. Spiracular peritremata (0) small and subcircular or (1) large and ovoid. Paired spiracular peritremata located on the eighth urotergite are less than the length of the segment when small. The spiracles are gouge-like and more than the length of several abdominal tergites in Ceratosolen and occasionally in Kradibia and Waterstoniella.

34. Hypopygium (0) without or (1) with a row of hyaline setae.

35. Ovipositor (0) shorter than the abdomen or (1) longer than the abdomen. Ovipositor lengths are generally correlated with fig breeding systems (Ramirez 1980) although there are some notable exceptions in Pleistodontes. Ovipositor lengths in pollinators of dioecious figs are generally 0.2-0.5 times as long as the abdomen. Ovipositor lengths in monoecious fig pollinators are typically 1.0-1.75 times as long as the abdomen.

36. Male head (0) less than as long as wide or (1) more than as long as wide. The male head is adapted to chewing and burrowing an exit from the syconium, as opposed to the female head and fore tibia which are involved in forcing entry through the bract-lined ostiole. In ripe syconia of some species, the males burrow through ostiolar bracts that appear to loosen when ripe. In other Ficus species, the males bypass the ostiolar bracts and burrow directly through the wall of the syconium (sects. Conosycea and Rhizocladus). Ceratosolen associated with subg. Sycomorus (sensu Ramirez 1977) have elongate heads.

37. Male head (0) without or (1) with dorsal spines. In some species, dorsal spines may function in burrowing through the ostiole or fig wall in a manner analogous to the ventral lamellae of the mandibular appendage in the female head except that the direction of passage is reversed. Note also that males in Wiebesia, Waterstoniella and Pleistodontes burrow an exit through the wall of the fig. Males in Ceratosolen, Kradibia and Liporrhopalum exit through the ostiole.
38. Epistomal margin or clypeus (0) without lateral lobes, (1) bilobate or (2) trilobate with a distinct medial prominence.
39. Male antennae (0) inserted in separate grooves on either side of prominent scrobes or (1) in a common medial groove toward the front of the head. When the antennal scrobes are deeply inserted, the toruli may be hidden in a common groove or separate grooves. Antennae are located in distinct grooves in Ceratosolen and Kradibia and in a common groove in Dolichoris, Waterstoniella and Wiebesia.
40. Male antennae with (0) four, (1) five or (2) seven segments.
41. Male antennae with (0) slender or (1) clavate (club-shaped) apical segments.
42. Male eyes (0) shorter than the cheek or (1) as long or longer than the cheek. Eyes are reduced or absent in males of many species. Eyes are equal to or more than twice as long the cheek (i.e. more than 0.2 as long as the head) in Dolichoris and some Blastophaga, Eupristina, Platyscapa, Pegoscapus, Tetrapus, Waterstoniella and Wiebesia.
43. Male mouthparts with (0) a distinct labium and maxillae, (1) with a reduced maxillolabial complex, or (2) without a labium and maxillae.
44. Male maxillolabial complex (0) without or (1) with setae. Paired setae on the maxillolabial complex represent the reduction of the maxillary or labial palps. Not applicable to taxa lacking mouthparts (see character 43).
45. Male mandibular glands (0) one or (1) two. Ramirez (1991) showed that the distribution of male mandibular glands is phylogenetically informative. Contrary to

Ramirez (1991), D. vasculosae and some species of Kradibia and Ceratosolen possess two mandibular glands.

46. Male pronotum (0) less than as long as wide or (1) more than as long as wide anteriorly. The male pronotum is elongate in Ceratosolen and Kradibia.

47. Male mesonotum (0) entire or (1) fused to the metanotum (i.e. the dorsal part of the metathorax). The metanotum may also be fused to the propodeum. All males are apterous.

48. Male metanotum or dorsal part of the metathorax (0) entire or (1) fused to the propodeum.

49. Male propodeal peritremata (0) less than half as long as the propodeum or (1) enlarged (as long as the propodeum). Enlarged propodeal peritremata in Ceratosolen subg. Strepitus may also protrude laterally.

50. Male fore tibia with (0) two, (1) three, (2) four or (3) five dorso-apical teeth. The fore tibia commonly has three or four dorso-apical and two ventro-apical teeth. Ceratosolen nanus, C. bisulcatus and Platyscapa fischeri have two dorso-apical teeth while Kradibia wassae and K. sp. "ohuensis" have five teeth.

51. Male fore tibia with (0) one, (1) two or (2) three ventro-apical teeth. The distribution of ventral teeth on the apex of the tibia are coded as a separate character because they are not correlated with the distribution of dorsal teeth.

52. Male fore tarsi (0) one, (1) two, (2) three, (3) four or (4) five. The reduced male fore tarsus is commonly bimerous although the two segments appear fused in Ceratosolen abnormis and C. grandii. The fore leg is complete in Pleistodontes and Waterstoniella while three and four tarsi occur in Ceratosolen vissali and Wiebesia pumilae, respectively.

53. Male mid leg (0) atrophied, (1) with oligomerous tarsi or (2) complete with five tarsi. A complete mid leg is common although reductions in the number of tarsal segments

occur in Ceratosolen nanus, Liporrhopalum and some Wiebesia. The mid leg is completely atrophied in some Kradibia.

54. Male hind leg with (0) three, (1) four or (2) five tarsi. Reductions in the number of tarsal segments on the hind leg are present in Eupristina verticillata, Kradibia and Liporrhopalum.

55. Armature of the male hind tibia (0) with two bicuspidate teeth; (1) with a bicuspidate axial tooth and an antaxial tooth; (2) without teeth or (3) with a single tooth.

56. Male tarsi (0) without or (1) with plantar protuberances. Protuberances on the plantar edges of the hind tarsi are present in some Ceratosolen and Kradibia species (Wiebes 1965).

57. Male genitalia (0) without or (1) with clawed claspers. Claspers bearing 2-5 claws may be observed in the fully extended male genitalia.

Appendix 6: Specimens included in a phylogenetic analysis of dioecious fig pollinators. Vouchers, host records, collectors and localities are listed for sources of molecular and morphological data. Except for specimens from Naturalis (RMNH) and the Bishop Museum (HSPA), vouchers are deposited at the Museum of Comparative Zoology of Harvard University. Abbrev: (A)pocryptophagus, (B)lastophaga, (C)eratosolen, (D)olichoris, (E)upristina, (K)radiba, (L)iporrhopalum, (Pla)tyscapa, (Ple)istodontes, (Wat)erstoniella, (Wie)besia) and (•) mitochondrial DNA voucher.

no	pollinator	host species	voucher	collector	locality
1	[A. spinitarsus]	variegata	B61•	B. Isua	Madang, PNG
			GW475.3	G. Weiblen	New Britain, PNG
2	B. intermedia	padana	GW1065•	G. Weiblen	Bogor, Indonesia
3	B. malayana	grossularioides	GW861•	G. Weiblen	Kalimantan, Indonesia
4	C. appendiculatus	variegata	GW475	G. Weiblen	New Britain, PNG
			B198	B. Isua	Madang, PNG
			RMNHs222	J. T. Wiebes	Bogor, Indonesia
			HSPA	F. X. Williams	Philippine Islands
			?	D. S. Hill	Hong Kong
5	C. bisulcatus	septica	G055	U. Koil	Madang, PNG
			B170	B. Isua	Madang, PNG
			RMNHs287	A. Hoogerwerf	Java, Indonesia
6	C. blommersi	botryoides	GW841•	C. Kerdelhue	Madagascar
7	C. capensis	sur	GW840•	C. Kerdelhue	Tanzania
8	C. emarginatus	auriculata	RMNH2723•	E. J .H. Corner	Penang Malaysia
			RMNH609	E. J .H. Corner	Selangor, Malaysia
9	C. fusciceps	racemosa	GW683	B. Panton	Darwin, Australia
			GW1075•	G. Brown	Darwin, Australia
			RMNHs83	J. T. Wiebes	Bogor, Indonesia
			RMNHs236	J. T. Wiebes	Bogor, Indonesia
			HSPA	C. Pemberton	Queensland, Australia
10	C. grandii	semivestita	GW700•	G. Weiblen	Madang, PNG
			RMNHs452	E. J. H. Corner	Morobe, PNG
11	C. medlerianus	mollior	B33•	B. Isua	Madang, PNG
			RMNH3330	J. T. Medler	Wau, PNG
12	C. nanus	pungens	B62	B. Isua	Madang, PNG
			G077	U. Koil	Madang, PNG
			RMNHs548	E. J. H. Corner	Lae, PNG
13	C. nexilis	nodosa	GW599	G. Weiblen	Madang, PNG
			GW829	G. Weiblen	Honiara, Solomon Isl.
			RMHN3326	J. T. Medler	Wau, PNG
			HSPA	C. Pemberton	Queensland, Australia

Appendix 6 (continued): Specimens included in a phylogenetic analysis of fig pollinators. Vouchers, host records, collectors and localities are listed for sources of molecular and morphological data.

no	pollinator	host species	voucher	collector	locality
14	C. "riparianus"	ochrochlora	GW735•	G. Weiblen	Crater Mountain, PNG
15	C. cf. nexilis	robusta	B195•	B. Isua	Madang, PNG
16	C. corneri	botryocarpa	GW468	G. Weiblen	New Britain, PNG
			B135•	B. Isua	Madang, PNG
			RMNHs405	E. D. Merrill	Mindoro, Philippines
			HSPA	F. X. Williams	Luzon, Philippines
17	C. dentifer	hispidioides	G051	U. Koil	Madang, PNG
			B133•	B. Isua	Madang, PNG
			RMNHs524	E. J. H. Corner	Lae, PNG
18	C. hooglandi	bernaysii	G093•	U. Koil	Madang, PNG
			RMNHs362	R. D. Hoogland	Madang, PNG
19	C. vechti	lepicarpa	GW1086•	G. Weiblen	Endau, Malaysia
			RMNHs98	J. H. de Gunst	Cibodas, Indonesia
20	C. abnormis	dammaropsis	G054	U. Koil	Madang, PNG
			B110•	B. Isua	Madang, PNG
			RMNHs408	Gjellerup	Irian Jaya, Indonesia
			RMNHs575	J. H. Ardley	Aiyura, PNG
21	C. armipes	itoana	GW622•	G. Weiblen	Madang, PNG
			RMNHs546	E. J. H. Corner	Wau, PNG
22	C. "kaironkensis"	microdictya	GW954•	G. Weiblen	Madang, PNG
			BISH	J. L. Gressitt	Mendi, PNG
23	C. vissali	theophrastoides	GW826•	G. Weiblen	Honiara, Solomon Isl.
			RMNH955	E. J. H. Corner	Guadal., Solomon Isl.
			RMNH962	E. J. H. Corner	Ysabel, Solomon Isl.
24	D. inornata	edelfeltii	GW821•	G. Weiblen	Madang, PNG
			B195	B. Isua	Madang, PNG
			RMNHs967	E. J. H. Corner	Solomon Isl.
25	D. "hombronianae"	hombroniana	GW953•	G. Weiblen	Madang, PNG
26	D. vasculosae	vasculosa	GW1084•	G. Weiblen	Endau, Malaysia
			RMNH406	D. S. Hill	Hong Kong
27	E. verticillata	microcarpa	G080	F. Dal	Madang, PNG
			F2	F. Dal	Madang, PNG
28	K. copiosae	copiosa	GW530	G. Weiblen	Madang, PNG
			G057	U. Koil	Madang, PNG
			RMNHs514	E. J. H. Corner	Lae, PNG
29	K. jacobsi	conocephalifolia	B66	B. Isua	Madang, PNG
			B154•	B. Isua	Madang, PNG
			RMNHs530	E. J. H. Corner	Lae, PNG

Appendix 6 (continued): Specimens included in a phylogenetic analysis of dioecious fig pollinators. Vouchers, host records, collectors and localities are listed for sources of molecular and morphological data.

no	pollinator	host species	voucher	collector	locality
30	K. "ohuensis"	trachypison	B136•	B. Isua	Madang, PNG
31	K."salembensis"	phaeosyce	GW610	G. Weiblen	Madang, PNG
			B179•	B. Isua	Madang, PNG
32	K. wassae	wassa	G051	U. Koil	Madang, PNG
			B176	B. Isua	Madang, PNG
			RMNH3313	J. T. Medler	Wau, PNG
33	L. gibbosae	tinctoria	G072	F. Dal	Madang, PNG
			F4•	F. Dal	Madang, PNG
			RMNH723	E. J. H. Corner	Sabah, Malaysia
34	L. virgatae	virgata	B146	B. Isua	Madang, PNG
			B166•	B. Isua	Madang, PNG
			RMNH980	E. J. H. Corner	Guadalcanal, Solomon Isl.
35	Pla. corneri	superba	GW851•	G. Weiblen	Bogor, Indonesia
			RMNH3267	E. J. H. Corner	Perlis, Malaysia
36	Pla. fischeri	prasinicarpa	GW827•	G. Weiblen	Honiara, Solomon Isl.
			G070	F. Dal	Madang, PNG
			RMNHs757	J. T. Wiebes	Mindanao, Philippines
37	Ple. plebejus	hesperidiiformis	GW624•	G. Weiblen	Madang, PNG
			B204	B. Isua	Madang, PNG
			AM349	A. Mack	Crater Mountain, PNG
			RMNHs442	R. D. Hoogland	PNG
			RMNHs556	E. J. H. Corner	Lae, PNG
38	Ple. rieki	xylosycia	G059•	U. Koil	Madang, PNG
			G066	U. Koil	Madang, PNG
			AM327	A. Mack	Crater Mountain, PNG
			RMNHs559	E. J. H. Corner	Bougainville Isl.
39	Ple. rigisamos	destruens	GW943•	G. Weiblen	Queensland, Australia
			RMNH5073	J. F. Addicott	Queensland, Australia
40	Wat. brevigena	pellucidopuncta ta	GW880•	G. Weiblen	Kalimantan, Indonesia
			RMNH3406	M. Leighton	Kalimantan, Indonesia
41	Wat. "dubium"	dubia	TL1021•	T. Laman	Kalimantan, Indonesia
42	Wie. "brusi"	baeuerlenii	B120•	B. Isua	Madang, PNG
43	Wie. "frustrata"	odoardi	B205•	B. Isua	Madang, PNG
			CRI638	M. Jebb (?)	Madang, PNG
			JE8	J. Ericho	Crater Mountain, PNG
44	Wie. punctatae	punctata	TL1022•	T. Laman	Kalimantan, Indonesia
			RMNH347	J. van der Vecht	Bogor, Indonesia
			RMNH307	J. H. de Gunst	Tijibodas, Indonesia

Appendix 7: Morphological characters for 44 fig wasp species. Characters and taxa are numbered as Appendices 5 and 6, respectively.

	1	2	3	4	5	6	7	8	9	10	11	12	13
1	0	0	0	0	2	1	1	0	0	0	0	1	0
2	0	0	3	1	1	0	0	1	2	0	0	1	1
3	0	0	3	1	1	0	0	1	2	0	0	1	1
4	0	0	3	1	0	0	0	1	2	0	0	0&1	1
5	0	0	3	1	2	0	0	1	2	0	0	0	1
6	0	0	3	1	1	0	0	1	2	0	0	1	0
7	0	0	3	1	0	0	0	1	2	0	0	0	0
8	0	0	3	1	0	0	0	1	2	0	0	0	0
9	0	0	3	1	0	0	0	1	2	0	0	0	1
10	0	0	3	1	2	0	0	1	2	0	0	0	1
11	0	0	3	1	2	0	0	1	2	0	0	0	1
12	0	0	3	1	2	0	0	1	2	0	0	0	2
13	0	0	3	1	1	0	0	1	2	0	0	1	1
14	0	0	3	1	2	0	0	1	2	0	0	0	1
15	0	0	3	1	1	0	0	1	2	0	0	1	1
16	0	0	3	1	2	0	0	1	2	0	0	0	1
17	0	0	3	1	2	0	0	1	2	0	0	0	0
18	0	0	3	1	2	0	0	1	2	0	0	1	0
19	0	0	3	1	2	0	0	1	2	0	0	1	0
20	0	0	3	1	1	0	0	1	2	0	0	0&1	1
21	0	0	3	1	1	0	0	1	2	0	0	1&2	1
22	0	0	3	1	1	0	0	1	2	0	0	2	1
23	0	0	3	1	2	0	0	1	2	0	0	1&2	0
24	0	0	3	1	2	0	0	1	2	0	0	2	0
25	0	0	3	1	2	0	0	1	2	0	0	1	0
26	0	0	3	1	1	0	0	1	1	0	1	1&2	0
27	0	0	1	1	2	0	0	1	2	0	1	1	1
28	0	0	2	1	1	0	0	1	2	0	0	1&2	1
29	0	0	3	1	1	0	0	1	2	0	0	1&2	0
30	0	0	3	1	2	0	0	1	2	0	0	0	1
31	0	0	3	1	1	0	0	1	2	0	0	0	1
32	0	0	3	1	1	0	0	1	2	0	0	1&2	1
33	0	0	1	1	1	0	0	1	2	1	1	1	1
34	0	0	3	1	1	0	0	1	2	1	0&1	1	1
35	0	0	3	1	1	0	0	1	1	0	0	2	1
36	0	0	3	1	2	0	0	0	1	0	1	0&1	0
37	1	0	0	0	2	1	1	0	1	0	0	1	0
38	1	0	0	0	2	1	1	0	1	0	0	0	1
39	0	0	1	0	2	1	1	0	1	0	0	0	1
40	0	1	1	1	2	1	0	1	1	0	0	0	1
41	0	0	1	1	2	0	0	1	1	0	1	2	1
42	0	0	3	1	1	0	0	1	2	0	0	0	1
43	0	0	3	1	1	0	0	1	2	0	0	0	1
44	0	0	3	1	2	0	0	1	2	0	0	1	1

Appendix 7 (continued): Morphological characters for 44 fig wasp species.

	14	15	16	17	18	19	20	21	22	23	24	25	26
1	0	-	1	-	-	-	-	0	0	0	0	0	0
2	2	1	1	0	1	0	0	1	0	0	0	1	0
3	2	1	1	0	1	1	0	1	0	0	0	1	0
4	2	1	1	0	0	0	0	1	0	0	1	2	0
5	1	1	1	1	1	1	0	1	0	0	1	1	0
6	0	1	1	0	0	1	0	1	0	0	1	0	0
7	0	1	1	0	0	0&1	0	1	0	0	1	1	0
8	0	1	1	0	1	1	0	1	0	0	1	1	0
9	1	1	1	0	1	1	0	1	0	0	1	1&2	0
10	1	1	1	0	3	3	0	1	0	0	1	1	0
11	2	1	1	0	3	4	0	1	0	0	1	2	0
12	2	1	1	0	0&1	2	0	1	0	0	1	0	1
13	0	1	1	0	1	2&3	0	1	0	0	1	2	0
14	0	1	1	0	3	4	0	1	0	0	1	2	0
15	0	1	1	0	1	2&3	0	1	0	0	1	2	0
16	2	1	1	0	1	1	0	1	0	0	1	2	0
17	1	1	1	0	2	1	0	1	0	0	1	2	0
18	1	1	1	0	0	1	0	1	0	0	1	2	0
19	1	1	1	0	1	2	0	1	0	0	1	2	0
20	0	1	1	0	2	2	0	1	0	0	1	3	0
21	0	1	1	0	4	4	0	1	0	0	1	3	0
22	0	1	1	0	4	4	0	1	0	0	1	3	0
23	0	1	1	0	4	3	0	1	0	0	1	2	0
24	1	1	1	0	1	6	1	1	0	1	1	1	0
25	1	1	1	1	1	1	0	1	0	1	1	1	0
26	0	1	1	0	0	6	1	1	0	1	1	0	0
27	0	1	1	1	1	6&7	1	1	0	1	1	0	0
28	2	1	1	1	1	0	0	1	0	0	1	2	0
29	2	1	1	1	1	3	0	1	0	0	1	3	0
30	2	1	1	1	0	0	0	1	0	0	1	2	0
31	2	1	1	1	0	3	0	1	0	0	1	2	0
32	2	1	1	1	0	0	0	1	0	0	1	2	0
33	0	1	1	0	0	1&2	0	1	0	0	1	1	0
34	0	1	1	0	0	2	0	1	0	0	1	0&1	0
35	0	1	1	1	1	5	1	1	0	1	1	0	0
36	0	1	1	1	1	5	1	1	0	1	1	0	0
37	0	0	1	1	4	8	1	0	0	0	0	0	0
38	0	0	1	1	1	8	1	0	0	0	0	0	0
39	0	0	1	1	4	7&8	1	1	0	0	0	0	0
40	0	1	1	1	1	2	1	1	0	0	0	0	0
41	0	1	1	1	4	7	1	1	0	0	0	0	0
42	1	1	0	0	1	6	0	2	1	0	0	1	0
43	1	1	0	0	1	6	0	2	1	0	0	0	0
44	1	1	1	0	0	2	0	2	1	0	0	1	0

Appendix 7 (continued): Morphological characters for 44 fig wasp species.

	27	28	29	30	31	32	33	34	35	36	37	38	39
1	0	0	-	0	0	1	0	0	1	1	0	2	-
2	0	0	0	0	0	1	0	1	0	0	1	0	1
3	0	0	0	0	0	1	1	1	0	0	1	0	1
4	0	0	0	0	0	1	1	1	0	1	0	2	0
5	0	0	0	0	0	2	1	1	0	1	0	1	0
6	0	0	1	0	0	2	1	0	1	1	0	2	0
7	0	0	0	0	0	2	1	1	1	1	0	2	0
8	0	0	0	0	0	2	1	0	0	1	0	2	0
9	0	0	0	0	0	2	1	0	1	1	0	2	0
10	1	1	0	1	0	0	1	1	0	1	0	2	0
11	0	0	1	0	0	1	1	1	0	1	0	2	0
12	0	0	0	0	1	2	0	1	0	1	0	2	0
13	0	0	1	0	0	2	0	1	0	1	0	2	0
14	0	1	1	0	0	1	1	1	0	1	0	2	0
15	0	0	1	0	0	2	0	1	0	1	0	2	0
16	0	0	0	0	0	3	1	1	0	1	0	2	0
17	0	0	0	0	0	2	1	1	0	1	0	2	0
18	0	0	0	0	0	2	1	0	0	1	0	2	0
19	0	0	0	0	0	2	1	0	0	1	0	2	0
20	0	0	1	0	0	2&3	1	0	0	1	0	2	0
21	0	1	1	0	0	1&2	1	1	0	1	0	2	0
22	0	1	1	0	0	2	1	1	1	1	0	2	0
23	0	0	0	0	0	2	1	1	0	1	0	2	0
24	0	0	0	0	0	1	0	0&1	1	0	1	0	1
25	0	0	0	0	0	1	0	1	1	0	1	0	1
26	0	0	0	0	1	1	0	1	1	0	1	0	1
27	0	0	0	0	1	1	0	0	1	0	0	1	1
28	0	0	0	0	0	0	0	1	0	0&1	1	0	0
29	0	0	0	0	0	2	1	1	0	0	1	2	0
30	0	0	0	0	0	2	0	1	0	0	1	0	0
31	0	0	0	0	0	3	0	1	0	0	1	2	0
32	0	0	0	0	0	1	0	1	0	0	1	0	0
33	0	0	1	0	1	4	0	1	0	0	1	0	0
34	0	0	1	0	1	1	0	1	0	0	1	0	0
35	0	1	1	1	1	1	0	0	1	0	1	0	1
36	0	1	0	1	1	1	0	0	1	0&1	0	0	1
37	0	0	0	1	0	1	0	0	0	1	1	0	1
38	0	0	1	1	0	2	0	0	0	1	1	0	1
39	0	0	1	1	0	2	0	0	0	1	1	0	1
40	0	0	1	1	1	1	0	0	1	1	0	0	1
41	0	0	1	0	1	1	1	0	1	0	0&1	0	1
42	0	0	0	0	1	2	0	0	0	0	1	0	1
43	0	0	0	0	1	2	0	0	0	0&1	1	0	1
44	0	0	1	1	0&1	2	0	0	0	0&1	1	0	1

Appendix 7 (continued): Morphological characters for 44 fig wasp species.

	40	41	42	43	44	45	46	47	48	49	50	51	52
1	1	1	1	0	1	1	1	1	0	0	-	-	4
2	1	1	1	1	1	0	0	0	0	0	1	1	1
3	1	1	1	1	1	0	0	0	0	0	1	1	1
4	1	0	0	2	1	0	1	0	1	1	1	1	1
5	1	0	0	1	1	0	1	0	0	1	0	1	1
6	1	0	0	2	0	0	1	0	1	0	1	1	1
7	1	0	0	2	0	0	1	0	1	1	1	2	1
8	1	0	0	0	1	0	1	0	0	1	1	1	1
9	0	0	1	0	1	0	1	0	1	1	1&2	2	1
10	0	0	0	0	1	1	1	0	0	1	1	2	0&1
11	0	0	0	2	0	1	1	0	0	1	2	0	1
12	1	0	0	2	0	0	1	0	0	1	0	0	1
13	0	0	0	1	1	1	1	0	0	1	1	1	1
14	0	0	0	2	0	1	1	0	0	1	2	1	1
15	0	0	0	1	1	1	1	0	0	1	1	1	1
16	1	0	0	1	0	0	1	0	1	1	2	1	1
17	1	0	0	1	1	0	1	0	1	0	2	2	1
18	1	0	0	1	1	0	1	0	1	1	1	0	1
19	1	0	0	1	1	0	1	0	1	0	2	2	1
20	1	0	0	0	1	0	1	0	1	1	2	1	0
21	0	0	0	2	1	0	1	0	1	1	1	2	1
22	0	0	0	2	0	1	1	0	1	1	1	2	1
23	0	0	0	1	1	0	1	0	1	1	1	1	2
24	1	1	1	0	1	0	0	1	1	0	1	2	-
25	1	1	1	0	1	0	0	1	0	0	1	1	1
26	0	1	1	0	1	1	1	1	0	0	2	1	1
27	1	1	1	1	0	1	0	0	1	0	1	0	2
28	1	1	1	1	1	1	0	0	1	1	2	1	1
29	1	1	1	1	1	1	0	0	1	0	2	0	1
30	0	1	1	1	0	0	1	0	1	0	3	1	1
31	0	1	1	1	0	0	1	0	0	0	2	1	1
32	0	1	1	1	1	1	1	0	1	1	3	1	1
33	1	0	1	1	0	0	0	0	0	0	2	1	1
34	1	0	1	1	0	1	0	0	0	0	2	1	1
35	0	1	0	1	1	0	0	0	1	0	1	1	1
36	0	1	0&1	1	0	0	1	0	0	0	0	1	1
37	2	1	1	0	1	0	0	0	1	0	1	1	4
38	2	1	1	1	1	0	0	0	1	0	1	1	4
39	2	1	1	1	1	0	1	0	1	0	1	1	4
40	1	1	1	1	0	1	0	1	1	1	1	2	4
41	1	1	1	1	0	1	0	1	1	0	2	2	4
42	1	1	1	1	1	1	0	0	0	0	0	0	1
43	1	1	1	1	0	1	0	0	0	0	1	0	1
44	1	1	1	1	0	1	0	0	1	0	3	2	1

Appendix 7 (continued): Morphological characters for 44 fig wasp species.

	53	54	55	56	57
1	2	2	-	0	1
2	2	2	1	0	0
3	2	2	1	0	1
4	2	2	1	0	0
5	1	1&2	1	0	1
6	2	2	1	0	0
7	2	2	1	0	1
8	2	2	1	0	1
9	2	2	1	0	1
10	2	2	1	1	0
11	2	2	0	1	0
12	1	0	1	0	0
13	2	2	1	0	0
14	2	2	1	1	0
15	2	2	1	0	0
16	1	2	2	0	1
17	2	2	3	0	1
18	2	2	2	0	1
19	1&2	2	2	0	1
20	2	2	1	0	0
21	2	2	1	1	1
22	2	2	1	1	1
23	2	2	1	0	1
24	1&2	2	0	0	1
25	2	2	0	0	1
26	2	2	0	0	1
27	1	1	0	0	0
28	0	1	1	1	0
29	2	2	1	1	0
30	0	2	0	1	0
31	0	0	1	0	0
32	0	1	1	1	0
33	1	0	1	0	1
34	1	0	1	0	1
35	2	2	0	0&1	0
36	2	2	0	0	0
37	2	2	1	0	0
38	2	2	1	0	1
39	2	2	0	0	0
40	2	2	0	0	0
41	2	2	0	0	0
42	0	2	1	0	0
43	0	2	1	0	1
44	0	2	1	0	1

Appendix 8: Feeding records for herbivorous insects on Ficus in New Guinea. Only herbivores known from two or more feeding records are listed. Taxonomic names are available at www.bishop.hawaii.org/bishop/natsci/ng/ngecol.html. Feeding guilds are abbreviated as follows: larval (LF), adult (AF), lifetime (LT), xylem (XF), phloem (PF) and mesophyll (MF). Host species are numbered as in Table 1 (Chapter 5).

species code	guild	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
FCURC001	LT	0	0	8	11	0	2	0	0	0	12	0	0	0	11	0
FCURC002	LT	0	6	1	14	0	2	0	18	2	0	0	0	0	1	1
FCURC003	LT	0	0	0	5	1	2	0	1	0	0	1	2	0	2	3
FCURC005	LT	2	0	7	28	0	3	0	1	0	6	17	0	0	0	5
FCURC007	LT	0	0	2	4	0	0	0	0	0	0	0	0	0	0	0
FCURC008	LT	0	0	2	0	1	0	0	0	1	2	0	0	0	0	1
FCURC010	LT	1	0	7	1	2	4	0	0	0	0	1	0	0	1	1
FCURC012	LT	3	3	4	19	6	9	0	15	3	11	3	0	0	16	11
FCURC014	LT	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0
FCURC017	LT	0	0	0	0	0	0	0	0	5	6	0	0	0	0	0
FCHRY001	LT	16	22	3	138	35	16	5	6	14	12	21	180	42	63	52
FCHRY002	LT	51	121	341	162	227	44	0	154	27	108	20	8	225	199	99
FCHRY003	LT	18	0	0	7	0	0	0	0	0	0	0	267	0	0	24
FCHRY004	LT	100	1	1	0	0	6	0	1	0	0	16	0	0	0	0
FCHRY006	LT	0	1	12	1	0	0	0	0	0	0	0	0	0	41	23
FCHRY007	LT	0	0	0	12	0	0	0	1	3	0	0	0	0	0	0
FCHRY009	LT	8	1	57	3	6	7	0	4	28	4	11	0	12	0	17
FCHRY012	LT	0	0	2	0	0	0	0	1	1	1	0	0	0	0	0
FCHRY014	LT	4	1	3	2	1	0	0	0	0	0	0	0	0	1	2
FCHRY016	LT	7	5	22	14	9	0	0	4	0	5	0	0	6	42	0
FCHRY017	LT	0	0	1	0	0	0	0	0	0	0	0	0	0	0	2
FCHRY018	LT	0	0	0	0	0	0	0	2	0	0	0	0	0	0	1
FCHRY019	LT	2	3	0	8	5	0	0	2	0	1	0	0	3	0	8
FCHRY020	LT	0	0	6	3	3	0	0	25	8	1	0	0	2	7	31
FCHRY021	LT	0	0	2	8	7	7	0	0	5	1	0	0	5	8	3
FCHRY023	LT	0	1	2	0	1	0	0	1	1	1	0	3	4	1	0
FCHRY024	LT	0	0	0	1	0	0	104	0	0	0	0	1	0	0	0
FCHRY025	LT	0	0	0	4	1	0	141	0	0	1	0	0	0	0	0
FCHRY026	LT	0	1	0	3	0	0	0	0	1	1	0	0	0	0	0
FCHRY027	LT	0	0	0	0	0	0	25	7	0	0	0	48	0	20	2
FCHRY029	LT	0	0	0	0	0	0	0	1	0	0	0	5	0	0	0
FCHRY030	LT	52	42	94	78	76	20	0	75	23	69	15	0	283	12	10
FCHRY031	LT	2	0	2	11	0	1	1	1	2	1	1	0	5	7	6
FCHRY032	LT	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0
FCHRY033	LT	0	0	6	0	1	0	0	0	1	0	1	0	0	0	2

Appendix 8 (continued): Feeding records for herbivorous insects on Ficus.

species code	guild	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
FCHRY037	LT	0	0	15	54	22	17	0	2	1	0	2	1	4	1	2
FCHRY038	LT	0	0	2	0	2	0	0	1	1	0	0	0	0	0	1
FCHRY039	LT	2	0	6	0	4	0	0	2	4	0	0	0	2	0	1
FCHRY040	LT	2	0	1	0	0	0	0	0	2	0	0	0	0	0	0
FCHRY041	LT	0	0	0	16	0	0	0	0	0	0	0	0	0	77	0
FCHRY043	LT	0	0	0	0	0	0	0	21	0	0	0	0	0	0	0
FCHRY044	LT	0	0	0	0	1	0	0	0	0	4	0	0	0	0	1
FCHRY046	LT	1	2	3	1	0	0	0	0	0	0	0	0	1	1	6
FCHRY051	LT	0	0	0	0	0	28	3	0	0	0	0	0	0	0	0
FCHRY052	LT	3	0	5	0	0	0	0	13	0	2	0	0	1	0	0
FCHRY053	LT	0	0	0	0	0	0	0	5	0	0	0	0	1	1	0
FCHRY054	LT	0	0	7	0	7	0	0	0	0	0	0	0	0	1	0
FCHRY055	LT	0	0	0	0	0	8	0	0	0	12	0	0	8	1	0
FCHRY056	LT	0	0	1	12	3	5	0	12	10	1	0	0	17	1	9
FCHRY058	LT	0	1	0	0	0	0	0	0	8	0	0	0	0	0	0
FCHRY061	LT	0	1	0	0	0	0	0	0	0	0	0	2	1	0	0
FCHRY066	LT	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
FCHRY067	LT	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0
FCHRY068	LT	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0
FCERA002	AF	0	0	0	1	1	1	8	0	0	0	2	2	0	0	0
FCERA003	AF	0	1	2	2	2	0	0	0	0	0	0	0	1	1	1
FCERA004	AF	4	2	8	6	2	5	0	2	2	3	0	0	2	2	4
FCERA005	AF	4	3	2	5	6	2	0	3	4	6	2	0	5	4	3
FCERA006	AF	0	0	0	0	0	0	0	0	0	3	0	0	1	0	0
FCERA007	AF	0	1	2	0	0	0	0	0	0	0	0	0	0	1	0
FCERA008	AF	0	0	0	0	0	1	0	0	0	0	0	2	0	1	1
FCERA009	AF	3	5	2	2	4	3	0	2	7	4	0	0	4	11	1
FCERA010	AF	1	0	1	0	0	0	0	0	1	3	0	0	1	0	3
FCERA011	AF	3	4	5	3	6	0	0	1	6	6	3	0	3	3	5
FCERA012	AF	0	0	0	0	0	0	0	0	0	1	0	0	0	0	2
FCERA013	AF	0	1	0	0	0	0	0	0	0	0	0	0	0	2	1
FCERA015	AF	0	0	0	0	0	1	0	1	0	0	0	4	2	0	1
FCERA016	AF	1	0	0	2	0	0	0	0	0	1	0	0	1	1	1
FCERA017	AF	0	0	0	1	0	0	0	1	0	1	0	0	0	1	2
FCERA018	AF	1	1	1	1	2	1	0	2	1	2	0	0	0	4	3
FCERA020	AF	0	1	1	0	3	0	0	0	0	9	0	0	0	1	1
FCERA022	AF	0	0	0	0	1	0	0	1	2	2	2	0	0	2	1
FCERA024	AF	0	1	1	1	2	2	0	0	0	3	0	1	0	1	3
FCERA025	AF	0	0	1	0	3	1	0	0	0	1	0	0	1	1	0
FCERA028	AF	1	0	1	0	0	0	0	2	0	2	0	0	1	1	0
FCERA030	AF	1	0	4	3	3	4	0	1	2	5	2	0	5	5	1
FCERA033	AF	0	0	0	0	0	0	0	0	2	0	0	0	3	1	0
FCERA034	AF	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0

Appendix 8 (continued): Feeding records for herbivorous insects on Ficus.

species code	guild	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
FCERA035	AF	0	0	0	0	0	0	0	0	0	1	2	0	1	0	0
FCERA038	AF	2	0	1	0	0	0	0	1	0	0	0	0	0	0	1
FCERA041	AF	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0
FCERA049	AF	1	0	2	0	2	1	0	0	3	0	0	0	1	3	3
FCERA051	AF	0	0	1	0	0	0	0	2	0	1	0	0	0	0	0
FCERA052	AF	1	0	2	0	0	0	0	0	0	1	0	0	0	0	0
FCERA057	AF	2	4	1	1	2	3	0	1	2	3	1	0	1	0	1
FCERA059	AF	0	0	1	0	0	0	0	0	2	0	0	0	0	0	0
FCERA065	AF	0	0	0	0	0	2	0	0	0	0	0	0	0	2	0
TYP003	MF	2	6	9	6	15	22	0	13	90	11	17	0	7	22	5
TYP004	MF	4	3	1	5	8	4	0	5	5	1	1	0	7	2	6
TYP005	MF	39	14	14	15	2	6	0	0	18	12	4	0	5	0	72
TYP007	MF	0	2	3	0	1	0	0	5	0	0	1	0	0	11	2
TYP010	MF	2	6	5	7	1	0	0	4	4	4	3	0	17	1	0
TYP011	MF	0	2	7	10	0	3	0	8	3	2	2	0	1	0	20
TYP013	MF	0	2	115	0	0	3	0	2	10	0	0	0	0	0	2
TYP014	MF	0	3	1	0	0	0	0	0	3	0	0	0	1	0	0
TYP018	MF	0	0	0	1	0	0	0	0	0	0	2	0	0	0	0
TYP019	MF	0	0	0	0	1	42	0	0	0	2	0	0	0	0	0
TYP020	MF	2	0	0	0	0	0	0	0	0	0	0	0	20	0	2
TYP024	MF	0	0	0	0	18	15	0	0	10	1	0	0	12	1	0
TYP025	MF	2	0	6	0	0	3	0	51	0	0	14	0	50	80	0
TYP027	MF	7	1	58	0	0	4	0	1	1	1	0	0	1	11	6
TYP029	MF	74	42	163	5	0	16	0	15	65	12	0	0	0	2	4
TYP030	MF	1	1	1	0	0	1	0	2	3	0	0	0	0	0	1
TYP034	MF	0	0	0	0	0	0	0	0	0	29	0	0	0	0	0
TYP035	MF	0	4	5	0	0	0	0	0	7	1	2	0	10	0	3
TYP037	MF	3	0	0	0	0	0	0	0	0	0	0	0	0	0	2
TYP041	MF	0	0	0	0	1	13	0	0	8	0	0	0	0	0	0
TYP044	MF	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0
TYP045	MF	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0
TYP047	MF	1	0	4	0	0	0	0	2	0	0	3	0	0	0	0
TYP048	MF	3	0	1	0	0	1	0	0	4	0	0	0	1	0	0
TYP052	MF	0	0	0	0	1	0	0	0	2	8	0	0	6	4	0
TYP053	MF	0	0	1	0	0	0	0	0	0	0	0	0	0	0	2
TYP054	MF	3	0	1	0	0	249	0	9	0	99	6	0	0	0	1
TYP057	MF	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0
TYP059	MF	0	1	1	2	0	0	0	0	0	0	0	0	4	0	0
TYP059	MF	5	1	1	2	16	2	0	1	5	4	0	0	114	14	0
TYP064	MF	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0
TYP067	MF	1	0	0	0	0	1	0	0	0	0	4	0	0	0	0
TYP071	MF	0	2	0	0	0	0	0	11	0	0	0	0	0	88	0
TYP080	MF	0	0	0	0	0	5	0	0	0	0	0	0	0	6	0

Appendix 8 (continued): Feeding records for herbivorous insects on Ficus.

species code	guild	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
TYP081	MF	1	0	1	0	0	0	0	0	2	0	1	0	1	1	0
TYP094	MF	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0
TYP115	MF	1	0	0	8	0	0	0	0	2	2	0	0	0	1	3
TYP116	MF	1	0	0	5	0	0	0	0	0	0	0	0	1	0	0
TYP117	MF	1	0	4	1	1	0	0	0	0	1	0	0	2	0	10
TYP118	MF	2	3	1	8	1	0	0	0	10	0	2	0	1	0	0
TYP119	MF	6	5	3	40	5	7	0	0	19	5	1	0	1	1	125
TYP120	MF	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
TYP122	MF	0	14	0	0	0	0	0	0	0	0	0	0	0	0	0
TYP125	MF	0	0	11	0	0	0	0	0	7	0	0	0	0	0	0
TRO001	PF	11	12	25	128	48	27	0	3	15	18	94	0	7	18	32
TRO004	PF	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0
TRO005	PF	0	0	1	0	0	0	0	0	0	1	0	0	0	0	7
TRO007	PF	0	0	0	0	0	0	1	0	0	0	0	2	1	0	1
RIC001	PF	5	1	1	10	11	1	2	0	1	1	3	0	0	4	23
RIC002	PF	80	24	67	112	91	68	12	40	88	37	109	6	48	22	44
RIC003	PF	78	50	109	269	215	67	0	52	92	101	200	1	71	27	78
RIC004	PF	62	60	93	259	183	65	5	55	61	94	234	29	44	41	103
RIC005	PF	5	10	15	33	42	16	0	19	8	12	25	0	21	3	30
RIC006	PF	12	16	25	35	47	14	3	50	8	9	38	0	21	13	34
RIC007	PF	0	0	4	3	0	0	0	0	2	0	0	0	1	0	0
RIC008	PF	0	0	0	12	0	0	3	4	0	1	2	3	2	0	1
RIC010	PF	11	1	4	1	5	1	1	5	1	1	6	0	1	2	6
RIC012	PF	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0
RIC013	PF	0	1	0	1	1	0	0	1	0	2	1	12	0	0	0
RIC017	PF	4	0	0	4	6	0	0	1	1	0	0	0	3	0	0
RIC020	PF	0	0	0	0	0	0	0	0	0	0	1	8	0	0	0
RIC024	PF	0	0	0	0	0	0	0	0	0	0	0	8	0	0	0
NOG001	PF	11	6	10	15	6	4	5	5	6	10	1	12	4	5	14
NOG002	PF	1	3	0	2	4	1	0	0	7	1	2	0	0	1	4
NOG003	PF	0	6	2	4	6	1	1	1	2	1	0	1	1	2	2
MEM001	PF	2	4	8	9	7	1	0	2	5	2	7	0	8	1	14
MEM002	PF	35	15	24	29	24	11	2	15	38	19	54	9	11	20	37
MEM006	PF	1	0	2	1	0	2	0	0	0	0	1	0	0	1	0
MEM008	PF	0	1	1	0	0	0	0	0	0	0	2	0	0	2	0
MEM013	PF	0	0	12	1	0	0	0	0	0	0	0	0	0	0	0
MEM014	PF	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0
MEE001	PF	25	12	27	24	17	21	3	20	25	19	19	2	21	45	18
MEE002	PF	1	1	0	1	9	0	0	0	0	0	2	1	0	3	0
MEE003	PF	0	1	3	0	3	0	0	0	1	0	1	0	0	1	3
MEE006	PF	1	1	3	1	1	1	0	0	4	1	0	0	2	0	1
MEE007	PF	18	7	17	16	16	19	8	11	16	12	39	5	18	7	19
MEE008	PF	109	12	191	85	168	87	1	54	63	63	102	0	74	28	85

Appendix 8 (continued): Feeding records for herbivorous insects on Ficus.

species code	guild	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
MEE009	PF	0	0	2	3	1	0	0	3	2	0	2	0	8	2	2
MEE010	PF	0	0	0	0	0	0	0	0	2	0	1	0	1	0	1
MEE011	PF	0	1	1	0	2	0	0	0	3	1	0	0	1	0	1
MEE013	PF	1	11	4	3	1	2	0	0	18	0	0	0	2	0	1
MEE014	PF	0	6	0	1	0	0	0	0	0	0	0	0	0	0	0
MEE016	PF	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0
MEE017	PF	0	1	0	0	0	0	0	1	2	0	0	0	0	0	0
LOP001	PF	113	123	135	114	153	25	3	44	85	15	95	1	87	34	21
LOP002	PF	54	26	27	24	61	26	29	16	39	6	64	9	20	7	22
LOP003	PF	5	5	19	6	5	3	0	2	4	4	3	0	23	3	1
LOP004	PF	3	0	5	10	7	8	0	2	1	0	2	0	6	0	0
ISI002	PF	207	302	192	222	330	173	0	132	258	104	284	1	144	146	262
ISI004	PF	10	16	6	3	13	5	0	7	4	5	6	1	4	5	9
ISI007	PF	0	0	2	0	0	1	0	0	2	1	0	0	0	0	0
ISI009	PF	1	1	1	1	3	1	0	0	0	0	0	1	0	0	3
ISI011	PF	1	0	0	0	2	1	0	1	0	1	0	0	0	0	0
ISI014	PF	0	0	0	0	1	0	0	0	0	0	0	0	0	0	2
ISI018	PF	1	2	3	4	1	1	2	1	5	1	1	0	0	0	2
FUL001	PF	17	9	11	8	14	13	0	0	15	14	13	1	15	8	17
FUL002	PF	3	1	6	3	11	10	0	3	8	1	2	0	6	0	4
FUL004	PF	0	0	1	0	1	1	0	0	1	0	2	0	0	0	0
FLA001	PF	37	31	37	64	82	28	205	9	171	58	35	125	61	3	23
FLA002	PF	0	0	0	1	1	0	7	2	0	0	0	10	1	0	1
FLA003	PF	61	36	23	51	31	123	1	12	89	32	59	8	83	6	26
FLA004	PF	2	5	2	2	1	3	0	2	2	4	0	0	3	1	3
FLA005	PF	54	38	45	108	64	40	100	29	94	46	126	52	38	24	64
FLA007	PF	50	60	111	40	102	131	0	35	88	84	40	0	24	16	65
FLA008	PF	77	13	54	43	95	87	1	96	62	47	110	11	34	15	53
FLA009	PF	24	26	30	49	42	21	0	26	53	30	23	1	45	9	18
FLA010	PF	4	3	3	5	7	5	64	7	6	0	3	65	28	0	3
FLA012	PF	26	26	28	23	33	21	1	2	39	35	35	2	20	14	18
FLA013	PF	2	4	2	14	2	11	12	8	8	1	8	31	17	5	7
FLA014	PF	5	8	9	1	4	5	0	1	15	2	3	0	4	3	4
FLA018	PF	0	6	2	8	1	9	6	1	3	5	2	3	6	4	7
FLA021	PF	70	3	5	70	193	61	0	24	91	2	98	0	15	0	8
FLA025	PF	0	0	1	1	0	0	1	0	2	0	0	0	1	0	0
FLA028	PF	0	0	0	0	0	0	0	0	0	0	0	0	3	0	2
FLA030	PF	11	7	1	1	7	1	0	3	19	10	0	0	2	0	1
FLA033	PF	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0
DIC001	PF	8	16	16	7	28	22	0	3	34	0	5	0	4	5	1
DIC002	PF	9	0	1	2	9	2	0	3	2	3	1	0	0	3	2
DER001	PF	2	0	8	0	0	0	0	1	6	0	0	0	0	1	2
DER002	PF	2	5	6	0	1	0	0	1	9	1	0	1	2	2	7

Appendix 8 (continued): Feeding records for herbivorous insects on Ficus.

species code	guild	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
DER003	PF	91	33	127	45	58	72	0	17	155	15	22	1	72	18	104
DER005	PF	0	0	7	2	0	0	0	0	4	5	2	0	0	0	0
DER007	PF	0	0	0	0	0	0	0	0	3	0	0	0	1	0	1
DER008	PF	0	2	3	1	0	1	0	0	5	0	0	0	0	4	11
DER009	PF	4	0	1	1	0	0	0	0	4	1	0	0	0	0	1
DER010	PF	1	1	1	1	0	1	0	0	3	0	0	3	1	1	1
DER011	PF	0	0	2	2	0	37	0	19	0	66	22	4	2	2	0
DER012	PF	2	6	2	25	13	9	0	30	5	10	8	0	1	28	6
DER013	PF	98	35	32	81	64	48	0	58	48	42	69	0	87	51	60
DER014	PF	1	10	3	1	0	0	0	0	8	1	0	0	1	0	0
DER015	PF	0	0	3	1	0	1	1	0	2	0	1	1	0	1	0
DER016	PF	2	2	3	1	0	1	0	0	6	2	0	1	2	1	1
DER018	PF	0	0	0	0	0	0	2	2	0	0	0	0	0	0	0
DER019	PF	7	7	18	3	0	0	0	0	60	1	2	0	6	3	14
DER020	PF	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0
DER021	PF	1	0	9	1	0	12	0	4	1	0	0	0	4	0	2
DER022	PF	6	7	13	8	5	4	1	4	18	5	1	1	3	2	15
DER023	PF	0	0	6	2	1	0	0	0	1	1	0	0	0	0	0
DER024	PF	2	2	6	2	6	3	2	1	3	1	2	13	1	2	11
DER026	PF	0	0	0	0	0	0	0	0	0	0	0	0	2	1	1
DER027	PF	6	2	0	0	1	1	0	0	4	0	0	0	0	0	1
DER028	PF	7	12	4	2	1	1	0	0	4	1	1	0	3	0	5
DER029	PF	13	12	12	7	2	2	0	2	16	3	1	0	4	2	9
DER031	PF	0	1	0	0	0	0	0	0	7	0	0	0	0	0	0
DER032	PF	3	13	2	13	21	0	0	4	4	0	9	1	7	6	2
DER033	PF	8	7	4	3	2	1	0	1	5	3	2	0	2	0	5
DER034	PF	1	11	2	3	5	3	1	0	9	3	1	1	2	3	1
DER035	PF	0	0	0	0	0	0	0	0	2	0	0	0	0	0	1
DER037	PF	1	0	3	1	2	0	0	0	5	0	1	0	0	0	2
DER038	PF	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0
DER039	PF	1	0	3	1	0	0	0	0	2	0	0	0	0	0	0
DER040	PF	3	3	7	4	2	2	1	0	5	1	1	0	2	0	2
DER041	PF	1	0	5	1	0	0	0	0	1	1	0	0	0	0	0
DER043	PF	0	2	1	2	4	1	1	4	2	1	1	0	3	5	3
DER045	PF	3	4	1	7	2	2	0	0	3	0	1	0	2	2	4
DER049	PF	1	1	1	1	2	1	0	0	5	0	1	0	2	1	2
DER050	PF	1	1	1	0	0	2	0	0	0	0	1	0	4	4	0
DER053	PF	1	1	1	1	2	0	1	0	2	0	1	0	0	0	1
DER055	PF	0	0	1	0	4	0	0	0	3	0	1	0	0	0	2
DER060	PF	0	0	0	2	0	0	12	0	1	0	1	120	0	0	0
DER062	PF	1	1	0	0	0	0	0	0	3	0	0	0	0	0	2
DER063	PF	1	1	1	0	2	2	0	2	2	0	2	2	1	2	2
DER067	PF	1	0	5	0	1	0	0	0	0	1	0	0	0	0	4

Appendix 8 (continued): Feeding records for herbivorous insects on Ficus.

species code	guild	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
DER071	PF	1	1	2	0	3	0	0	0	2	1	0	0	1	0	1
DER074	PF	0	5	1	0	0	0	0	0	0	0	0	0	1	0	0
DER075	PF	0	1	0	1	2	0	0	0	1	0	0	0	1	1	1
DER078	PF	0	1	1	1	1	0	0	2	4	0	0	0	0	1	3
DER080	PF	0	0	0	0	1	0	1	8	1	0	1	1	0	2	0
DER082	PF	1	1	0	0	5	2	0	0	2	0	0	0	0	0	0
DER090	PF	0	0	0	0	3	0	0	1	0	0	1	0	1	0	0
DER096	PF	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0
DER101	PF	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
DER103	PF	0	0	0	0	0	1	0	3	0	1	0	0	0	0	0
DER105	PF	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0
DER107	PF	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0
DER112	PF	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0
DEL001	PF	20	12	25	8	11	4	0	5	27	14	14	0	46	6	7
DEL003	PF	0	50	51	0	2	8	0	1	23	10	1	0	20	10	1
DEL004	PF	10	30	2	2	30	11	0	2	6	15	1	0	10	7	1
DEL006	PF	0	0	0	1	1	2	0	8	1	0	1	0	0	0	1
DEL011	PF	2	0	0	0	0	1	0	2	1	1	0	0	0	1	0
DEL012	PF	5	0	2	0	0	2	0	0	1	2	6	0	2	0	1
CIX001	PF	3	2	2	4	1	0	0	1	4	1	0	0	5	1	6
CIX004	PF	1	1	1	3	0	0	0	0	1	1	2	0	3	0	0
CIX005	PF	2	0	1	1	1	0	0	0	0	1	0	0	0	0	2
CIX007	PF	1	0	2	1	2	0	0	0	4	0	0	0	0	0	0
CIX013	PF	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0
CIX015	PF	0	0	0	0	0	2	0	0	0	0	0	2	0	0	1
CCD001	XF	3	1	1	1	2	0	1	1	1	0	2	4	1	0	0
CCD002	XF	13	2	10	3	7	7	0	2	8	3	3	2	8	2	0
CCD005	XF	0	1	0	0	1	0	1	0	0	0	0	1	2	0	0
CCD008	XF	0	0	0	0	1	0	2	0	1	0	0	3	0	0	0
CIC001	PF	10	11	13	16	18	12	1	21	21	11	13	0	10	16	7
CIC002	XF	80	155	335	146	113	113	0	160	199	36	97	2	131	207	230
CIC003	PF	2	3	10	4	2	8	0	1	12	3	2	0	2	0	4
CIC004	PF	30	53	50	64	36	47	0	46	58	26	138	0	34	39	85
CIC005	PF	1	0	1	0	0	0	0	0	1	1	0	0	0	1	2
CIC011	PF	0	0	3	0	0	0	0	2	0	0	1	0	1	2	1
CIC013	XF	38	10	35	41	43	29	2	61	13	30	25	300	47	68	13
CIC014	XF	1	2	2	2	5	0	2	2	0	2	3	0	1	3	2
CIC016	PF	0	1	1	0	1	1	0	1	4	0	0	0	1	1	1
CIC019	XF	6	16	2	9	17	32	0	10	10	16	4	0	1	20	9
CIC020	PF	1	2	1	1	3	3	71	2	2	0	18	206	2	1	6
CIC021	PF	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0
CIC023	PF	11	8	15	10	6	8	0	19	24	3	10	0	8	3	2
CIC024	PF	1	1	1	7	9	0	9	3	1	1	4	29	0	2	7

Appendix 8 (continued): Feeding records for herbivorous insects on Ficus.

species code	guild	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
CIC025	PF	2	1	0	0	4	0	0	2	3	0	0	0	0	0	0
CIC026	PF	11	5	22	13	8	2	0	3	11	7	5	1	8	9	4
CIC027	PF	1	1	1	3	1	1	0	5	3	1	1	0	5	1	4
CIC028	XF	1	0	2	5	2	2	0	1	1	0	3	0	2	1	1
CIC030	PF	0	5	2	3	1	3	0	4	6	1	1	0	0	0	7
CIC033	PF	4	0	5	0	2	1	0	2	2	1	2	0	1	0	5
CIC035	PF	19	10	6	25	26	17	0	1	4	4	15	0	7	2	12
CIC038	PF	0	0	2	1	1	1	0	0	1	1	1	0	0	2	0
CIC039	PF	2	1	1	0	0	0	0	0	0	1	0	0	2	1	0
CIC040	PF	3	1	2	3	1	3	0	2	7	2	1	0	2	0	0
CIC042	PF	1	9	0	7	3	0	0	0	1	1	3	0	0	1	2
CIC043	PF	0	0	1	3	1	0	0	1	0	0	0	0	0	2	1
CIC045	PF	0	0	0	1	3	0	0	0	0	1	1	0	0	0	1
CIC048	XF	0	0	0	0	0	0	8	0	0	0	0	3	0	0	0
CIC051	PF	4	0	0	0	0	0	0	0	0	0	0	0	0	0	1
CIC052	PF	0	2	0	1	1	5	1	0	0	2	0	14	1	0	0
CIC053	PF	0	1	1	0	1	1	0	0	1	1	0	0	0	0	2
CIC059	PF	0	0	0	0	0	3	0	0	0	0	9	0	0	2	0
CIC076	PF	0	0	26	8	27	0	0	0	0	26	0	0	0	0	0
CIC081	PF	0	2	1	0	2	0	0	0	0	1	0	0	0	0	0
CIC099	XF	16	73	48	29	36	35	0	21	39	20	25	0	64	43	71
CIC100	XF	7	9	1	21	2	4	0	7	9	4	3	0	2	2	5
CIC101	XF	1	2	4	4	2	15	0	10	9	19	2	0	29	11	6
CER001	XF	3	5	7	2	6	2	0	4	35	5	25	0	9	3	2
CER002	XF	1	2	2	1	11	2	0	5	4	3	3	0	1	0	0
CER004	XF	9	8	1	2	7	9	0	4	9	6	6	0	1	2	2
CER005	XF	1	0	0	1	0	2	0	0	3	2	0	0	2	0	0
CER006	XF	1	2	2	2	2	1	0	0	1	2	0	0	1	1	0
CER007	XF	0	2	0	0	3	0	0	0	1	0	0	0	4	0	0
APH001	XF	363	586	419	280	465	358	34	685	377	261	379	47	401	640	288
APH002	XF	20	44	64	33	35	37	0	55	34	17	49	0	36	48	48
APH003	XF	25	103	22	18	22	18	0	12	15	11	24	0	17	24	5
APH004	XF	8	71	27	9	31	8	0	28	62	11	21	0	10	21	26
APH005	XF	29	44	28	38	29	19	0	42	32	28	32	0	38	40	30
APH006	XF	20	7	10	4	23	11	0	11	8	63	23	0	10	11	2
APH007	XF	12	54	68	19	31	11	0	45	25	17	66	0	13	70	9
APH008	XF	0	1	1	0	1	2	0	3	1	1	1	0	1	1	0
APH009	XF	1	4	4	3	18	3	6	12	6	7	10	0	4	14	3
APH010	XF	4	3	7	1	4	2	14	4	0	1	6	63	3	8	9
APH011	XF	0	0	0	1	0	1	1	1	1	0	0	2	2	1	2
APH012	XF	13	11	6	9	13	1	0	8	12	11	13	0	1	10	11
APH013	XF	1	2	0	2	3	3	36	4	0	2	3	43	1	0	0
APH014	XF	0	2	0	0	1	0	0	0	0	0	0	26	0	1	4

Appendix 8 (continued): Feeding records for herbivorous insects on Ficus.

species code	guild	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
APH015	XF	0	0	1	0	0	0	135	0	0	0	12	277	0	0	0
FTORT008	LF	1	2	1	0	0	0	0	3	2	0	0	0	0	4	5
FTORT022	LF	0	17	7	7	7	3	0	8	5	0	2	0	0	11	5
FTORT034	LF	0	1	0	0	0	0	0	2	1	0	0	0	1	9	2
FTORT038	LF	0	2	0	0	0	0	0	1	0	0	0	0	0	0	0
FTORT040	LF	0	1	0	3	1	2	0	3	2	1	1	0	0	4	1
FTORT044	LF	0	0	0	0	3	0	0	2	3	0	0	0	1	0	0
FTORT048	LF	0	0	0	0	0	1	0	4	0	2	0	0	0	0	0
FTORT049	LF	0	1	0	1	2	0	0	1	1	0	0	0	0	0	1
FTORT055	LF	0	0	3	0	1	0	0	0	0	0	0	0	0	0	0
FSPHI002	LF	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0
FTORT041	LF	1	0	2	0	0	0	0	0	6	0	1	0	7	0	6
FPSYC001	LF	2	1	0	0	1	0	0	0	0	0	3	0	0	1	0
FXXXX006	LF	2	0	1	0	4	3	3	5	4	0	0	1	0	4	2
FNYMP001	LF	3	1	5	11	1	1	0	0	4	0	0	3	1	0	78
FNYMP002	LF	4	3	5	5	3	0	7	0	10	0	15	31	8	16	49
FNYMP004	LF	0	0	0	0	4	8	0	1	0	0	0	0	1	3	0
FNOCT002	LF	0	30	5	0	3	124	0	0	0	10	68	3	0	12	2
FNOCT003	LF	0	0	0	0	0	0	14	0	0	0	0	9	0	0	1
FNOCT004	LF	0	2	0	0	0	20	0	0	0	0	0	3	0	0	0
FNOCT005	LF	0	1	0	0	0	3	0	0	0	2	0	0	0	0	0
FNOCT009	LF	0	0	0	0	0	0	0	8	0	0	0	0	0	0	0
FNOCT010	LF	0	1	0	0	0	9	0	0	0	0	0	0	0	0	0
FNOCT013	LF	0	0	0	1	0	0	2	0	0	0	0	0	0	0	0
FNOCT014	LF	0	2	0	0	0	0	0	0	0	3	0	1	0	0	0
FLYMA001	LF	0	0	0	0	0	0	0	2	0	2	0	0	0	2	1
FLYMA002	LF	0	0	0	2	1	0	0	0	6	0	0	0	0	0	9
FLYMA003	LF	1	0	0	1	0	0	1	1	1	0	0	0	3	2	5
FLYMA004	LF	0	0	0	0	0	0	3	1	0	0	1	0	0	1	0
FLYMA009	LF	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0
FLYMA011	LF	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0
FLYCA001	LF	1	0	0	0	0	35	0	0	0	5	0	5	7	0	0
FLIMA001	LF	10	0	0	0	0	0	0	0	1	0	0	0	0	0	0
FLIMA004	LF	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
FXXXX003	LF	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0
FXXXX009	LF	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0
FXXXX011	LF	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0
FXXXX012	LF	0	0	0	0	1	0	0	3	0	0	0	0	0	0	0
FTORT023	LF	0	0	0	1	0	0	0	2	0	0	0	0	0	0	0
FCRAM002	LF	10	3	13	239	6	0	12	6	6	4	8	58	121	4	60
FCRAM003	LF	14	31	44	12	0	0	0	36	20	3	54	0	8	91	13
FCRAM005	LF	18	0	26	7	8	1	24	8	72	18	5	599	53	16	7
FCRAM006	LF	6	7	5	49	30	34	0	22	22	32	4	0	16	50	52

Appendix 8 (continued): Feeding records for herbivorous insects on Ficus.

species code	guild	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
FCRAM008	LF	0	0	0	0	0	0	2	5	0	1	0	0	0	61	3
FCRAM009	LF	2	1	14	19	3	0	0	1	0	0	0	0	4	0	16
FCRAM010	LF	0	0	1	0	0	3	57	2	0	0	0	0	1	0	0
FCRAM011	LF	0	3	0	1	1	0	0	2	1	1	0	0	0	0	0
FCRAM012	LF	37	17	39	3	13	4	0	47	14	5	14	0	21	32	28
FCRAM013	LF	0	0	0	1	1	0	0	3	0	0	0	0	0	1	0
FCRAM014	LF	0	0	1	0	0	0	0	4	0	0	2	0	4	0	1
FCRAM015	LF	0	0	0	0	0	0	0	2	0	0	0	0	0	14	0
FCRAM016	LF	0	0	0	0	0	0	34	0	0	0	0	1	0	0	0
FCRAM017	LF	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0
FCRAM018	LF	0	0	0	0	0	0	0	1	0	2	6	0	0	0	0
FCRAM024	LF	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0
FCRAM028	LF	0	0	0	1	0	3	0	0	0	0	0	0	0	0	0
FCRAM030	LF	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0
FCRAM034	LF	0	0	0	0	0	0	0	1	0	0	2	0	0	0	0
FGEOM001	LF	0	3	5	0	0	3	0	3	0	0	0	0	0	1	0
FCHOR001	LF	2	0	11	13	1	0	0	0	2	0	0	0	0	0	2
FCHOR002	LF	20	7	6	0	62	19	0	3	0	60	0	0	0	22	1
FCHOR003	LF	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0
FTORT003	LF	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0
FTORT005	LF	171	114	585	78	87	51	0	46	339	82	47	0	104	102	28
FTORT006	LF	8	25	10	107	3	3	0	13	49	1	3	0	7	0	94
FTORT007	LF	0	0	2	0	0	0	0	0	2	0	0	0	0	0	0
FTORT009	LF	0	7	0	0	0	0	0	0	1	0	12	0	0	0	0
FTORT012	LF	2	14	9	0	4	4	0	1	1	1	55	0	0	0	1
FTORT013	LF	0	0	0	1	0	0	0	0	2	0	0	2	0	0	12
FTORT015	LF	0	2	0	0	0	0	0	34	1	1	0	0	0	65	1
FTORT016	LF	0	0	0	0	0	0	15	0	0	0	0	1	0	0	0
FTORT018	LF	0	0	0	0	0	0	50	0	0	0	0	1	0	0	0
FTORT021	LF	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
FTORT031	LF	0	0	1	0	0	0	0	0	0	0	0	0	14	0	0
FTORT039	LF	0	0	0	0	0	0	0	2	0	0	0	0	0	1	0
FTORT042	LF	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0
FTORT056	LF	0	0	0	0	2	5	0	0	3	0	0	0	0	0	0
FSPHI001	LF	0	0	0	0	0	0	0	0	0	0	0	0	1	3	1
FIMMA001	LF	0	0	0	0	0	0	0	0	2	0	1	0	0	0	0
FDREP001	LF	0	7	0	0	0	0	0	0	0	0	0	0	0	0	0
FCRAM020	LF	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0
FCRAM022	LF	0	0	0	0	1	0	0	0	3	0	0	0	0	0	0
FTORT036	LF	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0
FTETT001	LT	0	0	1	0	0	0	0	0	0	0	0	1	1	0	2
FTETT002	LT	0	1	2	0	0	0	2	0	1	1	0	0	0	1	2
FTETT004	LT	1	0	8	3	1	0	1	3	5	5	0	1	2	2	5

Appendix 8 (continued): Feeding records for herbivorous insects on Ficus.

species code	guild	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
FTETT006	LT	0	0	2	0	0	0	0	0	0	0	0	0	0	2	1
FTETT007	LT	0	1	0	0	0	0	0	1	5	0	0	0	0	0	1
FTETT011	LT	0	0	0	0	0	0	0	0	0	0	0	5	0	1	1
FTETT012	LT	0	1	2	3	0	0	0	1	2	4	1	0	2	0	0
FTETT018	LT	1	0	0	0	0	0	0	0	0	0	0	0	0	2	1
FTETT019	LT	0	1	1	1	0	0	0	0	0	2	1	0	0	1	0
FTETT020	LT	0	1	1	0	2	0	1	0	0	0	0	1	0	0	0
FTETT022	LT	1	1	2	4	0	0	2	1	1	1	2	2	1	0	0
FTETT023	LT	0	0	0	0	0	0	1	0	0	0	0	0	0	0	3
FTETT027	LT	1	0	0	1	0	0	1	0	1	0	0	0	0	0	2
FACRI001	LT	13	14	8	17	18	0	12	15	10	19	11	4	10	9	11
FACRI002	LT	0	9	3	7	4	0	1	5	4	2	11	3	10	2	3
FACRI011	LT	1	0	2	0	0	0	1	1	3	2	0	0	6	1	3
FACRI020	LT	1	0	0	1	0	1	7	2	1	2	1	0	9	1	2
FACRI021	LT	2	0	1	0	2	0	0	1	0	1	0	0	0	0	0
FACRI005	LT	1	0	0	1	2	3	1	0	1	1	0	0	0	0	1
FACRI004	LT	1	1	0	5	1	0	1	2	0	3	0	0	2	4	0
FACRI013	LT	0	5	1	6	2	0	2	1	0	0	1	0	0	3	2
FACRI014	LT	1	0	0	0	0	1	0	0	0	0	0	0	2	0	0
FACRI015	LT	1	3	0	1	1	0	0	1	1	1	0	0	2	8	1
FACRI016	LT	0	2	0	0	0	0	0	0	0	0	0	0	3	2	0
FACRI017	LT	1	0	1	1	1	0	1	0	2	1	1	0	1	0	3
FACRI018	LT	2	1	0	2	0	0	3	1	0	3	0	0	0	0	1
FPHAS001	LT	0	0	1	1	0	0	0	1	0	0	0	0	0	1	2
FPHAS002	LT	5	0	5	3	3	0	3	5	1	2	0	0	5	3	6
FPHAS004	LT	0	0	0	2	0	0	0	2	0	3	0	0	0	0	0
FPHAS007	LT	1	1	0	1	0	0	1	0	1	4	1	0	0	1	0
FPHAS013	LT	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0