

# Cophylogeny of the *Ficus* microcosm

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## ABSTRACT

The various mutualistic and antagonistic symbioses between fig trees (*Ficus*: Moraceae) and chalcid wasps comprise a community in microcosm. Phylogenetic estimates of figs and fig wasps show general topological correspondence, making the microcosm a model system for cophylogeny. Incongruence between phylogenies from associated organisms can be reconciled through a combination of evolutionary events. Cophylogeny mapping reconciles phylogenies by embedding an associate tree into a host tree, finding the optimal combinations of events capable of explaining incongruence and evaluating the level of codivergence. This review addresses the results of cophylogeny analysis concerning *Ficus* and discusses the plausibility of different evolutionary events. Five different associations encompassing fig-pollinator, fig-parasite and pollinator-parasitoid interactions are reconciled. The method improves on previous comparisons by employing ‘jungles’ to provide an exhaustive and quantitative analysis of cophylogeny. A jungle is a mechanism for inferring host switches and obtaining all potentially optimal solutions to the reconciliation problem. The results support the consensus that figs codiverge significantly with pollinators but not non-pollinators. However, pollinators still appear to have switched between hosts in contradiction to the traditional model of faithful codivergence. This emphasises the growing realisation that evolutionary transitions in the microcosm are more flexible than previously thought and host specificity is necessary but not sufficient for codivergence. The importance of sampling strategy is emphasised by the influence of taxon set on the fig-pollinator and fig-parasite jungles. Spurious significant results for fig-parasite and fig-parasitoid jungles indicate that the choice of congruence measure influences significance; the total number of events required to reconcile two trees (‘total cost’) is not a good measure of congruence when switches cannot be realistically weighted.

*Key words:* *Ficus*, Agaonidae, plant–insect interactions, cophylogeny mapping, reconciliation, codivergence, jungles.

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## I. INTRODUCTION

### (1) The *Ficus* microcosm

The invertebrate community surrounding *Ficus* (Moraceae) is among the best examples of the genetic and ecological manifestation of coevolution. There are around 800 species of this pan-tropical genus of hemi-epiphytic and free-standing trees, interacting with both invertebrate mutualists and antagonists that collectively form a community in microcosm (Berg, 1989; Kerdelhué, Rossi & Rasplus, 2000). The unique role of *Ficus* within tropical communities derives from an enclosed reproductive structure – the syconium, or ‘fig’ – a fruit-like receptacle, within which the florets are arranged around a hollow lumen. *Ficus* is pollinated by Agaonid wasps (Chalcidoidea: Hymenoptera), which have evolved within a context of progressive internalisation of the flowers (Murray, 1985). This has promoted an exquisite array of coadaptations (Herre, 1989); not least for gaining entry to the syconium, which is achieved through a bract-lined pore – the ostiole – in the apex of the fig. Such is the level of coadaptation between the wasp, dependent upon the tree for oviposition sites, and host tree, dependent upon the wasp for the delivery of pollen, that they comprise a highly specialised and obligate mutualism (Herre *et al.*, 1999), described in Fig. 1. There is traditionally thought to be a one-to-one specificity between fig tree and pollinator (Ramirez, 1970; Wiebes, 1979; Bronstein, 1987), although exceptions continue to appear (Michaloud, Carriere & Kobbi, 1996; Kerdelhué, Hochberg & Rasplus, 1997; Molbo *et al.*, 2003). Figs are also hosts to a suite of non-pollinating chalcid wasps, fulfilling a variety of niches parasitising the fig (i.e. competing for oviposition sites without pollinating, e.g., *Apocryptophagus*) or parasitising the pollinator larvae (Bronstein, 1991; Boucek, 1993), which may be the strategy of *Sycoscapter*.

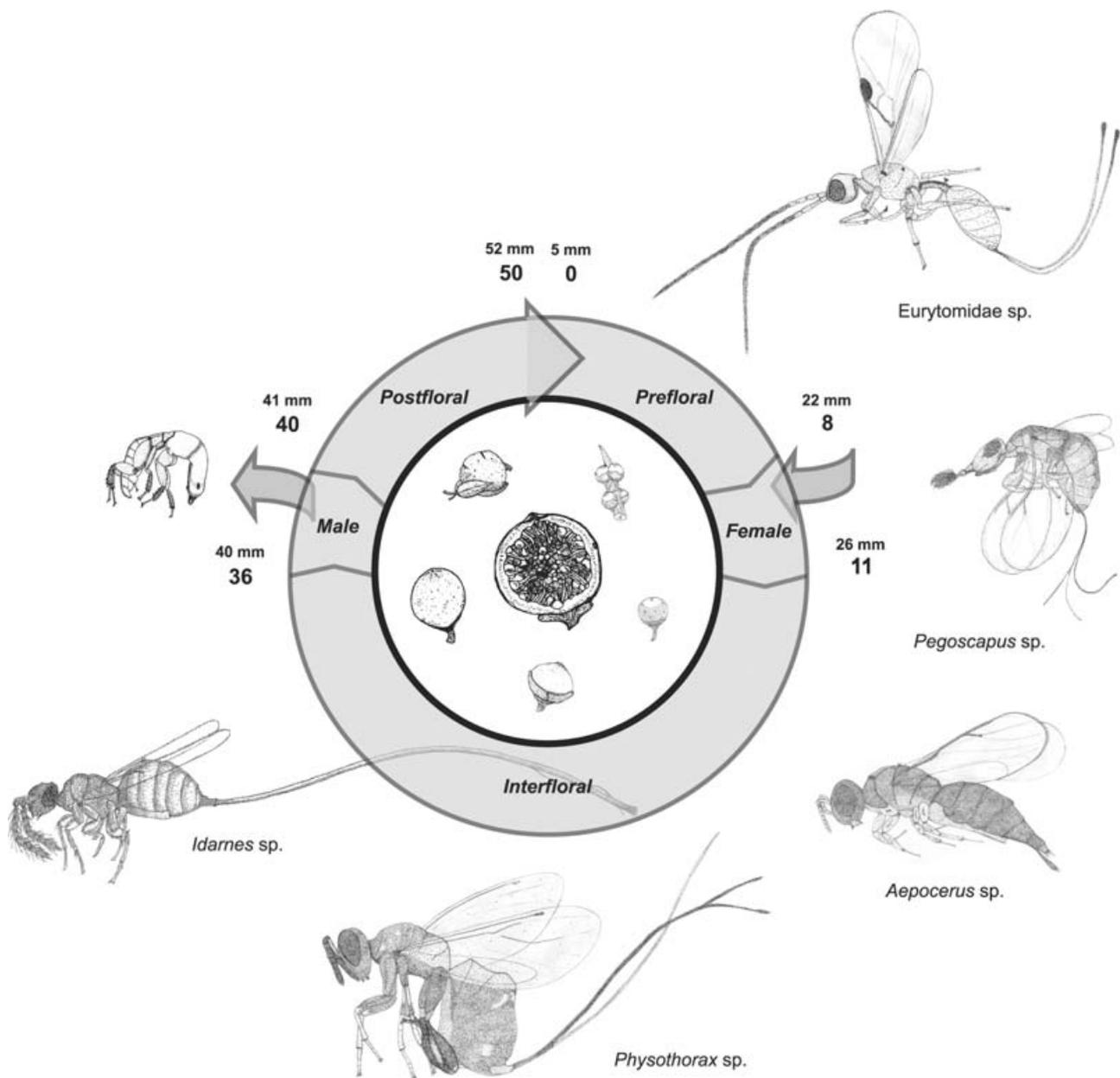
### (2) Coevolution and cophylogeny

The remarkable microcosm within *Ficus* invites hypotheses of cospeciation to support its morphological and ecological complementation (Ramirez, 1970; Janzen, 1979; Wiebes, 1979). Phylogenetic congruence was anticipated as morphological and, later, molecular phylogenies of *Ficus* and its associates became available. Taxonomic studies have

stressed that related figs are pollinated by related wasps (Berg, 1989; Wiebes, 1989; Berg & Wiebes, 1992). For example, pollinator genera *Kradibia*, *Blastophaga* and *Dolichoris* are restricted to *Ficus* sections *Sycidium*, *Ficus* and *Oreosyceae*. However, a substantial list of exceptions and anomalies also exist. Furthermore, subsequent to the seminal *Ficus* classification by Corner (1965), there was always the possibility that references to wasp taxonomy, and the implicit hypothesis of cospeciation, might have introduced circularity and so produced artefactual correspondence. The spectre of taxonomic artefact was largely exorcised by global molecular phylogenies of *Ficus* and the Agaonidae, which resolved many of the anomalies of morphological phylogenies and indicated correspondence of fig and pollinator clades (Weiblen, 2000, 2001). Taxonomic and early phylogenetic studies made qualitative comparisons of fig phylogenies with pollinators (Herre *et al.*, 1996; Weiblen, 2000, 2001) and parasitoids (Ulenberg, 1985; Machado *et al.*, 1996). These supported the prevailing view that the microcosm had developed through a long period of reciprocal evolutionary changes and codivergence (Janzen, 1979; Wiebes, 1979). Recent studies have begun quantitative descriptions of these changes, with parsimony and likelihood-based methods for statistically assessing whether the trees are significantly congruent or identical (Lopez-Vaamonde *et al.*, 2001; Weiblen & Bush, 2002).

### (3) Phylogenetic reconciliation through cophylogeny mapping

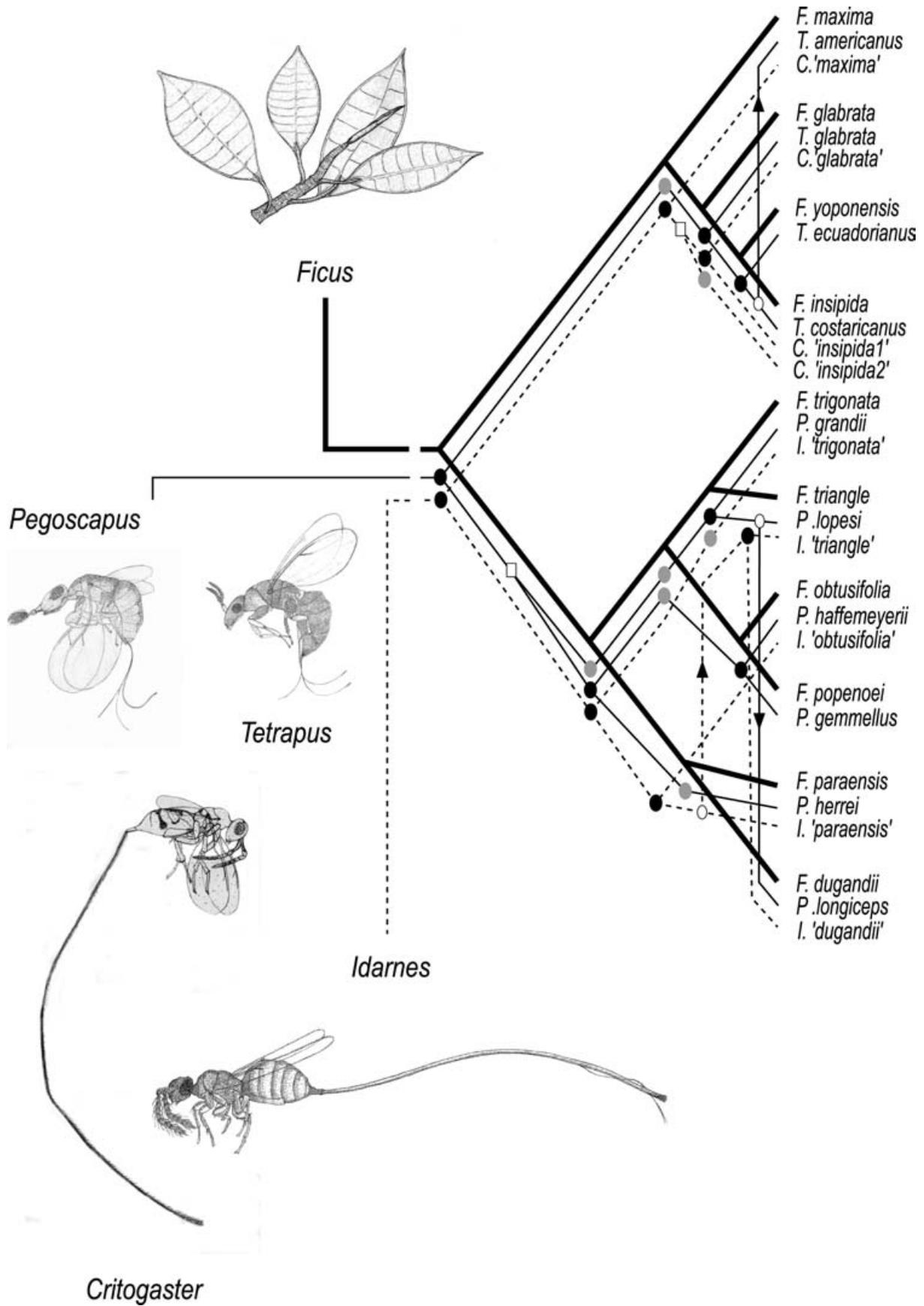
Lopez-Vaamonde *et al.* (2001) and Weiblen & Bush (2002) have used cophylogeny mapping in TREEMAP v1.0 (Page, 1995) to compare their phylogenies. Cophylogeny mapping is a means of embedding an associate tree within a host tree, mapping components of the former on to corresponding components of the latter (Page, 1988, 1990, 1994a). With the suggestion of four types of evolutionary event: codivergence, duplication, loss (extinction, omission or lineage sorting) and switching, the incongruence between associated phylogenies can be reconciled. One asks, if the trees share the same history, how can one account for their differences? The combination of events that provide a solution is known as a reconciled tree. Fig. 2 provides an example concerning neotropical figs from sections *Pharmacosyceae* and *Americana*,



**Fig. 1.** The *Ficus* microcosm. A 50-day developmental cycle [timescale (in days) and syconial diameters are shown in black], typical of *Ficus sycomorus* (Galil & Eisikowitch, 1968), but equally applicable to figs of the section *Americana*, to which the wasp images relate. The placement of wasp images indicates the periods when those guilds oviposit. The syconium remains unreceptive ('prefloral') until the female flowers are fully developed when it enters the 'female' stage. Attractant chemicals are released, intended for pollinators although probably attractive to non-pollinators also (Bronstein, 1991). Prior to, and during, the female stage gall-making parasites oviposit, usually to the exclusion of other wasps. Pollinator wasps of the family Agaonidae follow, as do non-pollinating competitors that also enter the syconium (Kerdelhué *et al.*, 2000). After pollination, the fig increases in diameter whilst seeds and larvae develop. Non-pollinating wasps arrive during the 'interfloral' stage and oviposit through the syconial wall with extended ovipositors. Such species may be parasitoids, inquiline or competitors of pollinator and parasite larvae (Kerdelhué & Rasplus, 1996). The male florets mature as the wasps complete development and emerge; during this 'male' phase female pollinators collect pollen and disperse, along with non-pollinator females. The fig ripens during a 'postfloral' stage, acquiring new colours, aromas and textures attractive to dispersal agents.

their pollinators (*Pegoscapus* and *Tetrapus*) and parasitoids (*Idarnes* and *Critogaster*), where the incongruence between *Pegoscapus* and *Idarnes* can be explained with the supposition of a switch and a loss.

There may be numerous solutions to any single reconciled tree. Optimal solutions are selected – or filtered – from all the possible solutions. Parsimony is used as a general optimising criterion and so is easily defined in some



situations, for example, a solution that includes unnecessary duplications and losses is non-optimal. However, the filter may be customised with specific costs and bounds for specific events, where evidence exists to support this. Solutions are then considered non-optimal if they exceed the customised bound for a particular event or total cost. The solutions produced by reconciliation can be considered as macroevolutionary hypotheses and used to diagnose past evolutionary events. Hence, the method can be used to restrict the set of potentially optimal solutions ('POpt') to hypotheses with particular features.

This 'diagnostic' function can be compared with a 'synoptic' function, which, rather than producing macroevolutionary hypotheses, seeks to evaluate general correspondence across POpt as a whole according to a similarity measure. Significant congruence is evaluated through randomisation and resampling of the associate set. This tests the hypothesis that two trees have greater congruence than expected by chance. All comparisons, even random tanglegrams, display a non-zero level of codivergence. Equally, very few associated trees are identical, even when taken from obligate mutualists. When evaluating congruence between trees the question should not be 'is there similarity' or 'are the trees identical' but 'is the similarity greater than that expected by chance'? Therefore, likelihood methods, such as the likelihood ratio and Shimodaira–Hasegawa tests (Heulsenbeck, Rannala & Yang, 1997; Lopez-Vaamonde *et al.*, 2001) or parsimony tests such as the partition homogeneity test (Johnson, Drown & Clayton, 2000), were not used here for comparing host and associate trees. Whilst they are useful for testing explicit hypotheses of cospeciation (Heulsenbeck *et al.*, 1997), such a test is too restrictive where one suspects other events may occur without eliminating a tangible pattern of congruence.

The correct inference of switches was sufficiently challenging that initial cophylogeny mapping did not allow them (Page, 1988). TREEMAP v1.0 was able to include switches but only those that required no additional sorting events to make the source and destination contemporary ('compatible' switches) (Page, 1994*b*). The deficiency in the solutions produced by TREEMAP v1.0 was solved through the invention of jungles (Charleston, 1998). A jungle is a directed graph of all possible mappings of one tree into another and, when solved, guarantees to deliver all potentially optimal reconstructions (Page & Charleston, 1998; Charleston, 1998). Furthermore, it is able to accommodate weakly incompatible switches, i.e. those that require subsequent losses to ensure that source and destination are contemporary. Cophylogeny mapping using jungles is a method for exhaustive and quantitative analysis of phylogenetic congruence. This review aims to reconsider cophylogeny in the *Ficus microcosm* using TREEMAP v2.0 (Charleston &

Page, 2002) to provide a general consensus on the degree of codivergence across published comparisons: (1) between *Ficus* and its Agaonid pollinators on a global scale (two data sets); (2) between *Ficus* and the pollinator *Ceratosolen* spp. of the Indo-Australasian region; (3) between *Ficus* and *Apocryptophagus* spp. of the Indo-Australasian region and (4) between *Pleistodontes* spp. and *Sycosapter* spp. of the Australasian region. Application of the method reveals more about three factors influencing the degree of statistically significant congruence: (i) host specificity, (ii) taxonomic level (i.e. sampling strategy) and (iii) different measures of similarity, i.e. the number of nodes in two trees that agree or that conflict.

## II. MATERIALS AND METHODS

Reconciliation analysis requires two fully resolved phylogenies, as TREEMAP cannot interpret polytomies. Finding POpt for a jungle is computationally intensive and the number of possible solutions increases exponentially with the number of taxa. Rather like finding the optimal phylogenetic tree in an exhaustive search, the process quickly becomes insoluble as the number of taxa involved passes a threshold of around 20 associations. Nine phylogenies were reconstructed using published molecular sequences, retrieved from GenBank (details of sequences and alignments are available from the author). Phylogenies were reconstructed *de novo* to obtain maximum resolution, which was not present originally.

### (1) Phylogenetic estimation

Availability determined the choice of molecular marker for the analyses, which are described in Table 1. Sequences were aligned by eye after initial multiple alignment in ClustalX (Thompson *et al.*, 1997). The completed alignments were edited in SeAl 1.0 (<http://evolve.zoo.ox.ac.uk/software>), the ends being levelled off to make all sequences of equal length. Phylogenetic estimation was carried out using PAUP\* v4.0b (Swofford, 1998). It should be remembered that the results of reconciliation analysis are contingent on the quality of the source phylogenies, especially when using the diagnostic function, i.e. trying to reconstruct actual transitions, and that, as a hypothesis, the reconciled tree can be falsified by rejecting the original trees. Hence, in estimating the source trees a balance was struck between ensuring complete resolution (a requirement of jungles analysis) and producing an estimate that was robust. To this end, the method of choice was maximum parsimony (MP), weighted by serial approximations. Serial approximation

**Fig. 2.** Phylogenetic reconciliation using cophylogeny mapping. *Pegoscapus* and *Tetrapus* are pollinators of *Ficus* (subgenus *Urostigma* and *Pharmacosycea* respectively). *Idarnes* and *Critogaster* are non-pollinators which utilise the microcosm to the detriment of the pollinators. Insect phylogenies [taken from Machado *et al.* (1996)] are mapped on to the host phylogeny to produce a reconciled tree. One of nine solutions to this jungle is shown. There is a significant degree of congruence ( $P < 0.05$ ). Evolutionary events: codivergence (●), duplication (□), loss (●) or switch (♣).

Table 1. Phylogenies used in reconciliation analysis. Nine phylogenies were estimated and combined in five analyses. For each phylogeny the taxon, sample size ( $n$ ) and molecular marker (locus) are stated (original sources are referenced). Available information is expressed as the total number of characters, the percentage of characters that were parsimony informative and the percentage of these characters that were fully weighted after serial approximation reweighting (SAR, see text)

Taxon	$n$	Locus	Total characters	Informative component (%)	Fully weighted informative characters (%)	Reference
1a† <i>Ficus</i> (I) <sup>#</sup>	12	Nuclear rRNA ITS	651	22.0	51.0	Weiblen (2000), Jousselin <i>et al.</i> (2003)
Agaonidae (COI)	12	Cytochrome c oxidase I	737	40.4	10.1	Weiblen (2001)
1b† <i>Ficus</i> (II) <sup>#</sup>	12	Nuclear rRNA ITS	723	8.7	34.9	Weiblen (2000), Jousselin <i>et al.</i> (2003)
Agaonidae (COI–II)	12	Cytochrome c oxidase I, tRNA <sub>Ieu</sub> , Cytochrome c oxidase II	1584	34.3	7.9	Weiblen (2001)
2 <i>Ficus</i> (III) <sup>#</sup>	16	Nuclear rRNA ITS	732	8.5	51.6	Weiblen (2000)
<i>Ceratosten</i>	16	Cytochrome c oxidase I, tRNA <sub>Ieu</sub> , Cytochrome c oxidase II	1442	42.2	11.5	Weiblen (2001)
3 <i>Ficus</i> (III) <sup>#</sup>	16	Nuclear rRNA ITS	*	*	*	Weiblen (2000)
<i>Apocryptophagus</i>	18	Cytochrome c oxidase I	398	43.2	7.0	Weiblen & Bush (2002), West <i>et al.</i> (2001)
4 <i>Pleistodontes</i>	13	28S rRNA, Nuclear rRNA ITS2	1473	57.2	76.7	Lopez-Vaamonde <i>et al.</i> (2001)
<i>Sycoscapter</i>	13	28S rRNA	766	31.1	79.8	Lopez-Vaamonde <i>et al.</i> (2001)

# Each *Ficus* phylogeny was estimated using the nuclear rRNA internal transcribed spacer but with different taxon sets (I, II and III) depending upon the particular associates with which it was compared.

\* Indicates that the same phylogeny was used in associations 2 and 3.

† Two agaonid phylogenies were estimated using alternative taxon sets and sequence data (see text); these are labelled COI and COI–II.

reweighting (SAR) excludes homoplastic characters through sequential reweighting, according to the rescaled consistency index of the previous estimate (Farris, 1969; Swofford *et al.*, 1996). Where the data are necessarily imperfect, this method produces a robust estimate, which cannot be said when using simple heuristic trees or other methods that enforce resolution, such as quartet puzzling (Strimmer & von Haeseler, 1996). Each input tree was reconstructed using the general programme settings: tree bisection-reconnection (TBR) swapping algorithm, initial tree obtained by ‘as-is’ stepwise addition, gaps were treated as missing data (except in association 3, see Table 1), ACCTRAN character state optimisation was employed, zero-length branches were collapsed, multiple states considered uncertain and the heuristic tree was allowed to proceed through branch rearrangement until completion. Confidence intervals for each estimate were obtained using 100 non-parametric bootstrap replicates (Felsenstein, 1985). To assess the effects of bias on the phylogenies, maximum likelihood (ML) and minimum evolution (ME) methods were also applied, although these always produced less-resolved bootstrap trees. ML trees, estimated with a fully parameterised general time reversible (GTR) model, were compared to MP topologies using a Shimodaira–Hasegawa (SH) test (Shimodaira & Hasegawa, 1999) to assess their disagreement. ME trees

were estimated with log-determinate corrections to check the effect of base composition imbalance.

## (2) Cophylogeny mapping

TREEMAP v2.0 is able to accommodate customised ‘costs’ and ‘bounds’ to specify the conditions under which a solution is considered ‘potentially optimal’. In the present study, the conditions were relaxed because little information exists to specify the relative probabilities of different events. For this reason, we do not use methods that require explicit macroevolutionary models (Huelsenbeck, Rannala & Larget, 2000) or evaluate total cost of solutions (Ronquist, 1995). The ‘costs’ of each event were set to default: codivergence (0), duplication, loss and switch (1). The costs had no bearing on the result since the total cost was not used to filter solutions or to evaluate those in POpt. Initially, the bounds defining permissible solutions were set to identify the maximum codivergence (or minimum duplication), that is, the number of codivergences was set high and reduced until a potentially optimal solution was returned. The conditions were then progressively relaxed until all solutions (POpt) were found. In cases where there is little congruence it may prove impossible to obtain these solutions due to computational complexity. Hence, it is useful to proceed as

described, starting with strict bounds and making them more permissive.

### (3) Significance testing

TREEMAP evaluates the similarity shared by two trees through randomisation tests. For each jungle, 100 randomised associate trees were generated through resampling of the associate taxa and the level of similarity, expressed either as the number of codivergences ( $N_{CE}$ ) or the number of non-codivergence events ( $N_{NCE}$ ), was compared to the numbers seen in these randomised jungles. Often it is not possible to obtain values for very common occurrences, i.e. low  $N_{CE}$  or high  $N_{NCE}$ . Here, it is enough to obtain the least significant value possible and declare the missing value  $P$  equal to or more than this.

## III. RESULTS

### (1) Phylogenetic estimates

The main requirement for reconciliation analysis is fully resolved phylogenies. For each of the nine phylogenies described in Table 1 and shown in Figs 3–6, the majority of nodes were resolved after SAR. For two unresolved nodes, in the Agaonidae (I) and *Ficus* (III) phylogenies, we rely on consensus trees for resolution. The resolution achieved in the bootstrapped MP tree was not matched by bootstrapped ML and ME trees; the percentage of dichotomous trees ranged between 8–64 and 33–74% respectively; this emphasises the need for SAR in this instance. ML trees agreed with MP topologies where they were resolved at the tips but the lack of general resolution perhaps accounted for a significant difference between ML and MP topologies detected by the SH test in all cases, except the *Ficus* phylogeny in association 2 and the *Apocryptophagus* phylogeny in association 3. Bias caused underestimation of the furthest distances in uncorrected estimation of Agaonidae (I), Agaonidae (II), *Ceratosolen* and *Apocryptophagus* phylogenies. ME topologies supported MP where they were resolved, suggesting no substantial effect by base composition bias.

### (2) Cophylogeny mapping

#### (a) General features of POpt

The result of cophylogeny mapping was an exhaustive set of reconciled trees. Each solution to the jungle was viewed as an equally valid hypothesis *a priori*. Representative solutions for each of five jungles are shown in Figs 3–6. The ranges for  $N_{CE}$  and  $N_{NCE}$  values appear in Table 2. These jungles are typical in that they produce a range of solutions that vary widely in  $N_{CE}$  and  $N_{NCE}$  and in the combination of events used to resolve incongruence. In Fig. 7 the solutions generated by each jungle are shown together. The diagrams emphasise the spectrum of solutions produced, ranging between all duplications and losses to all switches. Significant solutions, i.e. those with most codivergences, tend to have most losses and fewest switches.

Generally, all jungles produce a solution significant for the minimum number of non-codivergence events ( $N_{\min NCE}$ ), even when all other solutions are non-significant; these are composed entirely of switches. Significant solutions (using the maximum number of codivergence events,  $N_{\max CE}$ ) produce a variety of other solutions that are non-significant. So each jungle presents an equivocal view of association through time, with some solutions favouring codivergence as the predominant mechanism, others favouring non-codivergence. The distinct measures of similarity –  $N_{\max CE}$  and  $N_{\min NCE}$  – used to test significance did not always concur. Where a solution was significant under  $N_{\max CE}$  it was also significant using  $N_{\min NCE}$ ; but, for non-pollinators, the converse was not true and significant similarity was rejected using  $N_{\max CE}$ .

#### (b) *Ficus–Agaonidae*

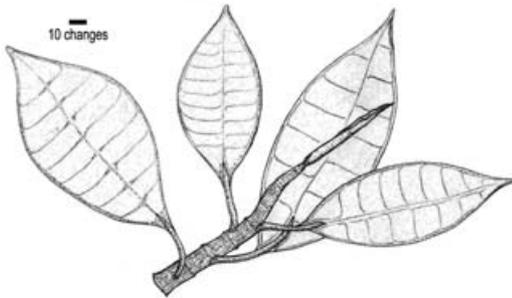
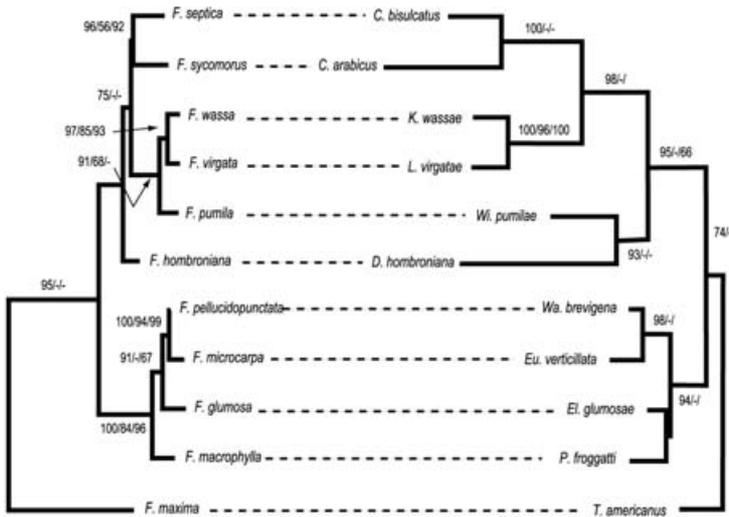
The two jungles combining *Ficus* and its pollinators present conflicting results. The first data set, using cytochrome oxidase I, is significant for both  $N_{\max CE}$  (16) and  $N_{\min NCE}$  (8). Solutions with  $N_{\max CE} = 16$  are those that posit an association between the common ancestors of both fig and pollinator (i.e. those that ‘tie the roots’). Fig. 3A–B offers a typical example. The basal fig subgenus *Pharmacosyceae*. In fact, *Tetrapus* appeared in two consistent positions, as the sister taxon to all other pollinators and grouping most basally with the clade including *Ceratosolen* and others but both permutations result in  $N_{\max CE} = 16$ . A number of solutions, including four with  $N_{CE} = 10$ , are non-significant but do not tie the roots. Instead, these solutions invoke colonisations of *Ficus* by Agaonidae after the initial diversification of the host plant.

The second data set (cytochrome oxidase I–II), shown in Fig. 3C–D, produced a non-significant result with both  $N_{\max CE}$  (12,  $P=0.78$ ) and  $N_{\min NCE}$  (19,  $P=0.08$ ). Two features detract from further agreement between the trees. First, the close relationship of *Ceratosolen* with *Kradibia* and *Liporrhopalum* (seen in both data sets) is not matched by the distant relationship between their hosts; section *Sycidium* is closer to sections *Rhizocladus* and *Ficus* than to the hosts of *Ceratosolen* (*Sycocarpus*, *Sycomorus* and *Neomorphe*). Second, there are three terminal triplets that are mismatched, i.e. in a set of three associations, the two sister pollinators do not correspond with the two sister figs. One change to either tree would result in two further codivergence events. Across the whole jungle such revisions would add six more codivergence events, making  $N_{\max CE} = 18$  and  $P < 0.01$ . However, these mismatches are the best-supported nodes in the tree; the incongruence appears to be real.

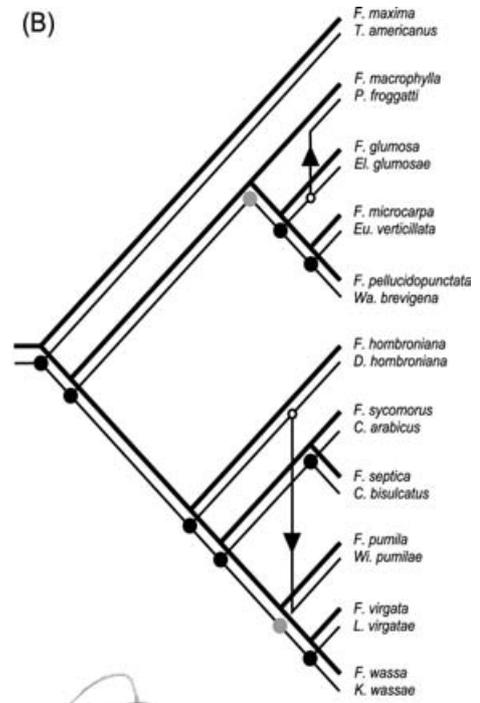
#### (c) *Ficus–Ceratosolen*

$N_{CE}$  ranges between 16 and 22 for the *Ficus–Ceratosolen* jungle (2). From 44 solutions in POpt, 24 are non-significant (where  $N_{CE} < 20$ ). Those solutions with  $N_{\max CE}$  are significant using both measures of congruence ( $P < 0.01$ ) and, once again, these solutions tie the roots, as shown in Fig. 4. The relationships between the three clades identified in the *Ceratosolen* tree, i.e. those containing *C. grandii*, *C. arabicus*

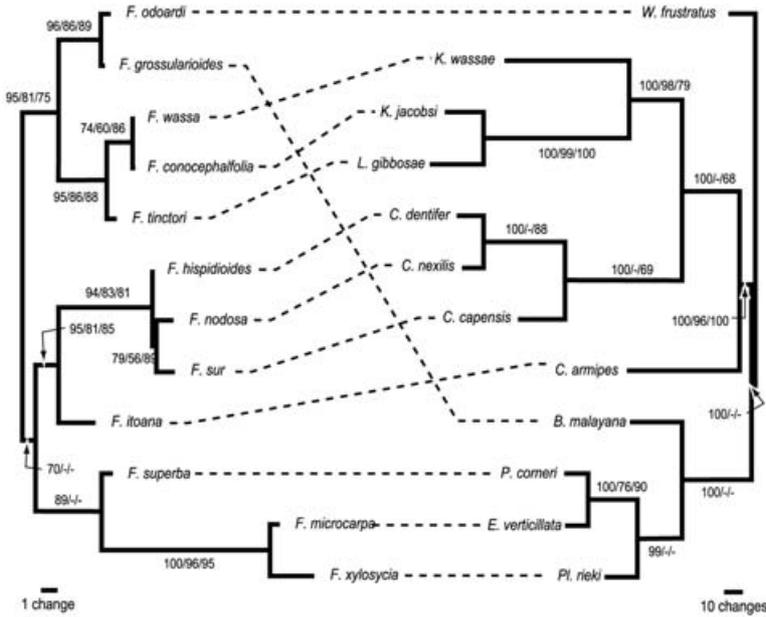
(A)



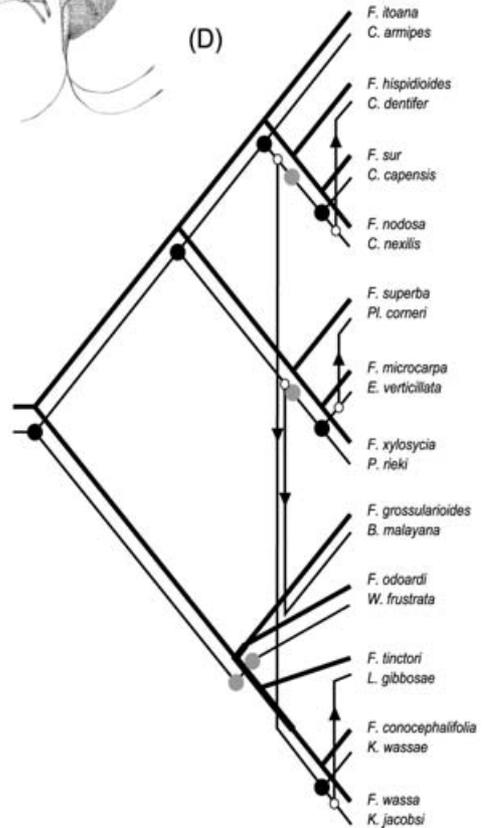
(B)

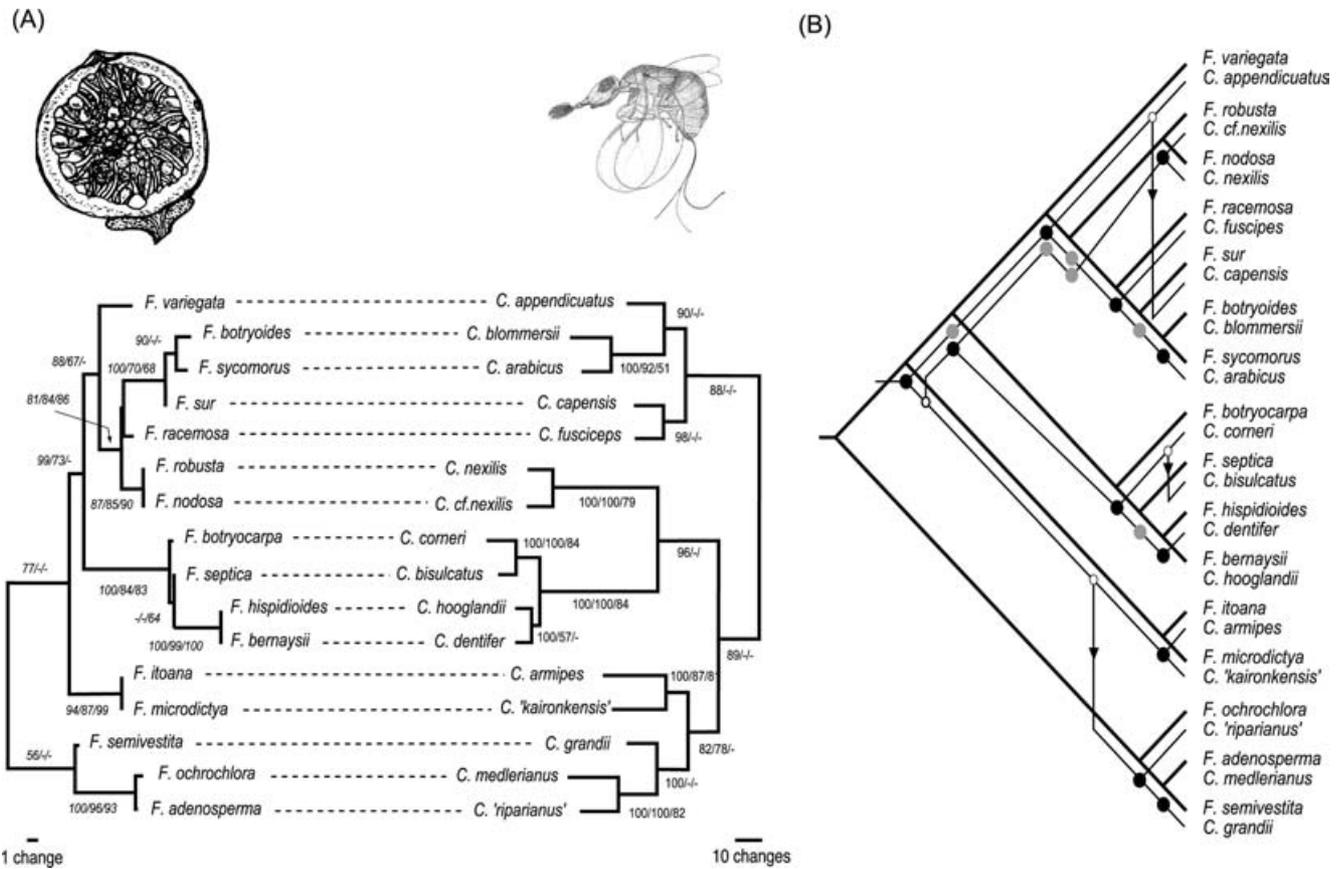


(C)



(D)





**Fig. 4.** Tanglegram (A) and reconciled tree (B) for association 2 (see Table 1) involving *Ficus* and its *Ceratosolen* spp. pollinators (POpt = 44,  $P_{\max\text{CE}} < 0.01$ ).

and *C. comeri* respectively, are the most fluid. The grouping *C. comeri* and *C. grandii*, to the exclusion of *C. arabicus* is shown in Fig. 4 but the other occasional grouping of *C. comeri* with *C. arabicus* produces the same  $N_{\max\text{CE}}$  and significance. The equivocal resolution of this node has no effect on the results. The third grouping of *C. grandii* and *C. arabicus*, which would maximise congruence with the parasitoid tree, is never seen. Taking these three clades and their respective host clades together, this jungle is essentially a mismatched triplet, demanding a set of losses or a switch early in the tree to be reconciled.

#### (d) *Ficus*–*Apocryptophagus*

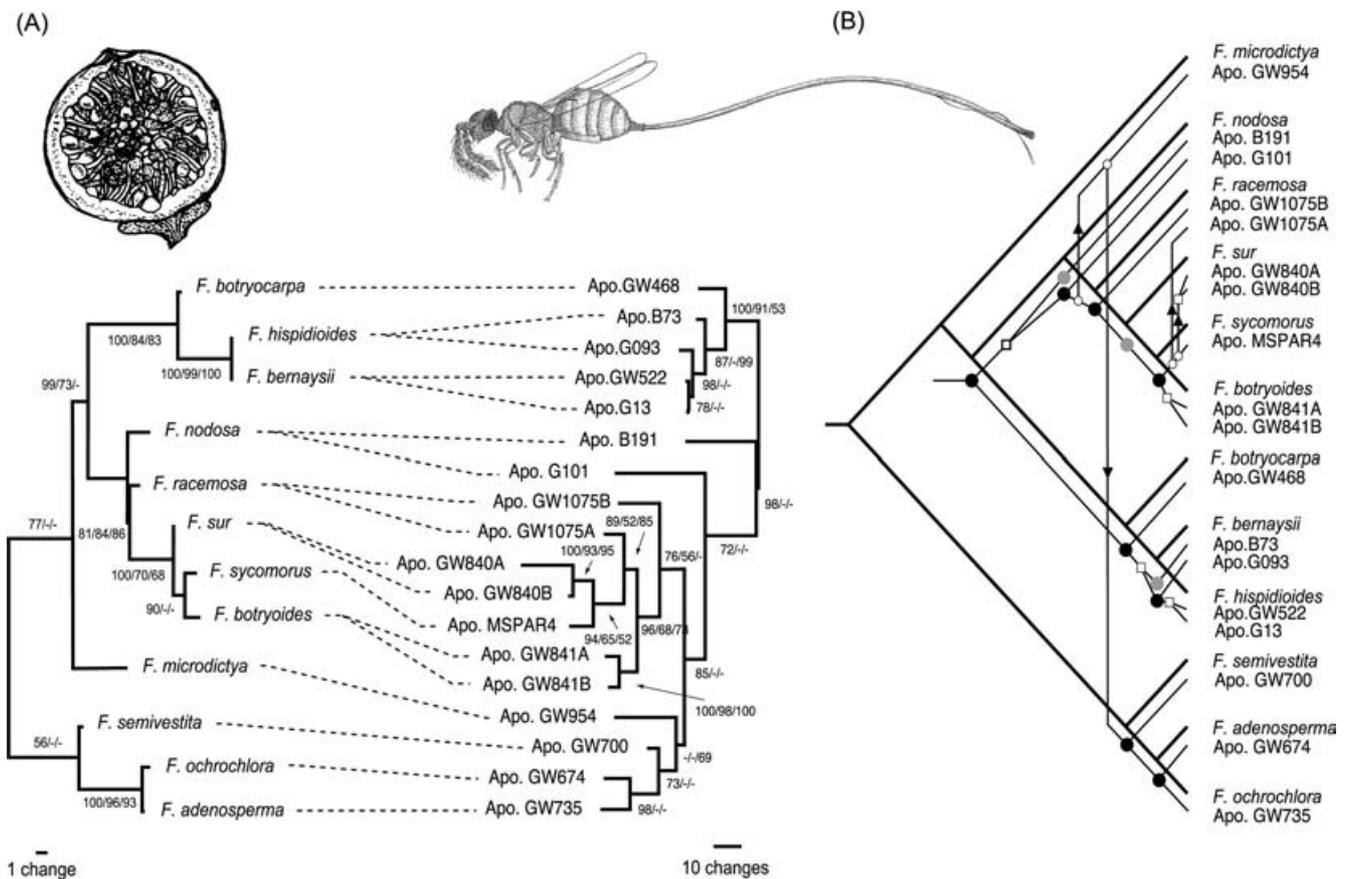
*Apocryptophagus* is not significantly congruent with its plant host using  $N_{\max\text{CE}}$  ( $P_{\max\text{CE}} = 0.2$ ). However, the jungle is significantly congruent using  $N_{\min\text{NCE}}$  ( $P < 0.01$ ) for those solutions with  $N_{\max\text{CE}}$ . This may reflect the similarity that is

evident from the tanglegrams in Fig. 5. Again, particular *Ficus* species groups (corresponding to the *C. comeri*, *C. arabicus* and *C. grandii* clades) have distinct non-pollinator clades. This effect can be captured in removing the four terminal duplications from the analysis, which results in a significant match ( $N = 13$ ,  $N_{\max\text{CE}} = 16$ ,  $P_{\max\text{CE}} = 0.04$ ).

#### (e) *Pleistodontes*–*Sycoscapter*

The jungle for association 4 (Fig. 6) produced a maximum of 12 codivergences, fewer than some random assemblages ( $P_{\max\text{CE}} > 0.44$ ). Despite the incongruence between these trees  $N_{\min\text{NCE}}$  is significant ( $P < 0.01$ ) for a solution with  $N_{\max\text{CE}}$  and a high number of switches, i.e. a solution in which extinction is not permitted. This jungle had the largest POpt (45) due to a large number of switches, each of which has multiple permutations due to variable export and import points.

**Fig. 3.** Tanglegrams and reconciled trees for associations between *Ficus* and its pollinators of the Agaonidae. (A–B) association 1a (cytochrome oxidase I) (POpt = 12,  $P_{\max\text{CE}} < 0.01$ ). (C–D) association 1b (cytochrome oxidase I–II and tRNA<sub>Leu</sub>) (POpt = 27,  $P_{\max\text{CE}} = 0.78$ ). Node support is given in each tanglegram in the form of bootstrap proportions using maximum parsimony, maximum likelihood and minimum evolution methods (MP/ML/ME). Genus abbreviations: C, *Ceratosolen*; K, *Kradibia*; L, *Liporropalum*; Wi, *Wiebesia*; D, *Dolichoris*; Wa, *Waterstoniella*; Eu, *Eupristina*; El, *Elisabethiella*; Pl, *Pleistodontes*; T, *Tetrapus*; P, *Platyscapa*; B, *Blastophaga*.



**Fig. 5.** Tanglegram (A) and reconciled tree (B) for associations 3 (see Table 1) involving *Ficus* and its *Apocryptophagus* spp. parasites ( $PO_{pt}=38$ ,  $P_{maxCE}=0.2$ ). *Apocryptophagus* species are undescribed and appear with their original identification number as catalogued in GenBank.

#### IV. DISCUSSION

This review has revisited cophylogeny within the *Ficus* microcosm using cophylogeny mapping to provide an exhaustive and quantitative reconciliation of partially incongruent trees. The results support the current consensus that pollinators show significant codivergence with their plant hosts but non-pollinators do not. Having said this, switches and losses are still required to reconcile figs and pollinators, indicating that codivergence is not a strict rule and suggesting that host associations can be labile. Furthermore, the *Ficus*–*Apocryptophagus* association has some phylogenetic structure and so plant divergence has played a part in determining parasite divergence, just not as much as insect ecology.

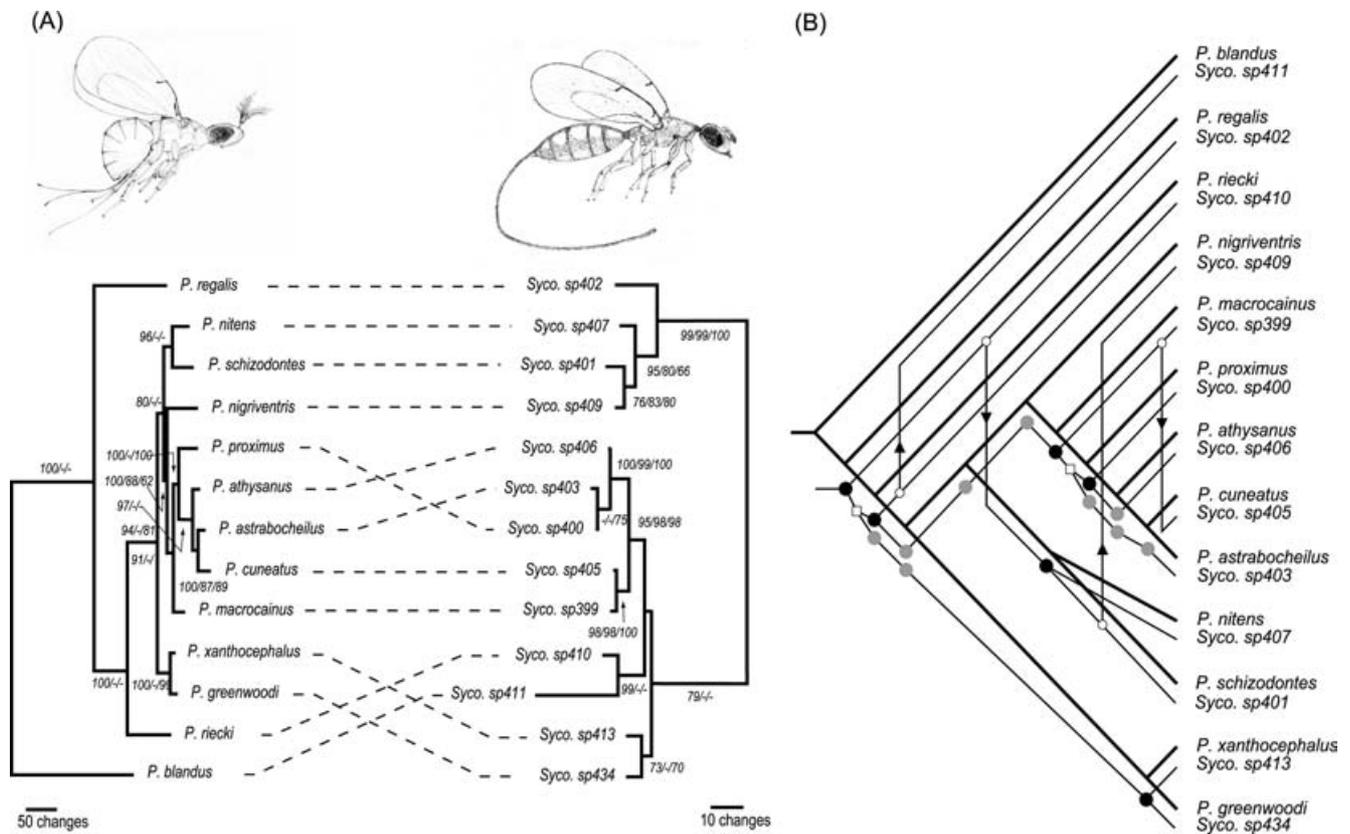
Codivergence and cospeciation are often held to be synonymous, yet synchronised cladogenesis is one of several explanations for codivergence. Correlations of genetic distances can indicate the relative timing of cladogenesis and rates of evolution between hosts and associates (Hafner & Nadler, 1990). Attempts were made here to compare distances corrected for bias and phylogeny but these were inconclusive, producing no significant trends. Comparisons of uncorrected genetic distances suggest that codivergence

by pollinators and parasitoids is synchronous with hosts, although evolutionary rates are faster in associates. Ideally, one would calibrate divergence times for host and associate but no rate estimate is available for ribosomal DNA in *Ficus*. However, previous estimates of fig wasps have indicated that the origin of Agaonidae could coincide with the appearance of *Ficus* in the fossil record between 60 and 80 million years ago (Machado *et al.*, 2001).

##### (1) Cophylogeny

###### (a) Codivergence

Most features of the *Ficus* microcosm suggest a long series of reciprocal adaptations and so codivergence was expected to characterise cophylogeny. The host specificity of fig wasps has become a central dogma, the very fact that exceptions to the ‘one-to-one rule’ can be catalogued individually indicating that it was robust (Ramirez, 1970; Wiebes, 1979). The adaptations of pollinators, for example, for entering the fig (Ramirez, 1974) and ensuring efficient pollination (Ramirez & Malavasi, 1997; Joussetin, Rasplus & Kjellberg, 2003) are widespread across the Agaonidae, strengthening the argument that specificity derives from an ancient dependency. Rearing pollinator wasps detracts from the



**Fig. 6.** Tanglegram (A) and reconciled tree (B) for association 4 (see Table 1), involving the pollinators *Pleistodontes* spp. and the non-pollinators *Sycosapter* spp., which are resident on the same host figs ( $PO_{pt}=45$ ,  $P_{maxCE} > 0.44$ ). *Sycosapter* species are undescribed and appear with their original identification number as catalogued in GenBank.

female reproductive function of the fig (Herre & West, 1997) but this can be tolerated because wasps never occupy all available ovaries. This apparent stability suggests that the mutualism is self-regulated by intrinsic, historical factors (Dufay & Anstett, 2003), for example, wasp entry may be limited by ostiolar closure, wasp occupation of ovaries may be egg-limited (Nefdt & Compton, 1996) or some ovaries may be unsuitable for rearing wasps (Anstett, 2001).

A stable, obligate mutualism of considerable age is an excellent candidate for codivergence. Taxonomic affinities were vindicated by phylogenetic studies which suggest phylogenetic congruence between *Ficus* and pollinators (Herre *et al.*, 1996; Weiblen & Bush, 2002). Research into volatile chemical attractants released by receptive syconia suggests that species-specific blends are responsible for attracting pollinators to their host (Ware *et al.*, 1993; Hossaert-McKey, Gibberman & Frey, 1994; Grison, Edwards & Hossaert-McKey, 1999; Grison-Pige *et al.*, 2002) with considerable precision. And so sufficient evidence for codivergence and its mechanisms existed to allow cophylogeny within the microcosm to be described as 'strict, parallel cladogenesis' (Anstett, Hossaert-McKey & Kjellberg, 1997) or at least predominantly so (Cook & Rasplus, 2003). Perhaps most surprising is that codivergence is not as pervasive as anticipated. With the necessary caveats regarding phylogenetic uncertainty notwithstanding, this

study has emphasised a role for switching and duplication in *Ficus* cophylogeny despite significant codivergence in some analyses.

Codivergence was not automatically expected for parasites and parasitoids because less information is available concerning their life histories. It is known that non-pollinators have negative effects on fig and pollinator fitness and that they are host specific (West *et al.*, 1996; Kerdelhué & Rasplus, 1996). It is not known if non-pollinators target several closely related figs or how many species they compete with or prey upon (Compton, Rasplus & Ware, 1994). Both figs and other wasps can be resources for non-pollinators (West *et al.*, 1996), and it is also unclear which should be most influential through evolutionary time. Phylogenies have suggested codivergence between pollinators and non-pollinators in the New World (Machado *et al.*, 1996), albeit with inadequate sampling, and for *Sycosapter* (Lopez-Vaamonde *et al.*, 2001). Lopez-Vaamonde *et al.* (2001) concluded that 'partial co-cladogenesis' characterised the *Pleistodontes*–*Sycosapter* cophylogeny. Association 4 reanalyses this data, although with several unresolved nodes removed and finds no significant codivergence. *Sycosapter* may track the *Ficus* phylogeny to a greater extent. Weiblen & Bush (2002) found no significant codivergence between *Ficus* and *Apocryptophagus*, although the present study has emphasised that fig divergence has still influenced parasite phylogeny.

Table 2. Results of cophylogeny mapping using jungles. The following statistics are given for each of five jungles: the numbers ( $n$ ) of host and associate taxa, the number of potentially optimal reconstructions (POpt), the maximum and minimum number of codivergence events posited ( $N_{\max\text{CE}}$  and  $N_{\min\text{CE}}$  respectively), along with their  $P$  values and estimation errors; the same quantities are provided for the minimum and maximum total non-codivergence events ( $N_{\min\text{NCE}}$  and  $N_{\max\text{NCE}}$  respectively). Significant values ( $P > 0.05$ ) are shown in bold

Host	$n$	Associate	$n$	POpt	$N_{\min\text{CE}}$	$P$	$\pm$	$N_{\max\text{CE}}$	$P$	$\pm$	$N_{\min\text{NCE}}$	$P$	$\pm$	$N_{\max\text{NCE}}$	$P$	$\pm$
1a <i>Ficus (I)</i>	11	Agaonidae (COI)	11	12	10	0.77	0.04	<b>16</b>	<b>&lt;0.01</b>	0.007	<b>8</b>	<b>&lt;0.01</b>	0.007	15	0.2	0.014
1b <i>Ficus (II)</i>	12	Agaonidae (COI-II)	12	27	8	>0.78		12	0.78	0.094	19	0.08	0.027	33	>0.62	
2 <i>Ficus (III)</i>	16	<i>Ceratosolen</i>	16	44	16	>0.05		<b>22</b>	<b>&lt;0.01</b>	0.007	<b>18</b>	<b>&lt;0.01</b>	0.007	<b>25</b>	<b>0.01</b>	<b>0.009</b>
3 <i>Ficus (III)</i>	12	<i>Apocryptophagus</i>	18	38	10	>0.2		16	0.2	0.13	<b>25</b>	<b>&lt;0.01</b>	0.007	42	>0.16	
4 <i>Pleistodontes</i>	13	<i>Sycoscapter</i>	13	45	6	>0.44		12	0.44	0.154	<b>20</b>	<b>&lt;0.01</b>	0.007	39	>0.54	

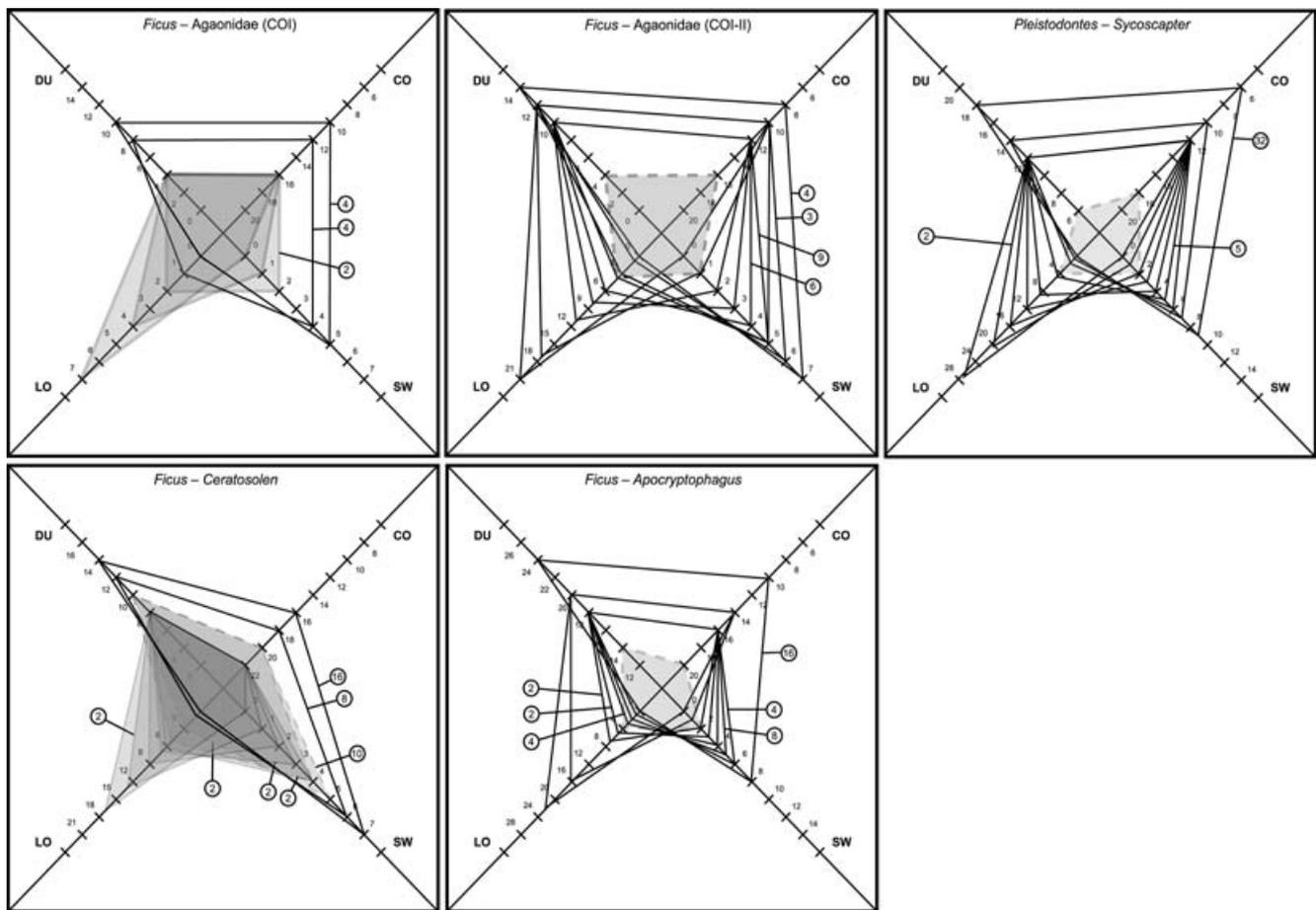


Fig. 7. Diagrams summarising the set of potentially optimal solution (POpt) for five jungles. Each line plots the number of codivergences (CO), duplication (DU), losses (LO) and switches (SW) for a single solution to form a quadrangle. Significantly congruent solutions have shaded quadrangles. For those jungles without significantly congruent solutions, the point of significance is marked with a dashed quadrangle. Circled numbers refer to multiple solutions with identical event combinations.

The diverse origins and life histories of non-pollinators means that the level of codivergence can be expected to vary case by case; indeed, significant codivergence between

non-pollinators and either fig or pollinator should be expected where the opportunities for utilising multiple species is minimised.

*(b) Duplication*

Duplication of wasp lineages within a single host has been documented in *Apocryptophagus* (Weiblen & Bush, 2002). Here it appears niche segregation separates sympatric species on the basis of oviposition sites within the syconium, similar to the seminal observation of niche segregation of oviposition depths in the parastoid *Megarhyssa* (Gibbons, 1979). Duplication by pollinators has been considered unlikely, given the emphasis placed on host fidelity and a unitary correspondence between host and insect. However, a recent comparison of microsatellite data by Molbo *et al.* (2003), discussed in detail below, showed that several neotropical pollinator species contained multiple lineages, demonstrating that duplication does happen within the fig.

*(c) Loss*

The loss of associates from figs seems particularly unlikely given the obligate nature of the mutualism. Whilst local extinction of a non-pollinator might seem plausible, a pollinator is surely indispensable. Non-pollinators have colonised introduced figs, for example, local *Sycophaga* and *Apocrypta* species have reproduced within the parthenocarpic figs of *F. sycomorus* introduced into the Mediterranean (Ramirez, 1970), suggesting that niches left vacant by extinction can be filled by local wasps. Local pollinators never colonise exotic figs but related figs often prove amenable. In their study of a *F. lutea* individual outside of its normal range, Ware & Compton (1992) found that foreign pollinators were not attracted to *F. lutea* but that if found accidentally, many different pollinators could gain entry through the ostiole. Successful reproduction was only observed in pollinators from closely related figs. Thus, features traditionally thought to ensure host specificity may account for this general rule but do not preclude the replacement of locally extinct pollinators by those of related figs. Coupled with the fact that figs can reproduce vegetatively in the absence of pollinators (Ramirez, 1970), this suggests that local extinction of pollinators could be tolerated in the short term. Extinction may be more probable where multiple pollinator lineages inhabit the same host. Competitive exclusion of one of these lineages could lead to extinction without negative effects on the host. Such extinctions may be important in accounting for the observations of Molbo *et al.* (2003), described below.

*(d) Switching*

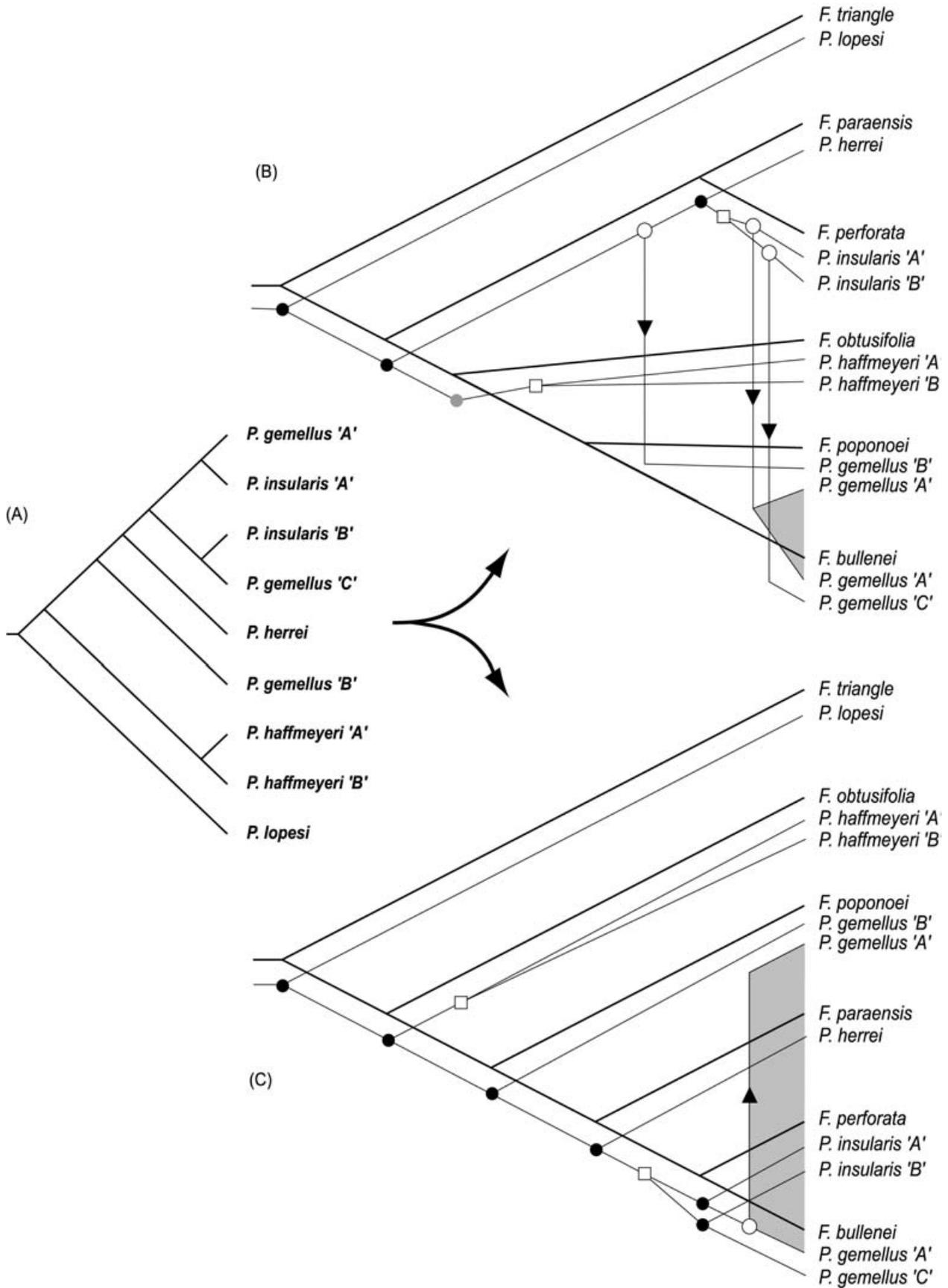
The ubiquitous specificity of fig wasps suggests that host switching seldom occurs. Furthermore, both pollinator and non-pollinator assemblages are substantially undersaturated (Hawkins & Compton, 1992); were switching a frequent ecological event, we might expect communities to become saturated as niches become occupied. Bronstein (1991) made the case for strict barriers to host switching, based mainly around the role of volatile chemicals in attracting both pollinating and non-pollinating fig wasps. For most figs the majority of foundress wasps are the 'right' species, with perhaps 1% being 'wrong' (Ramirez, 1970). Documented cases of copollination exist where a few figs share a complex

of pollinators, as described for *F. ottonifolia* and *F. sur* (Michaloud *et al.*, 1996; Kerdelhué, Le Clainche & Rasplus, 1999). These situations often require putative switches; indeed exceptions have always been observed but they have been considered exceptions to a robust general rule (Ramirez, 1970; Wiebes, 1979).

The robustness of this unitary correspondence has recently been scrutinised by Molbo *et al.* (2003). Analysis of microsatellite loci and COI sequence data of Panamanian pollinators (wasp images shown in Fig. 1) demonstrated that several fig species harboured cryptic pollinator species, contradicting the 1:1 rule. Furthermore, phylogenies of these species showed that pollinators sharing a host were not sibling species and may have switched from other hosts, or were interacting with multiple hosts. Clearly, these results suggest that our understanding of how pollinators diversify in the short term is, at best, incomplete and, at worst, a gross simplification. Can the idea that pollinators diversify in harmony with the host, consistent with strict host specificity and the 1:1 rule and inspired by phylogenetic correspondence, be reconciled with the knowledge that there are more lineages, more multiple interactions and more labile host selection than previously thought?

A jungle analysis of the Molbo *et al.* (2003) phylogeny is presented in Fig. 8. This analysis is provisional because the number of hosts is small [although Molbo *et al.* (2003) note that a similar situation may exist in the unrelated *Tetrapus*]. The null hypothesis of random association is addressed to the radiation of *Pegoscapus* on *Americana* figs, and so, with only six hosts, this is an incomplete view. Moreover, the host phylogeny is only poorly known. This being the case, two jungles were created. The first uses a host phylogeny that is reasonable on the basis of a previous phylogeny (Herre *et al.*, 1996) and morphological similarities; this demonstrates that there is no significant congruence with the wasp phylogeny ( $PO_{\text{opt}}=5$ ,  $N_{\text{maxCE}}=6$ ,  $P_{\text{maxCE}}=1$ ; Fig. 8(B)). Conversely, we can momentarily accept the traditional consensus that pollinators and figs codiverge and use this to predict host phylogeny. The second jungle tries to maximise the correspondence between the phylogenies and shows that, if this is the true host phylogeny, the observations of Molbo *et al.* (2003) are consistent with the traditional concept of unitary correspondence ( $PO_{\text{opt}}=2$ ,  $N_{\text{maxCE}}=12$ ,  $P_{\text{maxCE}}=0.03$ ; Fig. 8(C)). However, even this scenario requires two duplications (by *P. haffmeyer* and the ancestor of *P. gemellus* and *P. insularis*), a switch and a failure to speciate (each by *P. gemellus* 'A').

It remains to be seen which of these reconciled trees is most realistic. Yet, in all events, fig-pollinator cophylogeny is clearly more diverse than is evident in Fig. 1; the frequency of duplications, losses and switches could be much higher than indicated by the comparisons of ancient lineages made here. How can we reconcile the phylogenetic congruence at family and genus level with these data, suggesting that forces during speciation are undermining codivergence? The dynamics at work now present one picture of the diversification of a pollinator clade. However, this picture must be greatly influenced by another force: extinction. Any extant pollinator clade can be expected to lose a proportion of lineages through extinction as it diversifies, such that many



**Fig. 8.** Jungle analysis of the partial *Pegoscapus* phylogeny (A) from Molbo *et al.* (2003). (B) A reconciled tree constructed using the probable host phylogeny. (C) A prediction of the host relationships and reconciled tree required to accept a hypothesis of faithful codivergence between *Pegoscapus* and *Ficus*. Shaded areas between branches indicate a single taxon occupying multiple hosts.

historical traces will be lost and the picture presented by a phylogeny later will not mirror the complexity today. This ‘thinning out’ with time can explain why a simpler scenario, though not without its switches and duplications, emerges from older comparisons. With ancient patterns inconsistent with contemporary processes, the *Ficus* microcosm raises important questions as to how accurate a record of cophylogeny tree comparison is. It may be a salutary demonstration of how the pattern changes over time as lineages disappear, causing tree comparison to give an altered account of history.

## (2) Codivergence and host specificity

Intuitively, host specificity is associated with codivergent phylogenies, for example, pocket gophers and lice (Hafner & Page, 1995) and aphids and *Buchnera* (Clark *et al.*, 2000). But not all host-specific insects codiverge. Our ignorance of the exact life histories of non-pollinating fig wasps means that it is not clear that they should codiverge and if so, with what. However, in terms of largely polytrophic plant–insect interactions, fig parasites and parasitoids are oligotrophic and relatively specific, but associations 3 and 4 (see Table 1) showed no significant codivergence. In addition to specificity, associates must be denied regular opportunities for switching. Whilst gopher lice and *Buchnera* are restricted by being sedentary, fig wasps are highly vagile, at least during dispersal. Pollinators occupy a more restricted niche than non-pollinators; their reproductive success relies on successful pollination of the syconium to ensure resources for their developing larvae (Herre *et al.*, 1999). This puts pollinators under pressure to enter figs compatible with the pollen they carry. Most non-pollinators do not enter the syconium and, unlike the pollinators, have the option to oviposit within several syconia with which they are anatomically compatible; of course, these may not be related. A useful future study would address cophylogeny in the Sycoecinae and Otitisellinae, non-pollinators that enter the syconium; these might be better candidates for codivergence since they too are denied opportunities for switching but are free from the need to ensure effective pollination.

## (3) Effect of congruence measure

Fig. 7 shows that many solutions with fewest codivergences have the most switches. This itself is unsurprising but such solutions are often significant under the  $N_{\min\text{NCE}}$  criterion.  $N_{\text{NCE}}$  has a wider distribution of values across POpt (Charleston, 2003) and therefore provides a more permissive test of significant congruence than  $N_{\text{CE}}$ . Calculation of both values would provide maximum and minimum boundaries for the significance of any codivergence. Switches have a twofold effect in reducing the number of events required to reconcile two trees. First, they are considered a single event, whereas the alternative scenario of a duplication and subsequent losses comprises a minimum of two. Second, whereas a ‘large’ incongruence can be explained by a single switch across the host tree, the alternative basal duplication requires a large number of subsequent losses. So all-switch solutions require very few events, whilst switchless solutions

always require the greatest. The result is a solution composed entirely of switches being significantly congruent. Hence, we may discount the significance attached to associations 3 and 4 in Table 2 because these results refer to solutions with high numbers of switches.  $N_{\min\text{NCE}}$  indicates the most mathematically economical solution but  $N_{\max\text{CE}}$  ensures that a significant solution is biologically sensible.

## (4) Effect of sampling strategy

The significance of codivergence in associations 2 and 3 can be enhanced by small changes to the taxon sample. *Apo-cryptophagus* is significantly codivergent with *Ficus* if one discounts the most recent divergences, reflecting the influence of host phylogeny. Association 1b was non-significant for  $N_{\max\text{CE}}$  and was less representative than association 1a. By including representatives of more distinct lineages association 1a reduces the effect of particularly incongruent clades. Equally, it would be easy to inflate codivergence within association 1b by adding two or three *Ceratosolen* sp. to the sample, since this clade is significantly congruent. To define ‘representative sample’, we must ultimately refer to the hypothesis we wish to test. Certainly, the phylogenies of Weiblen (2000, 2001) show that other lineages could be added to this analysis (computational complexity permitting) and the majority of these would enhance the congruence. Association 1b demonstrates that restricted sampling, which happens to concentrate incongruent components of a wider cophylogeny, can give inconsistent results.

The ecological forces determining mutual descent are likely to be different depending upon the spatio-temporal scales being considered. Switching can be prevented by both local adaptation and historical contingency. For example, *Pharmacosycea* and *Americana* figs exist in secondary sympatry and are pollinated by the passive pollinator *Tetrapus* and the active pollinator *Pegoscapus* respectively. It is unlikely that *Tetrapus* would pollinate *Americana* because of historical factors – it lacks active pollination, cannot detect attractants from *Americana* figs, or fit into the ostiole or ovary. Certainly, *Tetrapus* would not switch to section *Sycomorus* since this is found thousands of miles away in Africa. Thus, any codivergence observed at this global scale may be of a different character to that of finer scales where sympatric wasps can interact with other hosts but do not due to contemporary selection pressures. For this reason – that mechanisms may depend on taxonomic level – sampling strategy makes a difference to the hypothesis being asked and the result of cophylogeny mapping.

## V. CONCLUSION

(1) Cophylogeny mapping with jungles has identified significant codivergence between the phylogenies of *Ficus* and its pollinators at different taxonomic depths. This is consistent with the consensus that they have codiverged throughout an ancient symbiosis.

(2) No significant codivergence was identified for non-pollinators and so there is a fundamental difference between

the cophylogenetic dynamics of the two groups, demonstrating that host specificity is necessary but not sufficient for significant codivergence. However, the diversity of life strategies within the non-pollinators means that the level of host specificity and codivergence is likely to vary.

(3) Whilst pollinators and non-pollinators may have fundamentally different cophylogenetic dynamics that reflect their different host orientations, this does not prevent incidents of switching by pollinators or codivergence by non-pollinators. It is becoming clear that the associates have more complex histories than previously thought but put simply, codivergence is more important than switching to pollinator phylogeny but the converse is true for non-pollinators. This is probably a direct reflection of the relative possibilities for utilising multiple hosts.

(4)  $N_{CE}$  is a better indicator of statistical significance than  $N_{NCE}$ .  $N_{NCE}$  attaches significance to solutions that have fewer events than expected by chance and produces significant results that may lack biological interpretation. It is ill advised to use total events as a significance criterion when the events cannot be weighted.

(5) Sampling strategy will affect the significance of codivergence in reconciled trees. One must use a sample that reflects the hypothesis being tested, e.g. codivergence within genera could be tested using the genus *Ceratosolen* but other, more distantly related taxa should not be included. Equally, distantly related clades should not be combined in a single jungle (as in Fig. 2). Effectively such combinations introduce older nodes and elements of different hypotheses (i.e. referring to familial divergences) and inflate the number of codivergences artefactually.

(6) Phylogenies of the *Ficus* microcosm have given a qualified affirmation of the consensus that figs and their insect associates have tracked one another through time. There does appear to be a fundamental difference in the level of codivergence between pollinators and non-pollinators but this should not be seen as an inviolable rule. The recent results of Molbo *et al.* (2003) demonstrate that switches and multiple interactions may routinely affect the fig-pollinator cophylogeny and, depending on the host phylogeny, that the dynamics at the contemporary and ancient scales may be at odds. This raises the question of whether extinction is responsible for 'thinning out' the pollinator clades and, to some extent, restoring a codivergent pattern in older comparisons.

(7) This and other recent work has indicated a more complex *Ficus* cophylogeny than previously thought, and points the way forward; we must bridge the gap between microevolution of species boundaries and host affiliations and cophylogeny. We must discover what happens at the 'frontier' of cophylogeny, that is, the moment when taxa and host associations diversify. We must examine the population structure of extant associations to (i) characterise the pressures, be they ecological or historical, that enforce codivergence and (ii) identify ecological pressures that cause wasps to make transitions independently of their hosts. This will demonstrate how limited cophylogenetic comparisons are as a record of history and will reveal the full complexity of transitions taking place as *Ficus* evolves with its assembled community.

## VI. ACKNOWLEDGEMENTS

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## VII. REFERENCES

- ANSTETT, M. C. (2001). Unbeatable strategy, constraint and coevolution, or how to resolve evolutionary conflicts: the case of the fig/wasp mutualism. *Oikos* **95**, 476–484.
- ANSTETT, M. C., HOSSAERT-McKEY, M. & KJELLBERG, F. (1997). Figs and fig pollinators: evolutionary conflicts in a coevolved mutualism. *Trends in Ecology and Evolution* **12**, 94–99.
- BERG, C. C. (1989). Classification and distribution of *Ficus*. *Experientia* **45**, 605–611.
- BERG, C. C. & WIEBES, J. T. (1992). *African Fig Trees and Fig Wasps*. North-Holland, Amsterdam.
- BOUCEK, Z. (1993). The genera of Chalcidoid Wasps from *Ficus* fruit in the new world. *Journal of Natural History* **27**, 173–217.
- BRONSTEIN, J. L. (1987). Maintenance of species-specificity in a neotropical fig – pollinator wasp mutualism. *Oikos* **48**, 39–46.
- BRONSTEIN, J. L. (1991). The non-pollinating wasp fauna of *Ficus pertusa* – exploitation of a mutualism. *Oikos* **61**, 175–186.
- CHARLESTON, M. A. (1998). *Jungles*: a new solution to the host/parasite phylogeny reconciliation problem. *Mathematical Bioscience* **149**, 191–223.
- CHARLESTON, M. A. (2003). Recent results in cophylogeny mapping. *Advances in Parasitology* **54**, 303–330.
- CHARLESTON, M. A. & PAGE, R. D. M. (2002). TREEMAP v2.0. Application for Apple Macintosh.
- CLARK, M. A., MORAN, N. A., BAUMANN, P. & WERNEGREEN, J. J. (2000). Cospeciation between bacterial endosymbionts (*Buchnera*) and a recent radiation of aphids (*Uroleucon*) and pitfalls of testing for phylogenetic congruence. *Evolution* **54**, 517–525.
- COMPTON, S. G., RASPLUS, J. Y. & WARE, A. B. (1994). African fig wasp parasitoid communities. In *Parasitoid Community Ecology* (eds B. A. Hawkins and W. Sheehan), pp. 343–368. Oxford University Press, Oxford.
- COOK, J. M. & RASPLUS, J. Y. (2003). Mutualists with attitude: coevolving fig wasps and figs. *Trends in Ecology and Evolution* **18**, 241–248.
- CORNER, E. J. H. (1965). Check-list of *Ficus* in Asia and Australasia, with keys to identification. *Gardens Bulletin of Singapore* **21**, 1–186.
- DUFAY, M. & ANSTETT, M. C. (2003). Conflicts between plants and pollinators that reproduce within inflorescences: evolutionary variations on a theme. *Oikos* **100**, 3–14.
- FARRIS, J. S. (1969). A successive approximations approach to character weighting. *Systematic Zoology* **18**, 374–385.
- FELSENSTEIN, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.
- GALLI, J. & EISIKOWITCH, D. (1968). On the pollinating ecology of *Ficus sycamorus* in East Africa. *Ecology* **49**, 259–269.
- GIBBONS, J. R. H. (1979). A model of sympatric speciation in *Megarhyssa* (Hymenoptera: Ichneumonidae): competitive speciation. *American Naturalist* **114**, 719–741.
- GRISON, L., EDWARDS, A. A. & HOSSAERT-McKEY, M. (1999). Interspecies variation in floral fragrances emitted by tropical *Ficus* species. *Phytochemistry* **52**, 1293–1299.

- GRISON-PIGE, L., HOSSAERT-McKEY, M., GREEFF, J. M. & BESSIERE, J. M. (2002). Fig volatile compounds – a first comparative study. *Phytochemistry* **61**, 61–71.
- HAFNER, M. S. & NADLER, S. A. (1990). Cospeciation in host-parasite assemblages – comparative-analysis of rates of evolution and timing of cospeciation events. *Systematic Zoology* **39**, 192–204.
- HAFNER, M. S. & PAGE, R. D. M. (1995). Molecular phylogenies and host-parasite cospeciation – gophers and lice as a model system. *Philosophical Transactions of the Royal Society of London, Series B* **349**, 77–83.
- HAWKINS, B. A. & COMPTON, S. G. (1992). African fig wasp communities – undersaturation and latitudinal gradients in species richness. *Journal of Animal Ecology* **61**, 361–372.
- HERRE, E. A. (1989). Coevolution of reproductive characteristics in 12 species of new world figs and their pollinator wasps. *Experientia* **45**, 637–647.
- HERRE, E. A., KNOWLTON, N., MUELLER, U. G. & REHNER, S. A. (1999). The evolution of mutualisms: exploring the paths between conflict and cooperation. *Trends in Ecology and Evolution* **14**, 49–53.
- HERRE, E. A., MACHADO, C. A., BERMINGHAM, E., NASON, J. D., WINDSOR, D. M., MACCAFFERTY, S. S., VAN HOUTEN, W. & BACHMANN, K. (1996). Molecular phylogenies of figs and their pollinating wasps. *Journal of Biogeography* **23**, 521–530.
- HERRE, E. A. & WEST, S. A. (1997). Conflict of interest in a mutualism: documenting the elusive fig wasp seed trade-off. *Proceedings of the Royal Society of London, Series B* **264**, 1501–1507.
- HOSSAERT-McKEY, M., GIBERNAU, M. & FREY, J. E. (1994). Chemosensory attraction of fig wasps to substances produced by receptive figs. *Entomologia Experimentalis et Applicata* **70**, 185–191.
- HUELSENBECK, J. P., RANNALA, B. & LARGET, B. (2000). A Bayesian framework for the analysis of cospeciation. *Evolution* **54**, 352–364.
- HUELSENBECK, J. P., RANNALA, B. & YANG, Z. H. (1997). Statistical tests of host-parasite cospeciation. *Evolution* **51**, 410–419.
- JANZEN, D. H. (1979). How to be a fig. *Annual Review of Ecology and Systematics* **10**, 13–51.
- JOHNSON, K. P., DROWN, D. M. & CLAYTON, D. H. (2000). A data based parsimony method of cophylogenetic analysis. *Zoological Scripta* **30**, 79–87.
- JOUSSELIN, E., RASPLUS, J. Y. & KJELLBERG, F. (2003). Convergence and coevolution in a mutualism: evidence from a molecular phylogeny of *Ficus*. *Evolution* **57**, 1255–1269.
- KERDELHUÉ, C., HOCHBERG, M. E. & RASPLUS, J. Y. (1997). Active pollination of *Ficus sur* by two sympatric fig wasp species in West Africa. *Biotropica* **29**, 69–75.
- KERDELHUÉ, C., LE CLAINCHE, I. & RASPLUS, J. Y. (1999). Molecular phylogeny of the *Ceratostenes* species pollinating *Ficus* of the subgenus *Sycomor* *sensu stricto*: biogeographical history and origins of the species-specificity breakdown cases. *Molecular Phylogenetics and Evolution* **11**, 401–414.
- KERDELHUÉ, C. & RASPLUS, J. Y. (1996). Non-pollinating Afro-tropical fig wasps affect the fig-pollinator mutualism in *Ficus* within the subgenus *Sycomor*. *Oikos* **75**, 3–14.
- KERDELHUÉ, C., ROSSI, J.-P. & RASPLUS, J.-Y. (2000). Comparative community ecology studies on old world figs and fig wasps. *Ecology* **81**, 2832–2849.
- LOPEZ-VAAMONDE, C., RASPLUS, J.-Y., WEIBLEN, G. D. & COOK, J. M. (2001). Molecular phylogenies of fig-wasps: partial cocladogenesis of pollinators and parasites. *Molecular Phylogenetics and Evolution* **21**, 55–71.
- MACHADO, C. A., HERRE, E. A., MCCAFFERTY, S. & BERMINGHAM, E. (1996). Molecular phylogenies of fig pollinating and non-pollinating wasps and the implications for the origin and evolution of the fig-fig wasp mutualism. *Journal of Biogeography* **23**, 531–542.
- MACHADO, C. A., JOUSSELIN, E., KJELLBERG, F. & COMPTON, S. G. & HERRE, E. A. (2001). Phylogenetic relationships, historical biogeography and character evolution of fig-pollinating wasps. *Proceedings of the Royal Society of London, Series B-Biological Sciences* **268**, 1–10.
- MICHALOUD, G., CARRIERE, S. & KOBBI, M. (1996). Exceptions to the one:one relationship between African fig trees and their fig wasp pollinators: possible evolutionary scenarios. *Journal of Biogeography* **23**, 513–520.
- MOLBO, D., MACHADO, C. A., SEVENSTER, J. G., KELLER, L. & HERRE, E. A. (2003). Cryptic species of fig-pollinating wasps: implications for the evolution of the fig-wasp mutualism, sex allocation and precision of adaptation. *Proceedings of the National Academy of Sciences of the USA* **100**, 5867–5872.
- MURRAY, M. G. (1985). Figs (*Ficus* spp.) and fig-wasps (Chalcidoidea, Agaonidae) – hypotheses for an ancient symbiosis. *Biological Journal of the Linnean Society* **26**, 69–81.
- NEFDI, R. J. C. & COMPTON, S. G. (1996). Regulation of seed and pollinator production in the fig fig wasp mutualism. *Journal of Animal Ecology* **65**, 170–182.
- PAGE, R. D. M. (1988). Quantitative cladistic biogeography: constructing and comparing area cladograms. *Systematic Zoology* **37**, 254–270.
- PAGE, R. D. M. (1990). Component analysis – a valiant failure? *Cladistics* **6**, 119–136.
- PAGE, R. D. M. (1994a). Maps between trees and cladistic-analysis of historical associations among genes, organisms, and areas. *Systematic Biology* **43**, 58–77.
- PAGE, R. D. M. (1994b). Parallel phylogenies – reconstructing the history of host-parasite assemblages. *Cladistics* **10**, 155–173.
- PAGE, R. D. M. (1995). TREEMAP v1.0. Application for Apple Macintosh.
- PAGE, R. D. M. & CHARLESTON, M. A. (1998). Trees within trees: phylogeny and historical associations. *Trends in Ecology and Evolution* **13**, 356–359.
- RAMIREZ, W. B. (1970). Host specificity of fig wasps (Agaonidae). *Evolution* **24**, 681–691.
- RAMIREZ, W. B. (1974). Specificity of Agaonidae: the coevolution of *Ficus* and its pollinators. Ph.D thesis, University of Kansas.
- RAMIREZ, W. B. & MALAVASI, J. (1997). Fig wasps: mechanisms of pollen transfer in Malvanthera and Pharmacosycea figs (Moraceae). *Revista de Biología Tropical* **45**, 1635–1640.
- RONQUIST, F. (1995). Reconstructing the history of host-parasite associations using generalised parsimony. *Cladistics* **11**, 73–89.
- SHIMODAIRA, H. & HASEGAWA, M. (1999). Multiple comparisons of log-likelihoods with applications to phylogenetic inference. *Molecular Biology and Evolution* **16**, 114–116.
- STRIMMER, K. & VON HAESLER, A. (1996). Quartet puzzling: a quartet maximum-likelihood method for reconstructing tree topologies. *Molecular Biology and Evolution* **13**, 964–969.
- SWOFFORD, D. W. (1998). PAUP\*. Phylogenetic Reconstruction Using Parsimony and Other Methods. Sinauer Associates, Sunderland.
- SWOFFORD, D. W., OLSEN, G. J., WADDELL, P. J. & HILLIS, D. M. (1996). Phylogenetic Inference. In *Molecular Systematics*, 2nd Edn. (ed. D. M. Hillis), pp. 407–514. Sinauer Associates, Sunderland.

- THOMPSON, J. D., GIBSON, T. J., PLEWNIAK, F., JEANMOUGIN, F. & HIGGINS, D. G. (1997). The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acid Research* **24**, 4876–4882.
- ULENBERG, S. A. (1985). The systematics of the fig-wasp parasites of the genus *Apocrypta* (Coquerel). North-Holland, Amsterdam.
- WARE, A. B. & COMPTON, S. G. (1992). Breakdown of pollinator specificity in an african fig tree. *Biotropica* **24**, 544–549.
- WARE, A. B., KAYE, P. T., COMPTON, S. G. & VAN NOORT, S. (1993). Fig volatiles – their role in attracting pollinators and maintaining pollinator specificity. *Plant Systematics and Evolution* **186**, 147–156.
- WEIBLEN, G. D. (2000). Phylogenetic relationships of functionally dioecious *Ficus* (Moraceae) based on ribosomal DNA sequences and morphology. *American Journal of Botany* **87**, 1342–1357.
- WEIBLEN, G. D. (2001). Phylogenetic relationships of fig-wasps pollinating functionally dioecious *Ficus* based on mitochondrial DNA sequences and morphology. *Systematic Biology* **50**, 243–267.
- WEIBLEN, G. D. & BUSH, G. L. (2002). Speciation in fig pollinators and parasites. *Molecular Ecology* **11**, 1573–1578.
- WEST, S. A., HERRE, E. A., WINDSOR, D. M. & GREEN, P. R. S. (1996). The ecology and evolution of the New World non-pollinating fig wasp communities. *Journal of Biogeography* **23**, 447–458.
- WEST, S. A., MURRAY, M. G., MACHADO, C. A., GRIFFIN, A. S. & HERRE, E. A. (2001). Testing Hamilton's rule with competition between relatives. *Nature* **409**, 510–513.
- WIEBES, J. T. (1979). Coevolution of figs and their insect pollinators. *Annual Review of Ecology and Systematics* **10**, 1–12.
- WIEBES, J. T. (1989). Agaonidae (Chalcidoidea, Hymenoptera) and *Ficus* (Moraceae): figs and their fig-wasps, III (*Elisabethiella*). *Proceedings of the Koninklijke Nederlandse Akademie Van Wetenschappen, Series C-Biological And Medical Sciences* **92**, 117–136.