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A RECONCILIATION ANALYSIS OF HOST SWITCHING IN PLANT-FUNGAL SYMBIOSES

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Abstract.—Plant-fungal symbioses include many familiar antagonistic and mutualistic associations and some model cases of coevolution. The relationship between coevolution at the different evolutionary scales has remained an open question. Widespread host specificity and documented host switches offer conflicting indications of what to expect from comparisons of plant and fungal phylogenies. This study sought to establish the role of plant phylogeny in determining fungal phylogeny and the relative contributions of codivergence and host switching by comparing tree topologies for 15 plant-fungal symbioses. Second it attempted to characterize the relationship between phylogenetic congruence and switching. Trees were estimated from published sequences and reconciliation analysis was applied in the form of cophylogeny mapping using ''jungles''. This provided an exhaustive account of all possible switches capable of reconciling two associated phylogenies. A continuum of cophylogenetic dynamics was identified, ranging from mostly codivergence (e.g., *Exobasidium*) to mostly switching, (e.g., *Erysiphe*). Surprisingly, congruent solutions do not necessarily have fewer switches when using cophylogeny mapping, but a significant negative relationship between congruence and the distance of switches proved to be a useful indicator. According to reconciliation analysis, the contribution of host phylogeny varies widely across plant-fungal symbioses, making host specificity and coadaptation poor indicators of macroevolutionary trends because they are necessary, but not sufficient, conditions.

Key words.—Codivergence, cophylogeny, fungal pathogen, mutualism, reconciliation analysis, symbiosis.

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Evolutionary biologists and agriculturalists alike have long appreciated that fungal life cycles are intricately and ubiquitously linked with those of plants. The fungal pathogens *Puccinia* spp. and *Melampsora lini* have provided seminal coevolutionary models, and lichens are a standard for mutualistic symbiosis (Barrett 1983). This study evaluated the correspondence between the phylogenies of plant and fungal symbionts.

It has been assumed that plants and fungi share a long evolutionary history and that, logically, this should be reflected in broad phylogenetic congruence (Hart 1988; Berbee 2001). This expectation was endorsed by corresponding taxonomy, although it may be a product of circular reasoning since fungi have been used in elucidating higher plant classificiation (Saville 1979). The considerable evidence for coadaptation between plants and their fungal pathogens—most eminently the reciprocal evolution of virulence and resistance between flax (*Linum usitatissimum*) and the rust *Melampsora lini* (Thrall et al. 2002)—seemed to offer a mechanism for the strict host specificity often observed and assumed to be maintained over evolutionary time scales. However, loss of adaptation in plants placed in enemy-free space and their subsequent general susceptibility suggests that specialization may occur due to local adaptation, acquired and lost over ecological time (Hijwegen 1988; Frank 1992).

The observation that primitive rust fungi were not found on primitive hosts (Hart 1988) indicated that rust evolution was not a simple case of inveterate cospeciation. Recently, cophylogenetic studies of *Puccinia* spp. and lichens have shown that coevolution and coadaptation are not translated into phylogenetic congruence (Piercey-Normore and DePriest 2001; Roy 2001). Rather, they are compatible with regular switching between hosts. Berbee (2001) asserts that variation should be expected in the phylogenetic outcomes of plant-fungal coevolution, due to variation in coevolutionary dynamics themselves, and suggests more systematic studies are necessary to test the significance of phylogenetic correspondence now that adequate phylogenies are available. This study addresses that need; the primary aim was to assess the relative contributions of codivergence and switching to cophylogeny in 15 plant-fungal symbioses through reconciliation analysis.

Phylogenetic reconciliation is a procedure for the comparison of two phylogenetic trees that are assumed to share a common history. Under this assumption, the two topologies should be congruent but there frequently will be differences. Evolutionary events from a prescribed model are used to explain the incongruities between their topologies (Page and Charleston 1998; Paterson et al. 2000). There are several methods for reconciling trees, but this study uses cophylogeny mapping (Page 1993; Page and Charleston 1998). The associate tree is embedded within the host tree and associate nodes are mapped to optimal positions within the host topology, based on known associations. Each reconciled tree has a particular combination of evolutionary events, and any particular analysis can produce several different combinations (Page 1990, 1994).

Figure 1 illustrates the reconciliation procedure: a model containing four events (a) is used to reconcile two phylogenies (b) with six associations (A–F). The events are: codivergence (synchronous cladogenesis or phylogenetic tracking), duplication (unilateral duplication of an associate), loss (the unilateral extinction or disappearance of an associate), and switching (the unilateral transfer of an associate to another host; Page 1990, 1994). Cophylogeny mapping yields seven solutions (Fig. 1c) ranging from one without switches (at left) through different combinations of duplications, losses, and switches.

Clearly, there are many ways to reconcile any particular pair of trees. Reconciliation analysis requires the location of

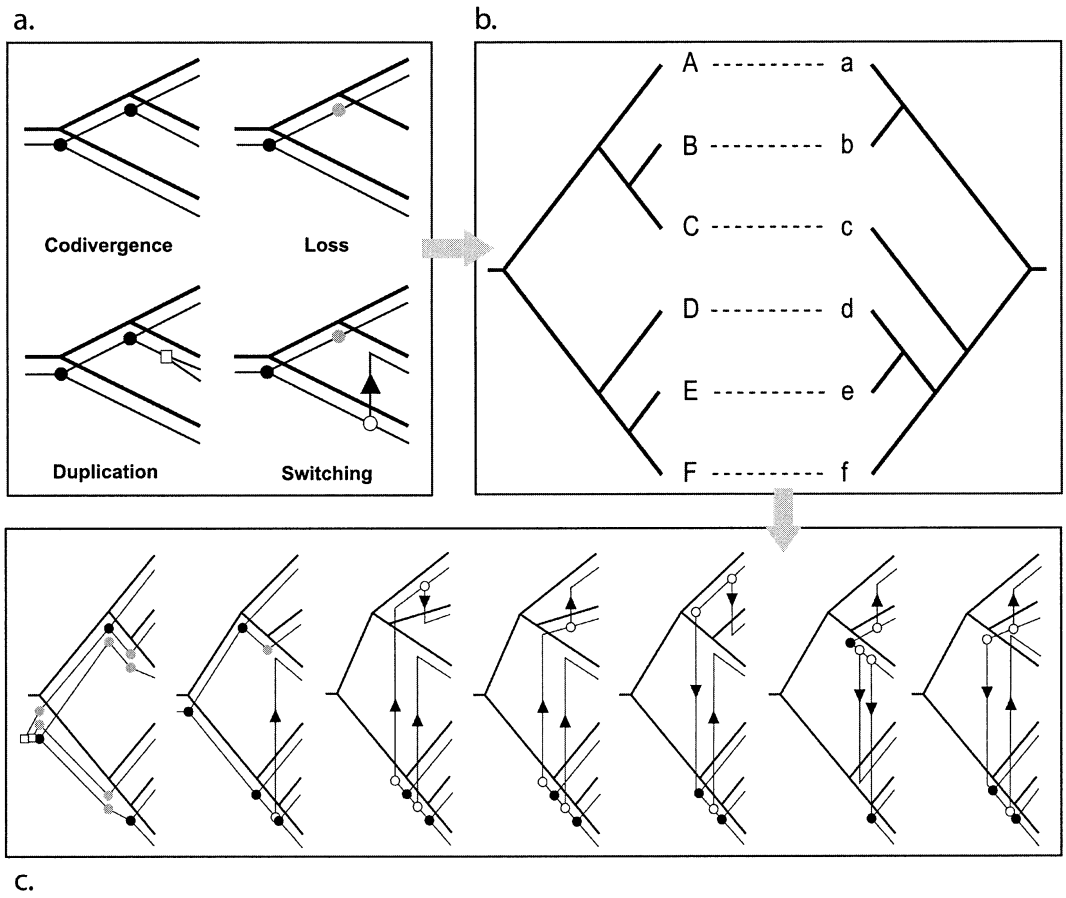

FIG. 1. Phylogenetic reconciliation by cophylogeny mapping. (a) A model comprising four evolutionary events is used to explain incongruence between two phylogenies. Evolutionary events have the following symbols: codivergence (black dot), duplication (open box), loss (gray dot), or switch (black triangle). (b) Phylogenies for six hosts (A–F) and associates (a–f) are compared and differ in a single respect: associate c is misplaced. (c) Application of the evolutionary model to explain the position of c has 847 possible solutions, of which seven are potentially optimal under some set of event costs.

all possible combinations of events and then the selection of optimal solutions from these. Until the addition of the ''jungle'' into cophylogeny mapping, this process was hampered by an inability to locate all possible switches (Page 1994; Charleston 1998). A jungle is a directed graph containing all the potentially optimal reconstructions (POpt) of the reconciled tree (Charleston 1998), that is, all solutions that could, under some set of costs, require fewest events for reconciliation. In Figure 1c, there are seven solutions in POpt, although there are 847 possible solutions; those shown at left would be optimal if switches were costly (or considered unlikely), while those at right would be optimal if loss was considered unlikely. Unlike previous algorithms, the jungle includes weakly incompatible host switches, that is those that require subsequent loss events to make the source and destination contemporary (Page 1994; Charleston 1998) and so provides both a quantitative and exhaustive solution to the reconciliation problem (Charleston 1998).

Cophylogeny mapping assumes that the component phylogenies are robust and that the host-associate interaction is asymmetric (i.e., the associate tracks the host). It is more conservative in its assumptions about the relative probabilities of evolutionary events than methods that use likelihood-based macroevolutionary models (Huelsenbeck et al. 2000) or that compare the total costs of solutions using weighted parsimony (Ronquist 1995). Differential costs can be applied to events but, typically, insufficient information exists to justify this and so non-codivergence events are treated equally.

Comparison of plant and fungal phylogenies and the significance of the observed codivergence was evaluated using the program TreeMap version 2.0 (Charleston and Page 2002), in which randomization tests were used to test the null hypothesis that any topological similarity could be accounted for by chance alone. In addition to the primary aim of assessing the role of plant phylogeny in determining fungal phylogeny, symbioses were compared to identify a useful indicator of significant switching. The observed congruence was correlated with the number of switches across all solutions, the distribution of switches across all lineages, and the distance passed by switches across all solutions to reveal the pattern of switches typical for a significant level of congruence.

METHODS

Sampling

Finding POpt for a jungle is computationally intensive, and the number of possible solutions increases exponentially

with the number of taxa (Charleston 2003). The process quickly becomes insoluble as the number of taxa passes an effective limit of about 20 associations. This constraint notwithstanding, taxa were selected for which monophyly and identity were well defined, as in the case of *Monilinia* or *Epichloë*. Taxa were only excluded if they were unresolved in the associate tree, if no information existed to place their host, or they possessed so many hosts that they prevented completion of the reconciliation analysis. Phylogenies for *Cintractia*, Exobasidiales, and *Tilletia* each had several taxa removed because their hosts could not be placed. The effect of these exclusions was investigated with alternative jungles where they replaced another taxon. The most closely related host to that of the excluded taxon was then assigned as a surrogate.

In some cases, historical associations seemed to transcend established taxonomy; for *Cintractia* and *Ustilago*, the taxon set was selected from a 28S rRNA phylogeny of the Ustilaginaceae. This suggested that *Ustilago* was paraphyletic with *Sporisorium* and *Cintractia* formed a clade with various genera (see Table 1), all of which were parasitic on Poales, mostly either Cyperaceae or Juncaceae. Similarly, using a larger Urediniomycetes 28S rRNA phylogeny, *Cronartium*, *Phragmidium*, and *Uromyces* were each found to be paraphyletic with other genera. Details of taxon and datasets are given in Table 1.

Phylogenetic Estimation

Thirty phylogenies were estimated using published molecular sequences, retrieved from GenBank (a catalogue of sequences and all alignments are available from the author). Sequences were aligned by eye after initial multiple alignment in ClustalX (Thompson et al. 1997). Phylogenies were estimated using PAUP* version 4.0b (Swofford 1998). The method of choice was maximum parsimony (MP) with serial approximation reweighting (SAR). This was chosen to maximize resolution with the best possible support, as TreeMap cannot interpret polytomies. SAR downweights homoplasic characters through sequential reweighting, according to the rescaled consistency index of the previous estimate (Farris 1969; Swofford et al. 1996). Each phylogeny was reconstructed using the general program settings: TBR swapping algorithm, initial tree obtained by simple stepwise addition, gaps were treated as missing data (except in association 7), Acctran character state optimization was employed, and multiple states considered uncertain. Confidence intervals for each estimate were obtained using 500 nonparametric bootstrap replicates (Felsenstein 1985). To assess the effects of bias in substitution rates across sites, the data was also estimated using maximum likelihood (ML), estimated with a full general time reversible model (Yang 1994), and neighbor joining (NJ) methods, estimated with log-determinate corrections to check the effect of base composition imbalance (Lockhart et al. 1994).

Cophylogeny Mapping

To exclude the possibility that sampling error could account for any differences between two trees, that is that they possess an identical underlying topology, two tests were performed. First, an incongruence length difference (ILD) test (Farris et al. 1994) was used to compare MP tree lengths; the two datasets were combined and compared using the partition homogeneity function in PAUP*. Second, a Shimodaira-Hasegawa (SH) test (Shimodaira and Hasegawa 1999) was used to compare ML scores in PAUP*.

Each pair of MP topologies was reconciled using TreeMap version 2.0, which allows the user to specify the conditions for optimality by placing explicit costs and bounds on each evolutionary event. The conditions were relaxed in this study because little information exists to specify the relative probabilities of different events. The costs of each event were set to default: codivergence (0); duplication, loss, and switch (1). This is necessary to place a pragmatic limit on the possible solutions, ensuring that if two trees are identical, the only optimal solution comprises codivergence events exclusively. Other than enforcing this constraint, the costs had no bearing on the results because total cost was not used to evaluate solutions. A complete jungle was constructed unless this exceeded memory capacity, which may happen with many multiple interactions or a high level of incongruence. In these cases, the least congruent solutions were often not recoverable. A few associates were present on widespread hosts, for example, in the association between *Cronartium* and *Pinus*, *C. ribicola* is associated with the *P. aristata*, *P. strobus*, and *P. lambertiana*, which are paraphyletic. Here, it was assumed that each host possessed a specific population of associate, which were treated as independent taxonomic units. For comparison with the MP topologies, ML trees were also reconciled.

Significance Testing

TreeMap evaluates the similarity, expressed in terms of the number of codivergences (N_{CE}), shared by two trees through randomization tests. For each jungle, 100 randomized associate trees were generated through resampling from the associate taxa. N_{CE} for these random trees was then compared to the observed N_{maxCE} in POpt. The null hypothesis that the level of similarity was due to chance alone was rejected if the given number of codivergences was seen in no more than five randomized jungles. Where it was not possible to obtain the significance of an event number, the significance value for the nearest event number possible was obtained and the missing value declared equal to or less than this. For example, the significance of N_{maxCE} (14) could not be found for *Erysiphe*, but the value for 16 was 0.16. Hence, the value for 14 must be equal to or greater than this. In evaluating the significance of congruence, TreeMap is more permissive than methods testing for a common tree topology using ML (Huelsenbeck et al. 1997; Legendre et al. 2002) or parsimony (Johnson et al. 2001) because these may reject cases where nonrandom similarity exists (Clark et al. 2000).

Correlations between Congruence and Switching Indicators

The percentage of codivergent nodes in the most congruent solution was correlated with three quantities. The first is the number of switches in the whole of POpt per solution. The second is the distribution of switches across all lineages and all solutions. To provide a synopsis of the distribution of

TABLE 1. Taxonomy, ecology, and phylogeny of 15 plant-fungal symbioses included in this study. Under Phylogeny, p.i. refers to the parsimony-informative component of a molecular character set.

	Class: Order	Family	Fungus	Plant hosts	Phylogeny
1	Basidiomycota: Ustilaginomycetes		Exobasidiales	Asteraceae/ Lauraceae	Associate phylogeny was estimated using nuclear rRNA sequences (562 bp, 142 p.i.; Begerow et al. 1997). Host phylogeny was estimated with nuclear rRNA sequences from various sources (810 bp, 401 p.i.). *Thea sinensis, Commelina* sp., and *Tradescantia* sp. were added to the host phylogeny on the basis of known taxonomic affinities.
2		Entylomataceae	*Entyloma*	Asteraceae	Associate phylogeny was estimated using nuclear rRNA (930 bp, 202 p.i., Begerow et al. 2002). Host phylogeny was estimated using nuclear rRNA (687 bp, 365 p.i.). *Arnoseris minima* was added on the basis of its placement in a second *ndhF* phylogeny that was consistent with rRNA.
3		Ustilaginaceae	*Cintractia*	Poales	Associates comprise a clade in the Ustilaginaceae phylogeny (see Methods): *Cintractia, Schizonella, Stegocintractia, Tolyposporium, Heterotolyposporium*. Their phylogeny was estimated using 28S rRNA sequences (542 bp, 72 p.i.; Begerow et al. 1997; Piepenbring et al. 1999). Host phylogeny was estimated using *rbcL* (1324 bp, 140 p.i.) from various sources.
4			*Ustilago*	Poaceae	Associate phylogeny includes *Ustilago* and *Sporisorium*, shown to be monophyletic in the Ustilaginaceae phylogeny, was estimated using rRNA ITS (771 bp, 221 p.i.; Roux et al. 1998; Bakkeren et al. 2000). Host phylogeny was estimated using nuclear rRNA sequences (634 bp, 222 p.i.) from various sources. *Sorghum bicolor* and *Scilla natalensis* were added on the basis of published grass phylogenies (Hilu et al. 1999; Mathews et al. 2000; Barker et al. 2001).
5		Tilletiaceae	*Tilletia*	Pooideae/ Panicoideae	Associate phylogeny was estimated using nuclear rRNA ITS (724 bp, 237 p.i.; Zhang et al., direct submission to GenBank). Host phylogeny was estimated using *ndhF* sequences from various sources. Fine relationships within the Aveneae were estimated using nuclear rRNA and checked against published grass phylogenies (Hilu et al. 1999; Mathews et al. 2000; Barker et al. 2001).
6	Basidiomycota: Urediniomycetes	Microbotryaceae	*Microbotryum*	Caryophyllales	Associate phylogeny comprises *Microbotryum,* including *Ustilago* on dicots and *Sphacelotheca* (Almaraz et al. 2002), and was estimated using rRNA ITS sequences (630 bp, 181 p.i.; Roux et al. 1998; Freeman et al. 2002). Host phylogeny was estimated using *rbcL* sequences (1299 bp, 181 p.i.), with fine relationships among the Asteridae being inferred using *ndhF* sequences (1222 bp, 182 p.i.).
7		Cronartiaceae	*Cronartium*	*Pinus*	Associates comprise a clade in the Urediniomycetes phylogeny: *Cronartium, Peridermium* and *Endocronartium.* The phylogeny was estimated using rRNA ITS, with gaps treated as informative characters (740 bp, 149 p.i.; Vogler and Bruns 1998). Two associates, *E. harknesii* and *C. ribicola,* were widespread on several hosts. Host phylogeny was estimated using *rbcL* sequences (1258 bp, 28 p.i.), with fine relationships among the *strobus* subgenus being inferred using rRNA sequences.

TABLE 1. Continued.

	Class: Order	Family	Fungus	Plant hosts	Phylogeny
8		Phragmidiaceae	*Phragmidium*	Rosaceae	Associates comprise a clade in the Urediniomycetes phylogeny, all parasitizing Rosaceae: *Phragmidium, Gymnosporangium, Thekospora, Tranzschelia, Kuehneola, Trachyspora,* and *Triphragmium.* Associate phylogeny was estimated using 28S rRNA sequences (531 bp, 95 p.i.; Maier et al. 2003). Host phylogeny was estimated using nuclear rRNA (670 bp, 183 p.i.), although *A. alpina, P. erectar* and *S. acupar* were placed using a second locus (rRNA ITS2, 275 bp, 113 p.i.).
9		Pucciniaceae	*Uromyces*	Rosidae	Associates comprise a clade in the Urediniomycetes phylogeny all parasitizing Rosidae: *Uromyces, Helicobasidium, Melampsoridium,* and *Septobasidium.* Associate phylogeny was estimated using rRNA ITS (738 bp, 280 p.i.; Pfunder et al. 2001). Host phylogeny was estimated using *rbcL* (1360 bp, 165 p.i.); *Betula* sp., *Alnus* sp., and *G. sagittalis* were each placed according to known taxonomic affinities.
10	Basidiomycota: Homobasidiomycetes		Homobasidiomycetes	Monotropoideae	Fungal phylogeny was estimated using rRNA ITS sequences (614 bp, 258 p.i.; Bidartondo and Bruns 2002). They are hosts to a closely related group of parasitic plants in the family Monotropoideae. Plant phylogeny was estimated also using rRNA ITS sequences (756 bp, 420 p.i). Note that *Monotropa hypopitys* is known from several *Tricholoma* hosts, which are grouped together in this analysis.
11	Ascomycota: Sordariomycetes	Clavicipitaceae	*Epichloë*	Pooideae	Associate phylogeny was estimated using a combined, and compatible, dataset of *tub2* and *EF1* (1433 bp, 199 p.i.; Craven et al. 2001). Host phylogeny was estimated using nuclear rRNA (612 bp, 135 p.i.), with certain taxa being placed using previous grass phylogenies (Hilu et al. 1999; Mathews et al. 2000; Barker et al. 2001).
12			*Claviceps*	Poaceae	Associate phylogeny was estimated using nuclear rRNA (626 bp, 103 p.i.; Pazoutova 2001). *C. purpurea* was excluded because it possessed many hosts and would prevent completion of the analysis. Host phylogeny was estimated using *ndhF* sequences (2095 bp, 230 p.i.).
13	Ascomycota: Leotiomycetes	Erysiphaceae	*Erysiphe*	Asteridae/ Rosaceae	Associate phylogeny included both *Erysiphe* and *Microsphaera,* which are monophyletic (Saenz and Taylor 1999). It was estimated using rRNA ITS (609 bp, 136 p.i.; Takamatsu et al. 1999; Saenz and Taylor 1999). Host phylogeny was estimated using *rbcL* (1292 bp, 185 p.i.).
14			*Golovinomyces*	Asteraceae	Associate phylogeny was inferred from nuclear rRNA (Matsuda and Takamatsu 2003). The majority of available *Golovinomyces* parasitic on Lactuceae were not included because it is known that these parasites have little host specificity. The hypothesis tested here is that significant congruence exists in the basal portions of the *Golovinomyces* phylogeny (sensu Matsuda and Takamatsu 2003), where host specificity is greater. Host phylogeny was estimated using rRNA ITS (709 bp, 404 p.i.), with tribal relationships enforced according to the Asteraceae phylogeny (Bayer and Starr 1998).
15		Sclerotiniaceae	*Monillinia*	Asteridae/ Rosidae	Associate phylogeny includes both *Monolinia* and *Scolerotinia priolae,* which are monophyletic (Holst-Jensen et al. 1997). It was estimated using nuclear rRNA (1321 bp, 162 p.i.; Holst-Jensen et al. 1997). *M. polycodii* was excluded because no sequence was available to place its host. Host phylogeny was estimated using nuclear rRNA (729 bp, 705 p.i.).

switches across all branches (either importing or exporting associates) of the host tree, the *D* statistic was employed. This index originally described the dispersion of a population of parasites among hosts (Poulin 1996) and is desirable since it can be compared between host populations (i.e., trees) of different sizes, whereas ordinary measures of overdispersion cannot. The index quantifies aggregation as the difference between the observed distribution of switches and that expected when all branches are equally involved and switches are uniformally distributed. It does this by comparing the cumulative numbers of switches and branches, and ranges between zero (underdispersion) to one (overdispersion):

$$D = 1 - \frac{2\sum_{i=1}^{N}\left(\sum_{j=1}^{i} x_j\right)}{\bar{x}N(N+1)}, \quad (1)$$

where x is the number of switches on branch j (where i branches are ranked according to increasing switching), $\bar{x}$ is the average number of switches on all branches and N is the total number of branches in a tree. The third quantity is the mean sum of switch distances across all solutions; each switch has a distance measured by the number of nodes circumvented and each solution has a sum distance for all switches. This quantity is the mean value for the sum distance across all solutions in POpt.

Results

Phylogenetic Estimation

Phylogenetic reconstruction using MP with SAR produced fully resolved input trees for most associates and hosts. For three hosts each in the Exobasidiales and *Uromyces* phylogenies these taxa were placed according to taxonomy (Table 1). These trees were robust and, where they could be resolved, ML and NJ topologies were concordant. Incorporation of adjustments for bias due to rate heterogeneity, invariant sites, and base composition into ML and NJ estimations failed to produce alternative topologies, suggesting that little bias was undermining the MP search. Associate and host topologies were significantly different according to both the ILD and SH tests ($P < 0.03$ in all cases). Therefore, sampling errors could not account for the differences between the phylogenies of any of these associations.

The Contributions of Codivergence and Host Switching to POpt

For each plant-fungal symbiosis, cophylogeny mapping produced an exhaustive set of potentially optimal reconstructions. Details of POpt and the significance of observed codivergence are given in Table 2. It was evident from Figure 1 that the number of solutions in POpt could be very large, ranging from predominantly codivergent solutions to those mostly characterized by switching. Summarizing this information is challenging and Figure 2a,b attempts to provide a consensus for each POpt. An individual diagram plots the number of each evolutionary event for each solution on four axes. This produces a series of overlapping quadrangles; those solutions with a significant level of codivergence have

TABLE 2. Results of reconciliation analysis for 15 plant-fungal associations. |POpt| describes the number of potentially optimal solutions produced by a jungle analysis; N_{maxCE}, P_{maxCE}, and $\%_{maxCE}$ denote the maximum observed number of codivergence events, the significance of that value, and expressed as a percentage of all nodes, respectively; No. refers to the number of solutions with significant codivergence; characters in bold refer to significant codivergence; an asterisk denotes an incomplete POpt, where not all solutions could be recovered.

Plant	*n*	Fungus	*n*	Maximum parsimony topologies					Maximum likelihood topologies			
				|POpt|	N_{maxCE}	p_{maxCE}	±	$\%_{maxCE}$	No.	|POpt|	N_{maxCE}	p_{maxCE}	±
Asteraceae/Lauraceae	17	Exobasidiales	17	69	**24**	**<0.01**	0.007	**75.0**	18	344*	**22**	**<0.01**	0.007
Asteraceae	12	*Entyloma*	12	156	**14**	**0.04**	0.02	**70.0**	20	29	**14**	**0.04**	0.02
Poales	12	*Cintractia*	12	9	**18**	**<0.01**	0.007	**81.8**	6	13	**18**	**<0.01**	0.007
Poaceae	11	*Ustilago*	13	24	12	0.345	0.088	50.0	0	77	12	0.345	0.088
Pooideae	13	*Tilletia*	13	57	14	0.310	0.086	58.3	0	23	14	0.310	0.086
Caryophyllales	14	*Microbotryum*	14	19	16	0.116	0.039	61.5	0	23	16	0.116	0.039
Pinus	10	*Cronartium*	16	11	16	0.186	0.059	53.3	0	62	14	>0.186	
Rosaceae	13	*Phragmidium*	15	69	**18**	**<0.01**	0.007	**64.3**	4	47	**18**	**<0.01**	0.007
Rosidae	13	*Uromyces*	13	60	14	0.260	0.062	58.3	0	22	16	0.12	0.045
Monotropeae	10	Homobasidiomycetes	10	2	**16**	**<0.01**	0.007	**88.9**	1	8	**14**	**<0.01**	0.007
Pooideae	13	*Epichloë*	13	69	12	0.58	0.099	50.0	0	478*	12	0.58	0.099
Poaceae	11	*Claviceps*	13	47	14	0.137	0.048	58.3	0	*	10	>0.137	
Asteridae/Rosaceae	14	*Erysiphe*	14	30*	14	>0.16		53.8	0	*	10	>0.16	
Asteraceae	16	*Golovinomyces*	16	38*	**24**	**<0.01**	0.007	**80.0**	24	35	**22**	**<0.01**	0.007
Asteridae/Rosidae	13	*Monilinia*	13	12*	**16**	**0.04**	0.02	**66.7**	4	22	**18**	**<0.01**	0.007

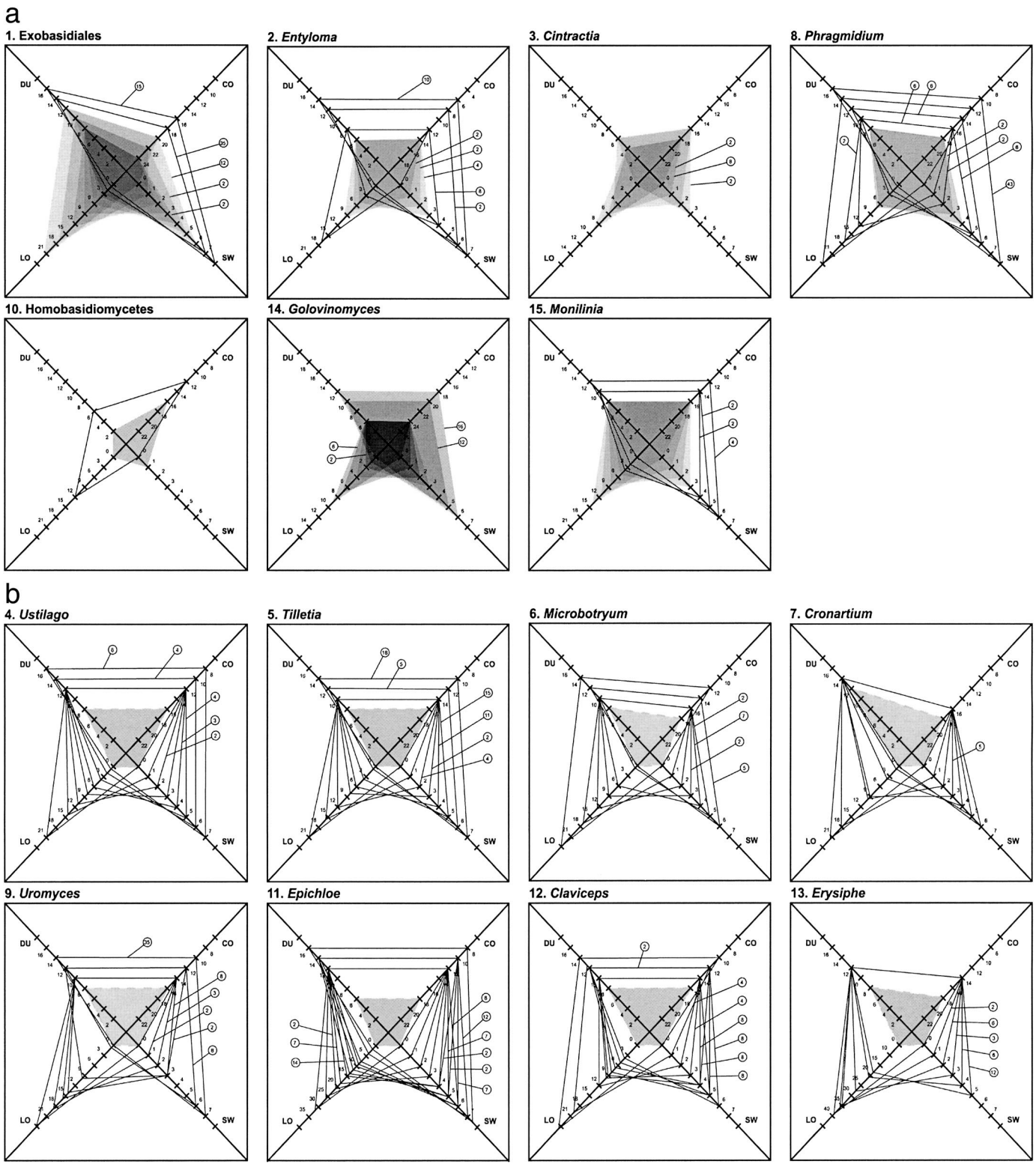

FIG. 2. Consensus diagrams showing all potentially optimal combinations of events for the reconciliation of (a) seven plant-fungal symbioses showing significant codivergence and (b) eight incongruent associations. Each solution is a combination of four evolutionary events: codivergence (CO), duplication (DU), loss (LO), and switching (SW). A given combination of events is depicted as a quadrangle drawn between four points coinciding with the number of each event. Circled numerals indicate multiple solutions with identical combinations. Significantly congruent solutions are shaded. For example, the outermost quadrangle in the figure for Exobasidiales indicates that there were 15 nonsignificant solutions with 16 codivergence events, 16 duplications, two losses, and eight switches.

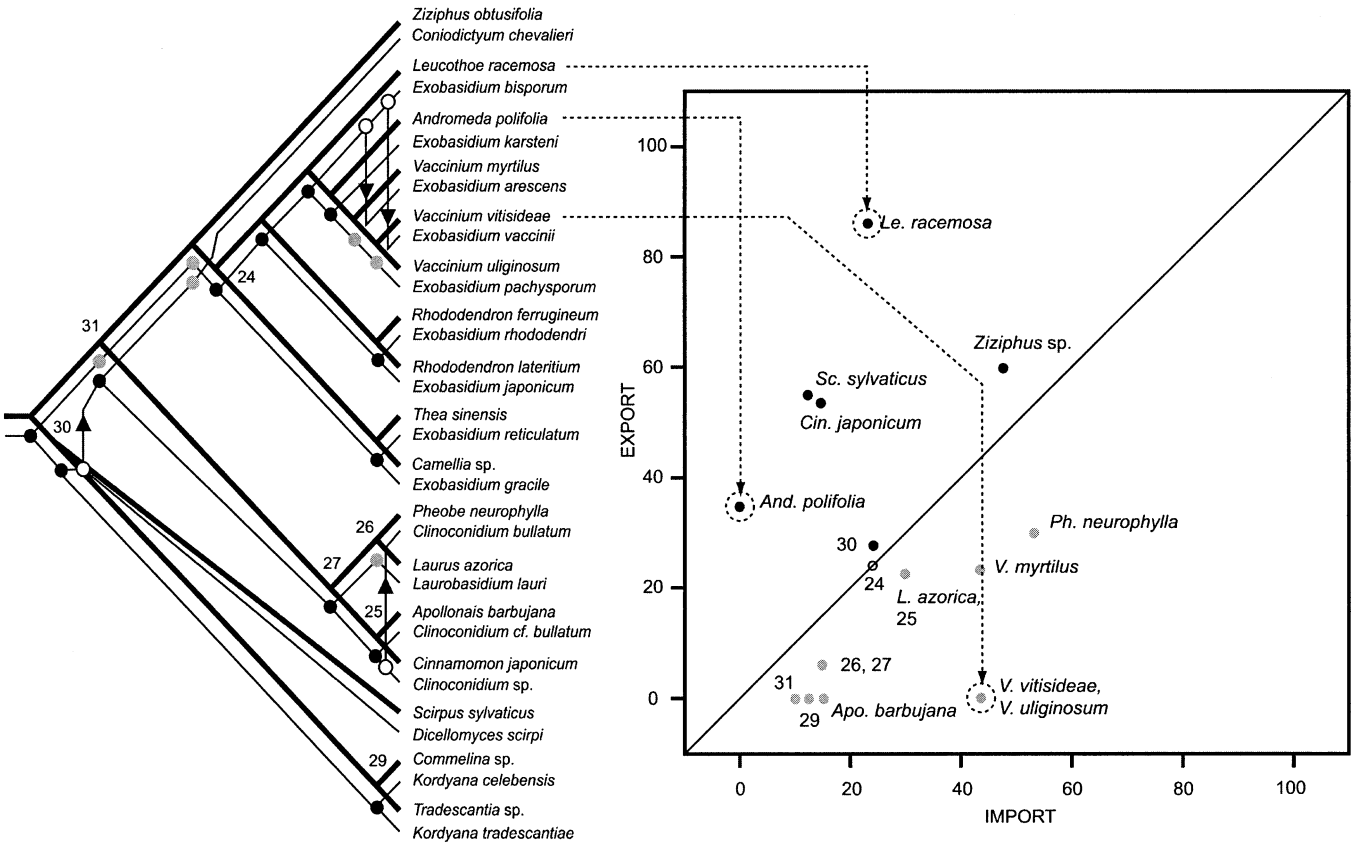

FIG. 3. (Left) One of 18 significantly congruent reconciled trees for Exobasidiales infecting Asteraceae and Lauraceae (association 1). Hosts and associates are top and bottom respectively in each pair of taxa at each terminal node. (Right) A switching diagram summarizing all solutions in POpt. The *y*-axis plots the total number of switches exported by a given host lineage across all solutions; the x-axis plots those switches imported. Black dots represent net exporters, gray dots are net importers, and open dots have no net polarity. Numerals refer to numbered internal nodes, that is, ancestral hosts.

shaded quadrangles. For instance, POpt for Exobasidiales contains 69 solutions; the outermost quadrangle in Figure 2a corresponds to the 15 nonsignificant solutions with 16 codivergence events, 16 duplications, two losses, and eight switches. Those POpt with significantly codivergent solutions have a core of shaded quadrangles that correspond to reconstructions where the two trees are corooted. In these solutions, the common ancestors of hosts and associates respectively were themselves associated. Other quadrangles surrounding this core represent solutions with more derived origins and necessitate switches to more basal lineages. Where there are several solutions with the same combination of events, this usually reflects unknown polarity in one or more switches; that is, a putative switch could have occurred in either direction between two branches.

The results of reconciliation analysis clearly rely on the quality of the trees provided. Using MP as opposed to ML topologies does not alter the status of any symbiosis with respect to congruence; although the result became less significant under ML for Exobasidiales, *Golovinomyces*, and Homobasidiomycetes, while *Monilinia* increased in significance. However, small differences between the topologies did affect the specific transitions invoked. Some associate tree topologies were affected by excluding certain taxa for which a host could not be placed. Including these taxa did not affect the overall correspondence when tested on Exobasidiales (N_{maxCE} remains at 24 and $P < 0.01$) or *Cintractia* (N_{maxCE} reduced to 16, $P = 0.02$) or *Tilletia* (where inclusion does not introduce further codivergence events).

Figure 2a presents consensus diagrams for associations with significant congruence (in relation to N_{maxCE}). In all cases, except *Golovinomyces* and *Cintractia*, this significance is contingent on associates and hosts being corooted; alternative scenarios are possible in which the level of codivergence is lower. Table 2 presents the significance of observed congruence for each association; the lowest percentage of codivergent nodes shown by a significantly codivergent system was 64% (*Phragmidium*). A typical reconciled tree with maximum congruence is shown in Figure 3 for Exobasidiales ($P_{\text{maxCE}} < 0.01$). Despite the level of congruence, the adjacent switching diagram suggests that *V. vitisideae* and *V. uliginosum* each received associates via host switches, whereas *L. racemosa* and *A. polifolia* consistently export associates.

Cintractia and *Golovinomyces* (each $P_{\text{maxCE}} < 0.01$) and *Entyloma* ($P = 0.04$) displayed significant congruence. Figure 4 shows a reconciled tree and switching diagram for *Phragmidium*; for four of 69 solutions (where $N_{\text{CE}} = 18$), the origin of these symbioses is placed at the base of the Rosoideae. The origin of the genus *Gymnosporangium* is suggested to be a switch to the Maloideae from an ancestor on Rosoideae.

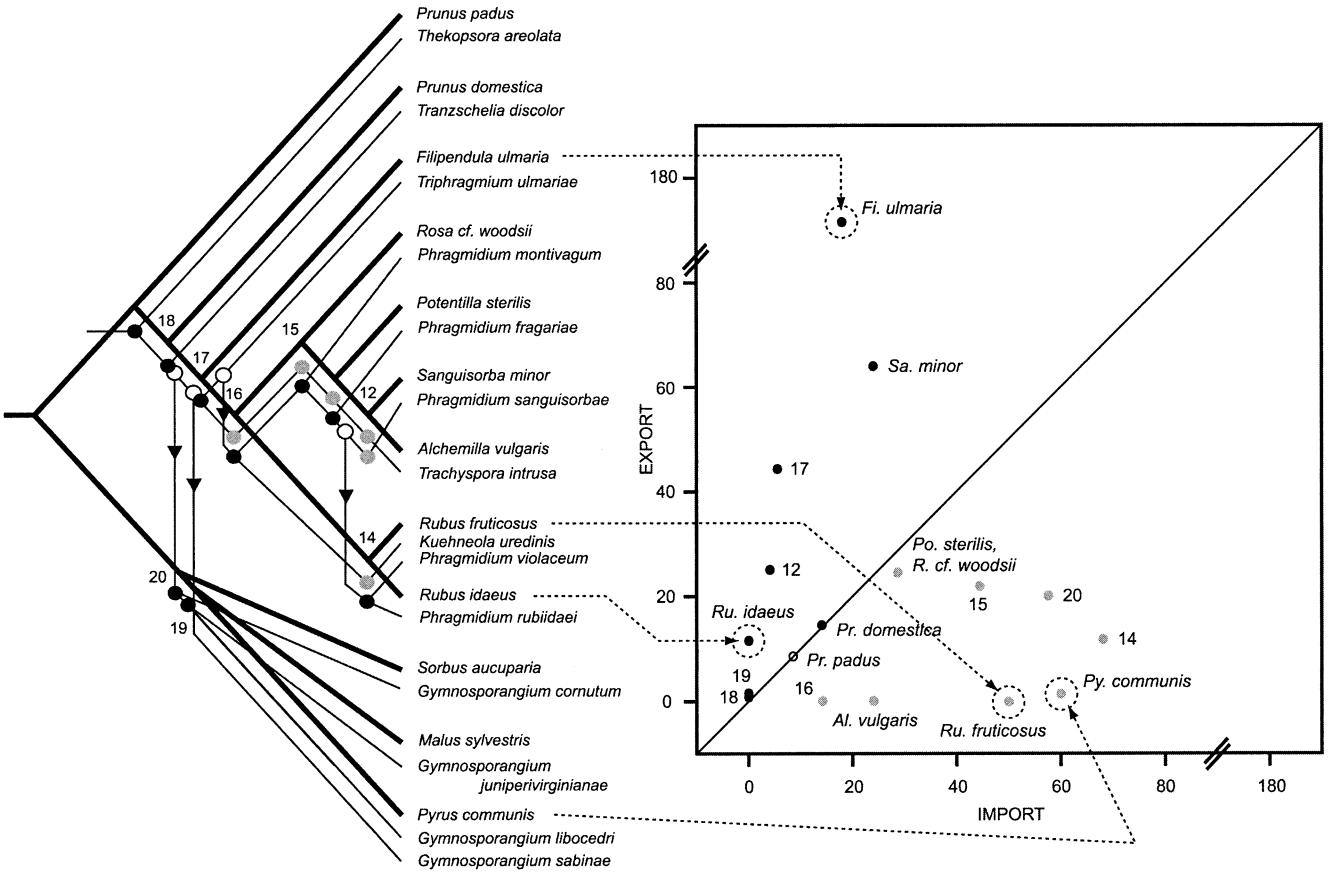

FIG. 4. One of four significantly congruent reconciled trees (left) and switching diagram (right) for *Phragmidium* and associated genera infecting Rosaceae (association 8).

Monilinia also displays significant congruence but includes substantial incongruence. A series of switches are suggested in Figure 5 and their polarity is clear, for instance, the internal lineage 13 always exports an associate to 14 and then the lineage leading to *V. corymbosum* always exports an associate to *G. baccata*. Finally, the Homobasidomycetes-Monotropoideae symbiosis was significantly congruent ($P_{\text{maxCE}} < 0.01$) in one of two solutions.

The remaining symbioses returned no solutions possessing significant congruence and are shown in Figure 2b. Of these, *Microbotryum* came closest to being significant (with 62% codivergent nodes). Indeed, it should be noted that lack of significant congruence does not preclude incidents of codivergence but does demand a more decisive role for loss or host switching. A large part of the incongruence between phylogenies of *Cronartium* and *Pinus* derived from multiple interactions. *Endocronartium harknesii* and *C. ribicola* are each present on multiple hosts that are not monophyletic; this introduces substantial incongruence and requires several switches, which are consistent across POpt.

POpt for the smut fungi *Ustilago* and *Tilletia*, the rust *Uromyces*, the endophytes *Epichloë* and *Claviceps*, and the mildew *Erysiphe* were each characterized by switching and/or lineage sorting. A reconciled tree for *Epichloë* is shown in Figure 6 and is typical in positing several switches. Notably, both *E. brachyelytri* and *E. glyceriae* are consistently implicated in switches, although the polarity is uncertain for the former.

Relationship between Congruence and Switching

This study investigated three possible indicators of significant congruence in reconciliation analyses: switch number, switch distribution, and switch distance. These quantities are reported in Table 3 and were correlated against the proportion of codivergent nodes. Figures 7a and 7b show that the number of switches per solution and the distribution of switches across solutions do not correlate with congruence ($P = 0.478$ and $P = 0.167$, respectively). However, in Figure 7c a significant negative correlation between congruence and the mean sum of switch distances across all solutions ($n = 15$, $P = 0.001$, $r^2 = 0.555$) is observed. The number and sum distance of switches for *Epichloë*, *Claviceps*, and *Erysiphe* are likely to be underestimated since not all solutions were found. Given the incongruence of these associations, their inclusion would probably enhance the relationship in Figure 7c.

DISCUSSION

Congruent Phylogenies: The Interpretation of Significant Codivergence

Related fungi frequently infect related hosts. This, and the typical host specificity of fungal associates, led several au-

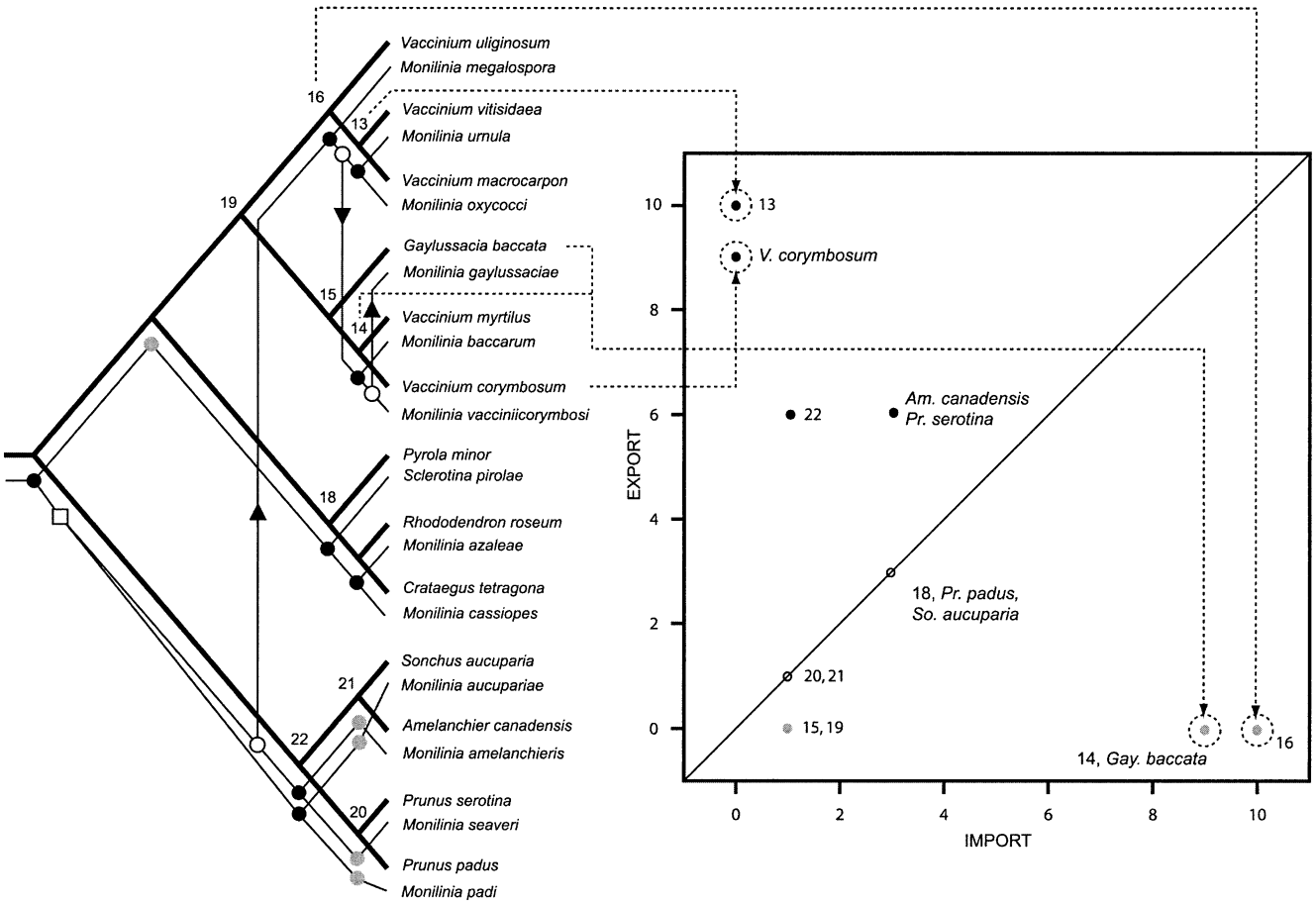

FIG. 5. One of four significantly congruent reconciled trees (left) and switching diagram (right) for *Monilinia* infecting Asteridae and Rosidae (association 15).

thors to consider the role of plant phylogeny in determining the phylogeny of fungal symbionts (Saville 1979; Barrett 1983; Humphries et al. 1986; Hart 1988; Roy 2001). More recently, it has been recognized that fungi do switch and often to unrelated hosts (Reddy et al. 1998; Takumatsu et al. 2000). The question now is to what degree host phylogeny restricts the frequency of switching. These results indicate that several genera have been influenced significantly by host phylogeny. However, with the exception of *Golovinomyces* and *Cintractia*, these patterns of significant codivergence are contingent on basal corooting; if one is prepared to accept that the ancestral associate was associated with the ancestral host, then all solutions are significant congruent for these genera. Therefore, in interpreting these results we require evidence that, first, we should favor an ancient association and dismiss more recent origins and, second, an interpretation of association by descent, as opposed to a pattern of host switching defined by host phylogeny.

Exobasidium has codiverged with Ericaceae and closely related Theaceae (Fig. 3), while more basal members of the Exobasidiales are specific to distantly related Lauraceae and Commelinaceae. Clearly, more sampling is required to identify Exobasidiales on intermediate hosts, to show that codivergence characterizes the expansion of the entire order, and to identify significant codivergence within genera such as *Kordyana* and *Clinocordium*. In this case, the large corrected genetic divergence between these fungi (1.6–53.7%, mean 23%) and the lack of multiple associates in contemporary species are consistent with an interpretation of association by descent, with minimal host switching, rather than a recent expansion (assuming approximately clocklike behavior in genetic divergence).

An alternative interpretation is that fungi have diversified in accordance, but not in synchrony, with the host tree. Begerow et al. (2002) reported the specificity of *Entyloma* on Asteridae and those on Ranunculales and suggested the radiation through the Asteridae to be a case of host tracking. In this study, taxa were sampled across the whole *Entyloma* genus and the observed significant codivergence was borderline ($P = 0.04$) and reliant on the Asteridae/Ranunculales definition previously observed. This radiation is consistent with the host tracking hypothesis, otherwise known as ''preferential host switching'' (Charleston and Robertson 2002). Greater sampling of more exclusive clades within the genus would be required to confirm codivergence as the characteristic dynamic here. The significant codivergence between homobasidiomycete mycorrhizae and parasitic monotropoids is notable because these fungi are quite unrelated. The most parsimonious mechanism to explain the apparent importance of plant phylogeny to fungal diversity is preferential host

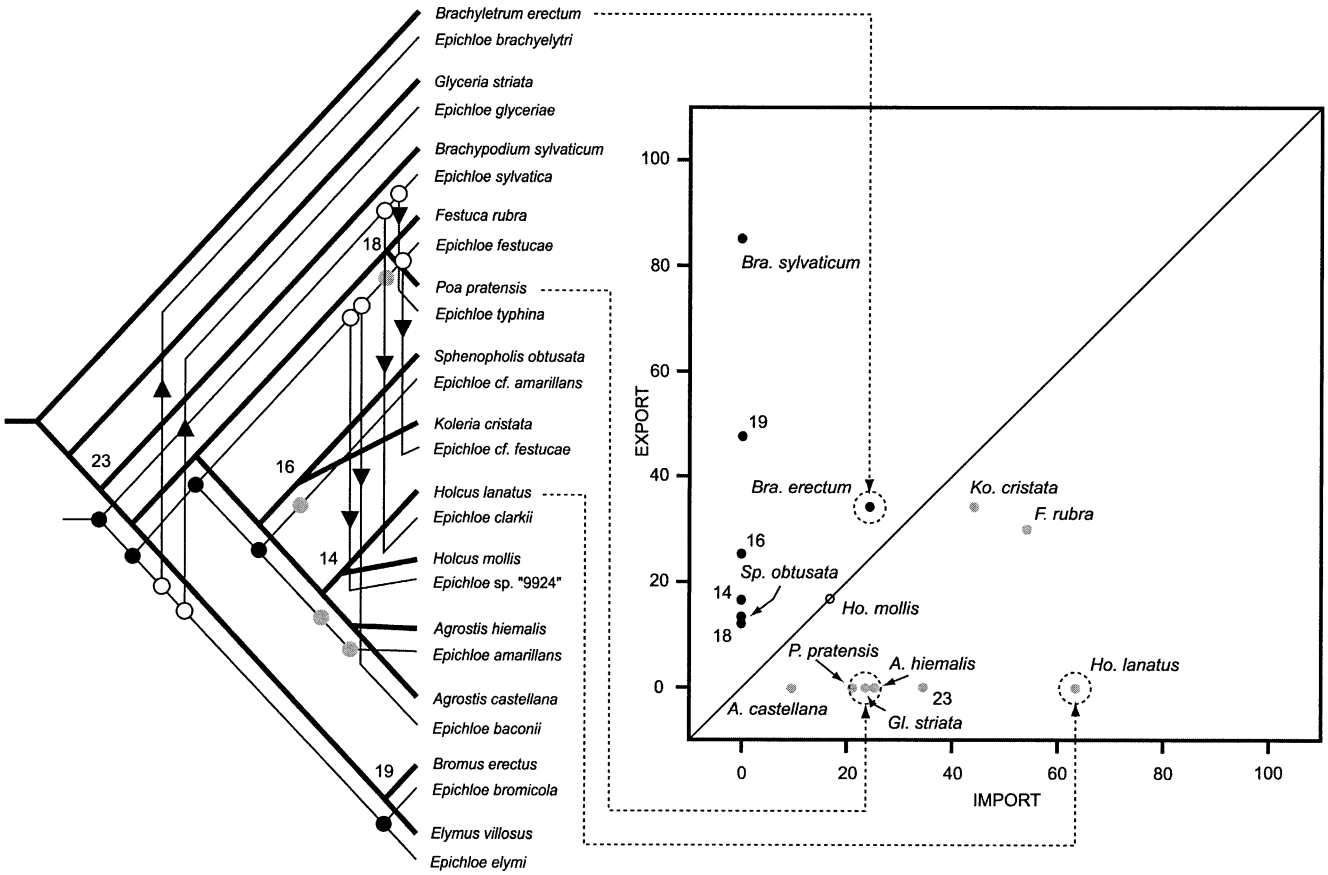

FIG. 6. One of 69 reconciled trees (left) and switching diagram (right) for *Epichloë* infecting Pooidae (association 11).

switching, yet the corrected genetic divergences of plants and fungi are consistent with contemporary descent when each estimated from ribosomal ITS sequences (Homobasidiomycetes: 0.4–54.5%, mean 26.3%; Monotropoideae: 7.7%–29%, mean 19.6%) and assuming roughly clocklike behavior by both. Were this topological correspondence to reflect cospeciation, there must be a great many unsampled mycorrhizal fungi that are parasitized by unsampled monotropoids.

In the case of *Cintractia* and *Phragmidium*, fungal clades specific to Poales (Cyperaceae and Juncaceae) and Rosaceae (Rosoideae, Maloideae and Amyddaloideae), respectively, were identified only after estimating the relationships of these two genera and their close relatives. The significant phylogenetic correspondence observed between these clades and their hosts emphasizes the need for thoughtful sampling when host affiliations and historical associations extend beyond established taxonomic boundaries. It is unclear whether the significant codivergence observed in these cases reflects as-

TABLE 3. Number, distribution, and distance of host switches interest by reconciliation analysis for 15 plant-fungal associations.

		Switches			Sum switch distance			Corrected distance		
Plant	Fungus	Total	Per solution	*D*	Mean	Min	Max	Mean	Min	Max
Asteraceae/Lauraceae	Exobasidiales	1130	16.38	0.64	14.4	2	22	0.90	0.13	1.38
Asteraceae	*Entyloma*	1516	9.72	0.47	11.0	6	16	1.00	0.55	1.45
Poales	*Cintractia*	40	4.44	0.71	10.3	4	17	0.94	0.36	1.55
Poaceae	*Ustilago*	240	10.00	0.62	20.3	3	30	1.69	0.25	2.50
Pooideae	*Tilletia*	580	10.18	0.67	14.0	2	23	1.17	0.17	1.92
Caryophyllales	*Microbotryum*	154	8.11	0.56	21.8	3	40	1.68	0.23	3.08
Pinus	*Cronartium*	66	6.00	0.64	17.4	6	28	1.16	0.40	1.87
Rosaceae	*Phragmidium*	892	12.93	0.51	21.2	3	27	1.51	0.21	1.93
Rosidae	*Uromyces*	675	11.25	0.68	17.4	5	27	1.45	0.42	2.25
Monotropeae	Homobasidiomycetes	1	0.50	1.00	4.0	4	4	0.44	0.44	0.44
Pooideae	*Epichloë*	634	9.91	0.63	21.1	6	33	1.62	0.09	0.05
Poaceae	*Claviceps*	388	8.26	0.64	13.0	2	19	1.08	0.17	1.58
Asteridae/Rosaceae	*Erysiphe*	214	7.13	0.73	16.7	3	26	1.28	0.23	2.00
Asteraceae	*Golovinomyces*	340	8.95	0.75	11.7	2	20	0.78	0.05	1.87
Asteridae/Rosidae	*Monilinia*	96	8.00	0.53	16.4	4	25	1.37	0.33	2.08

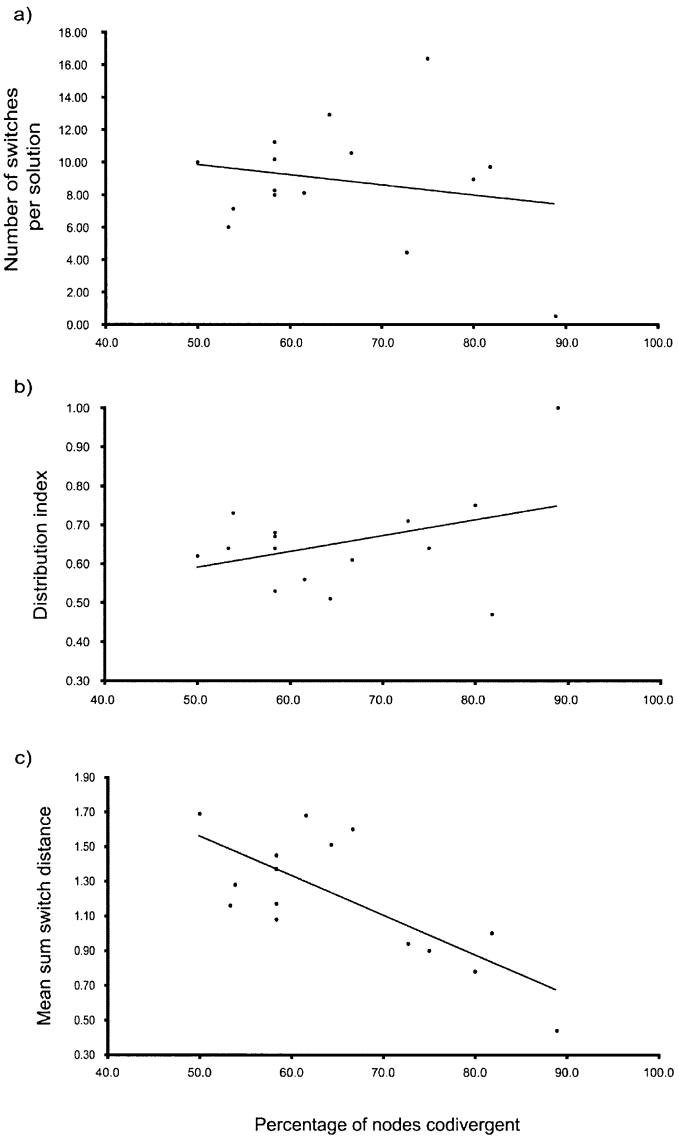

FIG. 7. Correlation of the percentage of nodes that were codivergent in each jungle analysis with: (a) the total number of switches in POpt, corrected for the number of solutions ($n = 15$, $b = -0.199$, $r^2 = 0.04$, $P = 0.478$); (b) the D index of switch distribution ($n = 15$, $b = 0.376$, $r^2 = 0.142$, $P = 0.167$); (c) the mean value for the sum of switch distances in each solution across POpt for each jungle analysis ($n = 15$, $b = -0.744$, $r^2 = 0.554$, $P = 0.001$).

sociation by descent or colonization. More sampling is required to substantiate these patterns, for instance, to establish that *Phragmidium* are specific to Rosaceae and codivergence characterises less known genera, such as *Gymnosporangium* and *Thekopsora*.

Even when substantiated with exhaustive sampling, the events suggested by reconciliation depend on a topological pattern and best represent a working hypothesis. To interpret processes from these patterns, ecological and genetic data are required to test the hypotheses. Indeed, improved phylogenies will continually require revision of the hypotheses formulated by reconciling trees. This study has taken a first step in showing, for those cases above, that plant phylogeny has played a significant role in shaping fungal phylogeny, on the basis of their shared topologies. It has also implicated certain taxa in host switching events, for instance, *Vaccinium vitisideae* and *Andromeda polifolia* are consistently shown importing and exporting fungal associates, respectively, in Figure 3. Likewise, reconciliation analysis indicates that *Rubus fruticosus* and *Pyrus communis* have received *Phragmidium* via host switches (Fig. 4). Hence, the role of association by descent is unlikely to be absolute, even when significant. Conversely, it may not be irrelevant even when two topologies are dissimilar.

Epichloë and Incidental Codivergence

Where two phylogenies are not significantly congruent, this does not preclude genuine cases of codivergence, it simply asserts that this is not the characteristic dynamic. The association between *Epichloë* and its grass hosts provides examples of such ''incidental'' codivergence, without greater congruence than expected by chance. Previous studies have suggested that codivergence was characteristic of this association, the *E. typhina–E. sylvatica* complex excepted (Schardl et al. 1997). The current analysis corroborates the codivergence observed by Schardl et al. (1997) at the split between *E. bromicola* and *E. elymi*. However, it also infers a number of lengthy switches, for instance, in Figure 6 between the lineage leading to the *Epichloë* on Triticeae to *Glyceria striata* and those involving *Agrostis hiemalis*. Overall, reconciliation analysis suggests that codivergence is less important than other forces promoting incongruence in determining *Epichloë* phylogeny, even after removing the *E. typhina–E. sylvatica* complex (for which the evolutionary dynamics have clearly been quite different to the remaining species and may have resulted in wider host usage). These other forces are explicitly described as loss or host switching by the current model but could take other forms. Hybridization is known to be possible between *Epichloë* strains (Chung and Schardl 1997) and has probably played an important role in fungal phylogeny (Tsai et al. 1994; Moon et al. 2002); coupled with subsequent sorting of associates, hybridization could have caused incongruities. The combination of incidental codivergence and incongruence may reflect the variable nature of transmission among *Epichloë* species. Asexual, mutualistic species are associated with vertical transmission, which would promote codivergence; sexual species are associated with pathogenicity and horizontal transmission, traits that may increase the opportunities for successful host switching (Brem and Leuchtmann 2001; Clay and Schardl 2002).

Incongruent Phylogenies: the Relationship between Coevolution and Codivergence

Several genera have shown no evidence of codivergence, but instead have required frequent host switching or substantial lineage sorting to be reconciled. This suggests that plant phylogeny has not been a primary cause of a fungal diversification, a view that is consistent with other evidence. For example, studies that suggest *Claviceps* switches frequently within the Poaceae (Pazoutova 2001). Incongruence involving *Erysiphe* is unsurprising because transitions be-

tween unrelated hosts of *Cystotheca* (Erysiphaceae) have been identified (Takamatsu et al. 2000). Yet, it contrasts with the codivergence observed for another mildew: *Golovinomyces*. The incongruence observed for *Uromyces* is consistent with the independence of infection rates from host phylogeny (Eckenwalder and Heath 2001) and the frequent extinction, recolonization, and ephemeral nature of *Uromyces* infections.

If these systems are characterized by evolutionary forces independent of host phylogeny, does it demand that associations are recently acquired? Furthermore, does this mean that there is no coevolution or coadaptation? Despite seemingly negotiable host specificity, the cophylogenies without significant codivergence have comparable genetic divergences to those that are congruent. Indeed, many are known to be old clades with ancient associations to particular hosts. For example, *Ustilago* and *Tilletia* are thought to have diverged around 200 million years ago (Berbee and Taylor 1993). The biogeography of *Claviceps* indicates that it has diversified in concert with its hosts as they have migrated, and yet this has not resulted in phylogenetic correspondence. Thus, a flexible—and possibly wide—host range does not necessarily indicate a young and unaccustomed symbiosis, but rather a long-established evolutionary strategy. Age need not bring specificity and codivergence.

Equally, coevolution need not result in codivergence over the long term. Many of these non-codivergent systems show evidence for coevolution, for example, through the interplay of virulence and resistance (Frank 1992). If these fungal pathogens can switch successfully to novel hosts, if they have formed ecotypes and have variable performance on different hosts, as is known for *Epichloë* (Brem and Leuchtmann 2002) and *Microbotryum* (Antonovics et al. 2002), then coadaptation may be just as important as in highly specialized symbioses. However, this coadaptation may be local adaptation, gained and lost as species associations change.

Patterns in Host Switching

The patterns in host switching observed across these cophylogenies have shown that switching is not inimical to significant codivergence. There is no relationship between either the number of switches per solution or their distribution between lineages and the level of codivergence. However, significantly codivergent solutions do have fewer long switches. From a methodological point of view, this provides a general understanding of what pattern of switching is characteristic of codivergence, when using TreeMap. A significant result is less likely when long switches are invoked because these circumvent nodes in the host tree by moving directly between terminal lineages. If these internal nodes are depicted without any associates, they cannot possibly contribute to N_{maxCE}. So, it is possible for significantly codivergent solutions to incorporate switching over short distances; this is seen in Figure 3 within the hosts, where *L. racemosa* and *A. polifolia* are consistently seen exporting associates, and in Figure 5, where *Monilinia* switches between its *Vaccinium* spp. hosts. While there are switches in Figure 4 between quite unrelated hosts of *Phragmidium* and its related fungi, these switches occur at the base of the reconciled tree and therefore circumvent few nodes. The contrast with Figure 6 (*Epichloë*), is clear; lengthy switches that traverse the tree are frequently required for reconciliation, a situation typical of other non-codivergent systems not shown.

It could be that this trend in TreeMap output reflects real patterns in host switching. Assuming that, in largely codivergent systems codivergence is enforced by specificity, and the legacy of specificity, any opportunities for switching may be restricted to hosts related to, and therefore phenotypically similar to, the original host. By contrast, in systems not characterised by codivergence, restrictions on host suitability might be lax.

Conclusion: Plant-Fungal Cophylogeny

Reconciliation analysis has revealed a variety of cophylogenetic dynamics among plant-fungus symbioses. They include systems characterized by significant codivergence, where we require extrinsic information of ecological and genetic interactions to interpret phylogenetic congruence as either association by descent or by colonization. They also include mixed dynamics, as in the case of *Epichloë*. These may reflect the action of opposing forces promoting codivergence and host switching, respectively. Finally, there are systems characterized by incongruence, where plant phylogeny seems not to direct fungal diversification. Because these systems can be long established and still display coadaptation or host specificity, such phenomena are unlikely to be effective predictors of codivergence or satisfying explanations for an observed topological pattern. Hence, having surveyed the diversity of cophylogenetic patterns in plant-fungal symbioses, the challenge now is to get information beyond the phylogeny to identify the factors regulating host associations and therein, promoting or precluding codivergence.

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