



Inferring processes of coevolutionary diversification in a community of Panamanian strangler figs and associated pollinating wasps*

Jordan D. Satler,^{1,2} Edward Allen Herre,³ K. Charlotte Jandér,⁴ Deren A. R. Eaton,⁵

Carlos A. Machado,⁶ Tracy A. Heath,¹ and John D. Nason¹

¹Department of Ecology, Evolution, and Organismal Biology, Iowa State University, Ames, Iowa 50011

²E-mail: jordansatler@gmail.com

³Smithsonian Tropical Research Institute, Unit 9100, P.O. Box 0498, Diplomatic Post Office, Armed Forces America 34002-9998

⁴Department of Ecology and Genetics, Evolutionary Biology Centre, Uppsala University, Norbyvägen 18D, 752 36 Uppsala, Sweden

⁵Department of Ecology, Evolution and Environmental Biology, Columbia University, New York, New York 10027

⁶Department of Biology, University of Maryland, College Park, Maryland 20742

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The fig and pollinator wasp obligate mutualism is diverse (~750 described species), ecologically important, and ancient (~80 Ma). Once thought to be an example of strict one-to-one cospeciation, current thinking suggests genera of pollinator wasps codiversify with corresponding sections of figs, but the degree to which cospeciation or other processes contribute to the association at finer scales is unclear. Here, we use genome-wide sequence data from a community of Panamanian strangler figs and associated wasp pollinators to estimate the relative contributions of four evolutionary processes generating cophylogenetic patterns in this mutualism: cospeciation, host switching, pollinator speciation, and pollinator extinction. Using a model-based approach adapted from the study of gene family evolution, our results demonstrate the importance of host switching of pollinator wasps at this fine phylogenetic and regional scale. Although we estimate a modest amount of cospeciation, simulations reveal the number of putative cospeciation events to be consistent with what would be expected by chance. Additionally, model selection tests identify host switching as a critical parameter for explaining cophylogenetic patterns in this system. Our study demonstrates a promising approach through which the history of evolutionary association between interacting lineages can be rigorously modeled and tested in a probabilistic phylogenetic framework.

KEY WORDS: *Ficus*, host switching, obligate mutualism, *Pegoscapus*, RADseq, ultraconserved elements.

Interactions among species contribute fundamentally to the diversification of life. Although many of these interactions are generalized and fleeting, some are specialized and persist over deep evolutionary timescales (e.g., Ehrlich and Raven 1964; Hafner and Nadler 1988; Thompson 1994). For long-term associations,

the relative importance of different ecological and evolutionary processes that have contributed to their persistence and diversification are not well understood. For example, in obligately associated lineages, processes that produce either more or less congruent diversification patterns (e.g., strict sense species specificity) are seldom estimated quantitatively for both groups, and therefore are poorly understood because there is no consistent basis for comparison among systems. Analyzing patterns of divergence of obligately interacting lineages in a probabilistic framework can

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reveal the relative importance of different processes that have influenced the evolution of biotic interactions. This can ultimately provide a better understanding into the roles interacting lineages play in generating and maintaining biodiversity.

For long-term interactions (*e.g.*, host–parasite, mutualisms), researchers often use cophylogenetic methods to compare diversification patterns between interacting lineages to infer the processes that produced these patterns. These approaches can be placed into two categories: global-fit methods and event-based methods (for review, see de Vienne et al. 2013; Cruaud and Rasplus 2016). Global-fit methods estimate the degree of congruence between two phylogenies, using either phylogenetic or genetic distance information (*e.g.*, Legendre et al. 2002; Balbuena et al. 2013), whereas event-based methods, generally implemented under the parsimony criterion, invoke various evolutionary events (*e.g.*, cospeciation, duplication, host switching, and loss) to allow mapping of one phylogeny onto another phylogeny (*e.g.*, Page 1994b; Ronquist 2003). Although both approaches can be useful for investigating coevolutionary associations, they are necessarily limited. For example, global-fit methods measure the degree to which two data sets match but do not estimate what processes could be contributing to the congruence or lack thereof between the groups. Event-based methods using the parsimony criterion require *a priori* costs to be assigned to the various events, with the reconciliation containing the lowest total cost selected, of which there may be more than one. Importantly, neither of these approaches directly model the processes that can generate the observed data in a probabilistic framework. As multiple processes can generate data with similar patterns, it is necessary to use a model-based approach where the relative contribution of these processes can be statistically evaluated with a likelihood function. Thus, the use of probabilistic methods that make explicit predictions about the processes important for generating observed patterns will provide an important contribution to our understanding of coevolutionary associations.

Obligate mutualisms are useful systems for understanding the relative importance of processes that contribute to the maintenance and diversification of interacting lineages. Figs (family Moraceae, genus *Ficus*) and their pollinating wasps (family Agaonidae, multiple genera) provide one of the best-known examples of an obligate mutualism, with both members of this mutualism solely dependent on the other for reproduction and survival. Figs are an ecologically and morphologically diverse genus composed of over 750 described species distributed across tropical and subtropical environments worldwide (Berg 1989). From the perspective of exchange of services that underlie their mutualistic interaction, figs require fig wasps for pollination services, and fig wasps require figs to provide a nursery for larval development (Janzen 1979; Wiebes 1979). Each species of fig

is typically pollinated by one, but sometimes more, specialized species of fig wasps that identify their appropriate hosts in part via volatile chemical signals produced by the hosts when receptive. Similarly, fig wasps are primarily associated with a single species of fig, and in addition to pollinating fig flowers, gall a subset of ovules that will support developing larvae. Together, figs and their pollinators are considered keystone species in tropical ecosystems because the generally aseasonal production of large crops of figs contributes heavily to the diets of many vertebrates, especially during seasons in which the fruiting activity of other tree species is low (Terborgh 1986; Lambert and Marshall 1991; Kissling et al. 2007). The obligate association between fig and pollinator, and its broader ecosystem services, has been maintained for upwards of 80 million years (Machado et al. 2001, 2005; Rønsted et al. 2005; Lopez-Vaamonde et al. 2009; Cruaud et al. 2012).

Although the long-held paradigm has been a one-to-one relationship between individual fig and fig wasp species, analyses of molecular data sets have revealed examples of multiple pollinator species associated with a single fig species as well as multiple fig species that share a single pollinator species (*e.g.*, Molbo et al. 2003; Haine et al. 2006; Marussich and Machado 2007; Darwell et al. 2014; Yang et al. 2015; Wang et al. 2016). In addition, while a signal of co-cladogenesis is often the dominant pattern recovered between sections of figs and genera of fig wasps (*e.g.*, Weiblen and Bush 2002; Rønsted et al. 2005; Cruaud et al. 2012), phylogenetic data collected at finer taxonomic scales has often revealed discordant diversification patterns within these groups (Machado et al. 2005; Jackson et al. 2008; Jousselin et al. 2008). These discordant patterns require confirmation utilizing robust phylogenies to determine the degree to which additional evolutionary processes (*e.g.*, host switching) are necessary to explain deviations from expectations under a strict one-to-one cospeciation history.

To date, phylogenetic reconstructions of both the figs and the wasps have been based on Sanger sequencing of only one or a few genes. The use of limited data sets often produces poorly resolved or conflicting phylogenetic estimates, which in turn limits efforts for inferring evolutionary processes responsible for generating patterns of codiversification. With advances in sequencing technologies and the ability to gather hundreds to thousands of loci for nonmodel organisms, large genomic data sets can be used to decrease sampling error and generate more accurate phylogenetic estimates with greater confidence. In addition, modeling the coalescent process during phylogenetic estimation provides a critical methodological advancement as incomplete lineage sorting can introduce substantial error if not taken into account (Kubatko and Degnan 2007). Improvement in phylogenetic estimates, both through large genomic data sets and better phylogenetic models, increases our ability to elucidate the processes important for producing cophylogenetic patterns.

Here, we use genome-wide sequence data to infer the processes producing cophylogenetic patterns in a sympatric community of Panamanian strangler figs (*Ficus* subgenus *Urostigma*, section *Americana*) and their pollinating fig wasps (*Pegoscapus* spp.). We gather restriction-site associated (RAD) loci from the host figs and ultraconserved element (UCE) loci from their associated pollinating wasps and estimate phylogenetic relationships to test how coevolutionary processes have contributed to the evolution of this mutualism. Using a probabilistic approach, we adapt a duplication-transfer-loss (DTL) model of gene family evolution (Szöllősi et al. 2012) to quantify the contribution of fundamental ecological and evolutionary processes to this mutualism, including cospeciation, host switching, pollinator speciation, and pollinator extinction. Although the available implementation of this approach already accounts for uncertainty in the fig wasp species tree, we extend the method to account for phylogenetic uncertainty in the host figs. In addition, we use simulations and a model selection approach to examine the sensitivity of this method within a cophylogenetic framework and aid in the biological interpretation of estimated model parameters. In sum, we present a framework for addressing cophylogenetic questions using a model-based method and demonstrate the benefits of utilizing this approach for quantifying the processes structuring the evolutionary history of long-term interactions.

Materials and Methods

SAMPLING, SEQUENCING, AND PHYLOGENETICS OF FIGS

More than 120 species of strangler figs have been described from the Neotropics. We sampled 31 individuals from 11 species of strangler figs (family Moraceae, *Ficus* subgenus *Urostigma*, section *Americana*) co-occurring in central Panama, centered around the Barro Colorado Island Nature Monument (Table S1). These 11 fig species include two pairs that share pollinator species, and another fig species that hosts two pollinator wasp species (Table 1). Genomic DNA was extracted using a modified CTAB protocol (Doyle and Doyle 1987) and sent to Floragenex Inc. (Eugene, OR) for library preparation using the *Pst*I restriction enzyme and the traditional RAD protocol (Baird et al. 2008). The resulting library was sequenced on two lanes of an Illumina HiSeq 3000 using 100 base pair single-end sequencing. Raw sequencing reads were processed in ipyrad version 0.6.20 (Eaton 2014) to generate alleles, loci, and single nucleotide polymorphisms (SNPs). Sequences were first demultiplexed, not allowing any mismatches with the barcode, and then filtered for Illumina adapter contamination. Base calls with a Phred score below 30 were replaced with an N and up to five Ns were allowed in any given sequence. Sequences above that threshold were removed from the data set.

Table 1. Sampling and ecological associations of figs and fig wasps. Species, number of individuals sequenced (N), and fig and pollinator associations are shown.

Fig species	N	Pollinator species	N
<i>Ficus citrifolia</i>	2	<i>Pegoscapus tonduzi</i>	5
<i>Ficus costaricana</i>	2	<i>Pegoscapus estherae</i>	6
<i>Ficus nymphaeifolia</i>	3	<i>Pegoscapus piceipes</i>	4
<i>Ficus paraensis</i>	3	<i>Pegoscapus herrei</i>	7
<i>Ficus near trigonata</i>	3	<i>Pegoscapus lopesi</i>	10
<i>Ficus trigonata</i>	3	<i>Pegoscapus grandii</i>	8
<i>Ficus obtusifolia</i>	3	<i>Pegoscapus hoffmeyerii A</i>	2
		<i>Pegoscapus hoffmeyerii B</i>	3
<i>Ficus colubrinae</i>	3	<i>Pegoscapus insularis</i> †	15
<i>Ficus perforata</i>	3		
<i>Ficus bullenei</i>	3	<i>Pegoscapus gemellus C</i>	10
		<i>Pegoscapus gemellus A</i> †	3
<i>Ficus popenoei</i>	3	<i>Pegoscapus gemellus B</i>	9

†Pollinating wasp species shared by two host figs. See Table S1 (figs) and Table S2 (wasps) for detailed sampling and sequence information.

A clustering threshold of 85% was used to assemble reads into loci. When clustering loci among samples, we allowed up to four individuals to be heterozygous for a shared site, up to eight indels in a locus, and up to 20 SNPs in a locus. All loci that contained at least four individuals were retained in the complete data set. We furthered filtered this pool to those loci that contained at least one individual per species, then subsampled a single SNP per locus at random for analysis.

Two approaches were used to generate species-level phylogenetic estimates of the sampled fig species. As both analyses use the multispecies coalescent model, individuals were assigned to their respective species *a priori*. First, SNAPP version 1.3 (Bryant et al. 2012) in BEAST version 2.4.7 (Bouckaert et al. 2014) was used to estimate a posterior distribution of species trees from biallelic SNP data under the multispecies coalescent model. Unlinked biallelic SNPs were recoded as “0, 1, 2,” with heterozygotes as “1,” major allele homozygotes as “0,” and minor allele homozygotes as “2.” Major and minor allele frequencies were calculated in BEAUTi informing priors on forward and reverse mutations; all other settings were left at default. SNAPP assumes a Yule prior on the species tree and draws branch rates from a strict clock model. Analyses were run for 2 million generations, sampling every 200 generations, resulting in 10,000 Markov chain Monte Carlo (MCMC) samples from the posterior distribution. To ensure consistency across runs, two independent Markov chains were run. Resulting log files were imported into Tracer version 1.6.0 (Rambaut et al. 2018) to assess nonconvergence. In addition, a species tree was estimated with SVDquartets (Chifman and Kubatko 2014) as implemented in PAUP* version 4.0a163 (Swofford

2003). SVDquartets uses site patterns in the nucleotide data to estimate a species tree under the multispecies coalescent model. All quartets were evaluated exhaustively with 100 bootstrap replicates run to evaluate nodal support values.

SAMPLING, SEQUENCING, AND PHYLOGENETICS OF FIG WASPS

We sampled 82 individuals from 12 lineages of pollinating wasps *Pegoscapus spp.* associated with the 11 focal strangler fig species sequenced above (Table S2). Wasps were allowed to emerge from mature fig fruit in the lab, where they were stored in 95% EtOH or RNALater for DNA collection and analysis. A single wasp was sequenced per fig fruit to ensure independence among samples. Genomic DNA was extracted with a Qiagen DNeasy Kit (Qiagen Inc., Valencia CA) and amplified using a Qiagen REPLI-g Mini Kit to increase total DNA yield. On average, 10–20 ng of DNA was used for whole genome amplification. Samples were standardized and sent to RAPiD Genomics (Gainesville, FL) for library preparation and sequencing. The hymenopteran probe v1 set of Faircloth et al. (2015) was used to target 1510 ultraconserved element (UCE) loci. UCE loci have been shown to be informative phylogenetic markers across diverse animal lineages, including wasps (e.g., Crawford et al. 2012; McCormack et al. 2012; Branstetter et al. 2017; Starrett et al. 2017). Following library construction, samples were sequenced on half a lane of an Illumina HiSeq 2000 using 150 base pair paired-end sequencing. Phyluce version 1.5 (Faircloth 2015) was used to process raw sequence reads and generate loci for each individual. Briefly, sequence reads were cleaned with Trimmomatic version 0.32 (Bolger et al. 2014) and assembled into contigs with Trinity version 2.0.6 (Grabherr et al. 2011). Contigs were aligned to the hymenopteran v1 UCE locus set to filter nonspecific sequences. Loci were then aligned with MAFFT version 7.130 (Katoh and Standley 2013), edge trimmed with trimAl (Capella-Gutiérrez et al. 2009), and ambiguously aligned internal sites were removed with Gblocks version 0.91b (Castresana 2000). We retained loci sampled for a minimum of 70% of individuals in the final data set.

We used two approaches to generate phylogenetic estimates of the pollinator wasps. First, we estimated a species tree using StarBEAST2 version 0.14.0 (Heled and Drummond 2010; Ogilvie et al. 2017) in BEAST version 2.4.7. StarBEAST2 coestimates the posterior distributions of gene trees and species trees under the multispecies coalescent model. This approach accounts for uncertainty in the tree topologies, branching times, and other model parameters using MCMC in a Bayesian inference framework. Due to the computational challenges of applying this method, loci were first filtered to those that had at least one sequence per OTU per locus. We then randomly subsampled *without replacement* 50 loci for analysis. Preliminary analyses containing more loci failed to reach stationarity. In addition, a maximum of three alleles per

species per locus was used to reduce computational burden, as this is sufficient for species tree estimation (Hird et al. 2010). For species at or below this allelic threshold for a given locus, all alleles were used, otherwise three alleles were randomly subsampled *with replacement* for analysis. We applied a birth-death model as a prior on the species tree, an HKY + I + Γ substitution model, and a strict clock for each locus. Analyses were run for two billion generations and sampled every 200,000 generations, yielding 10,000 samples from the target distribution. Two independent analyses were run and nonconvergence was diagnosed using Tracer. We also estimated a species tree with SVDquartets, using all UCE loci for analysis. In addition to estimating a species tree with individuals assigned to species (i.e., SVD_{ST}), we estimated a phylogeny using the individuals as tips in the tree (i.e., a lineage tree; SVD_{LT}). The lineage tree (SVD_{LT}) was used to confirm species association. For both analyses, quartets were evaluated exhaustively and 100 bootstrap replicates were run to evaluate nodal support values.

INFERRING COEVOLUTIONARY PROCESSES IN A MODEL-BASED FRAMEWORK

We are interested in inferring the evolutionary and ecological processes responsible for producing cophylogenetic patterns between interacting lineages. Using a model-based approach, we can evaluate the relative contribution of different parameters in a statistical framework. Here, we use a DTL model to test how various processes may have contributed to estimated phylogenetic patterns (Page 1994a; Doyon et al. 2011). The probabilistic DTL model of Szöllősi et al. (2012) estimates the maximum likelihood rates of gene duplication (D), horizontal gene transfer (T), and gene loss (L) of a gene family, conditioned on an ultrametric species tree with nodes ordered by speciation time. Based on the DTL rates, the probabilities of these events along the branches of the species tree can be calculated (Szöllősi et al. 2013b). Following likelihood calculation and maximization of parameters given the data and species tree, reconciled gene trees can be sampled from the species tree and event probabilities obtained using stochastic backtracking along the species tree (Szöllősi et al. 2013a). After sampling a set of reconciled gene trees, the average number of duplication (i.e., wasp speciation), transfer (i.e., host switching), and loss (i.e., wasp extinction) events, in addition to cospeciation, can be summarized providing estimates of the average number of these events for a gene family given a species tree. Thus, the DTL method of Szöllősi et al. (2012) allows us to estimate the process (ML rates of duplication, transfer, and loss) that generated our observed data, with the reconciled tree set providing estimates of the expected number of events (including cospeciation) from these processes.

An assumption of the DTL model is that each symbiont lineage is associated with a single host. The “widespread taxon”

problem has been a challenge with the application of DTL models to host–parasite (e.g., Page 1994b) and biogeographic systems (e.g., Sanmartín and Ronquist 2002). In our system, we have two examples where a pollinator species is shared by multiple hosts. *Pegoscapus insularis* pollinates *Ficus colubrinae* and *Ficus perforata*, whereas *Pegoscapus gemellus A* pollinates *Ficus bullenei* and *Ficus popenoei*. We used two approaches to address the widespread taxon challenge in our data set. In the first approach (referred to as *pruned*), we pruned the two wasp species (*P. insularis* and *P. gemellus A*) from the posterior distribution of species trees from StarBEAST2. As *P. insularis* is the sole pollinator of *F. colubrinae* and *F. perforata*, we also pruned those two fig species from the fig species tree distribution from SNAPP. This reduced our data sets to nine (figs) and ten (fig wasps) species, respectively. Although removing the shared wasp species reduced the size of our data set, it allowed us to satisfy the assumption of each pollinator species being associated with a single host species. In the second approach (referred to as *split*), following Ronquist (2003), we split the shared wasp tips into two sister tips for all fig wasp species trees sampled in the posterior distribution, and associated the newly split individual wasp samples with the host figs from which they were sampled. This approach makes the explicit assumption that the two shared wasp species are each composed of two cryptic species, and allows the DTL model to estimate the process that generated sister pollinator species that occupy different hosts. For example, if the host fig species are distantly related, it would likely be modeled as a recent host switch, whereas if the host figs are sister, it would likely be modeled as cospeciation. By violating the model’s assumption that each symbiont lineage is associated with a single host species, we are able to test model sensitivity when widespread taxa are included.

We applied the probabilistic DTL model as implemented in the dated version of the program ALEml version 0.5 (<https://github.com/ssolo/ALE>). ALEml takes as input an ultrametric species tree, with nodes ordered by speciation time, and a posterior distribution of gene family trees, accounting for uncertainty in the gene family tree estimates. In our application of the model, we treat the fig species tree as the host species tree, and the posterior distribution of fig wasp species trees as the gene family trees, explicitly accounting for phylogenetic uncertainty in the fig wasps. ALEml was then used to estimate maximum likelihood rates of wasp speciation (i.e., duplication), host switching (i.e., transfer), and wasp extinction (i.e., loss), generating a set of 1000 reconciled fig wasp species trees given the host species tree and probability of events along the branches. Averaging over this set of sampled reconciled fig wasp species trees provided average numbers of wasp speciation, host switching, wasp extinction, and cospeciation events. We extended the implementation of the method to also account for phylogenetic uncertainty in the fig

species tree by thinning the post-burnin SNAPP posterior distribution to 1000 species trees, and running the analysis across all 1000 trees sequentially. Results were then averaged across these 1,000 replicates. Implementation of the extension of this method is available as a Python script (autoALE.py) on GitHub (<https://github.com/jordansatler/autoALE>).

MODEL SENSITIVITY AND MODEL SELECTION

We explored the appropriateness of the DTL model as implemented in ALEml for the figs and fig wasps in two ways. First, simulations were used to evaluate the behavior and sensitivity of ALEml, and to contextualize empirical estimates by generating null distributions with which to compare estimated values. Specifically, trees were simulated with no explicit assumption of association between the tips of the simulated trees and the empirical host fig tree, providing an expectation of parameter values when there is no explicit history of cospeciation (Ronquist 2003). As these simulations approximate expectations under free host switching, this allowed us to understand how much cospeciation we would expect even with no explicit history of cospeciation between the phylogenies. We simulated 1,000 trees under a birth-death process ($\lambda = 1.0$, $\mu = 0.9$) in the R package TreeSim version 2.3 (Stadler 2011). Tree distributions were simulated matching the sampling properties of the fig wasps for both of our data sets (i.e., *pruned*: one fig tip species was randomly assigned two simulated tips, all other fig tips were randomly assigned a single simulated tip; *split*: three fig tip species were randomly assigned two simulated tips, all other fig tips were randomly assigned a single simulated tip). For the ALEml analyses, one simulated tree was analyzed with the fig maximum-clade credibility (MCC) SNAPP tree at a time (keeping the host tree constant across analyses) until all 1000 trees in the simulated set were independently analyzed. Using a single tree to represent the fig wasps removes phylogenetic uncertainty, but is sufficient for the purposes of the simulations. In addition, *F. colubrinae* and *F. perforata* were removed from the MCC tree when analyzing the *pruned* simulated tree set. Analyses were run under the full DTL model as outlined above. Parameter estimates across all replicates were then summarized as null distributions for the model parameters. For host switching, wasp speciation, and wasp extinction, *P*-values represent the proportion of simulations that produced an estimate equal to or below the empirical value. For cospeciation, the *P*-value represents the proportion of simulations that produced an estimate equal to or above the empirical value. We considered a *P*-value to be significant at the $\alpha = 0.05$ level.

Second, we used a model selection approach to test the relative fit of the DTL model and all nested submodels given our data. Although we can estimate parameter values under the full DTL model, a model selection approach allows us to identify what parameters of the model are most important in this system.

Table 2. Model set and model probabilities for the *pruned* data set.

Model	K	$\log \mathcal{L}$	AIC	Δ_i	w_i
T	1	-24.006	50.012	0.000	0.417
TL	2	-23.322	50.645	0.633	0.304
DT	2	-23.946	51.891	1.880	0.163
DTL	3	-23.285	52.570	2.558	0.116
DL	2	-30.352	64.704	14.693	0.000
L	1	-704.824	1411.648	1361.636	0.000
CoSp	0	-707.850	1415.700	1365.688	0.000
D	1	-707.850	1417.700	1367.688	0.000

The model name indicates estimated parameters; all other parameters were fixed to zero. “CoSp” represents the strict cospeciation model, where all parameters were set to zero. Δ_i reflects the AIC difference between the model and the best model. Model probabilities are represented by w_i .

For example, we can evaluate whether a model that only allows transfers is a better fit to the data than a model that only allows duplications and losses. The full DTL model and all subsets of parameter combinations comprise a set of eight models (Table 2). These models span from a strict cospeciation model, where the DTL rates are fixed to zero, to the full DTL model, where the three parameters are estimated. For each of the models, ALEml was run as outlined above. We then used Akaike information criterion (AIC; Akaike 1974) and calculated model probabilities following Burnham and Anderson (2002) to evaluate the relative fit of the models and identify those parameters most important for explaining the fig and fig wasp data. Following model selection, we generated model averaged parameter estimates by allowing each model to contribute to the parameter estimate in proportion to its model probability. The model selection approach was run on both data sets (*pruned, split*).

DESCRIBING COPHYLOGENETIC PATTERNS USING TRADITIONAL APPROACHES

In addition to our application of the DTL probabilistic method, two additional approaches were used. First, the degree of congruence between the fig and fig wasp phylogenies was assessed in the R package PACo version 0.3.2 (Balbuena et al. 2013; Hutchinson et al. 2017). PACo is a global-fit method that uses Procrustean superimposition to evaluate if two phylogenies are more similar than expected by chance, suggesting a shared evolutionary history. An eigenvalue correction of “lingoes”—a constant added to correct for negative eigenvalues—was used when transforming the phylogenetic distance matrices into principal coordinates. Rather than superimpose the fig wasp phylogeny on the fig phylogeny (as would be the case in a host and parasite system), both phylogenies were standardized to allow for the best-fit superimposition

independent of both phylogenies. We used 10,000 permutations to assess significance for the analysis.

Second, the parsimony event-based method Jane versus 4.0 (Conow et al. 2010) was used to estimate the number of events required to explain discordance in the two phylogenies. Jane takes as input host and symbiont phylogenies and reconciles the two trees to find the optimal reconciliation by minimizing the total number of event costs. Costs are applied a priori; default values were used with cospeciation = 0, host switching = 2, duplication = 1, loss = 1, and failure to diverge = 1. Following the reconciliation process, statistical support was generated by drawing 1,000 random symbiont trees from a Yule model (default beta value of -1.0) to generate a null distribution of event costs. As PACo and Jane can handle widespread taxa, MCC trees from SNAPP (figs) and StarBEAST2 (fig wasps) from the full data sets were used as input data.

Results

SAMPLING, SEQUENCING, AND PHYLOGENETICS OF FIGS

We generated 228,004,993 raw reads for the 31 sampled fig trees, with an average of 7,355,000 reads per individual ($\pm 9,704,999$). Following data processing in ipyrad, individuals had on average 18,444 loci ($\pm 9,023$). Requiring at least one individual to be sequenced per species per locus for phylogenetic analysis, 2529 unlinked biallelic SNPs were used to estimate a species tree for the figs. Phylogenetic results were consistent between SNAAP and SVDquartets. With SNAPP, species fell into two main clades, with generally good support throughout the tree although some nodes reflected greater uncertainty (Fig. 1). For the figs that share a fig wasp species, drastically different patterns emerge. *Ficus bullenei* and *F. popenoei* are sister species in the phylogeny. In contrast, while *F. colubrinae* and *F. perforata* fall in the same clade, they are not closely related. Similar interrelationships are recovered with SVDQ_{ST}, with varying levels of support across the tree (Fig. S1). One difference from SNAPP, however, is *F. colubrinae* and *F. perforata* are sister taxa in the SVDQ_{ST}, although this is weakly supported (BS = 67).

SAMPLING, SEQUENCING, AND PHYLOGENETICS OF FIG WASPS

We generated 164,703,731 raw reads for the 82 sampled pollinating wasp individuals, resulting in an average of 2,008,582 reads per individual ($\pm 577,848$). Following processing in phyluce, a pool of 1052 total UCE loci was recovered, of which 1017 contained at least three or more individuals per locus. On average, there were 628 loci per individual (± 162.60). Requiring a minimum of 70% taxon coverage to retain a locus, the final data set consisted of 550 loci. Loci in this data set had an average size

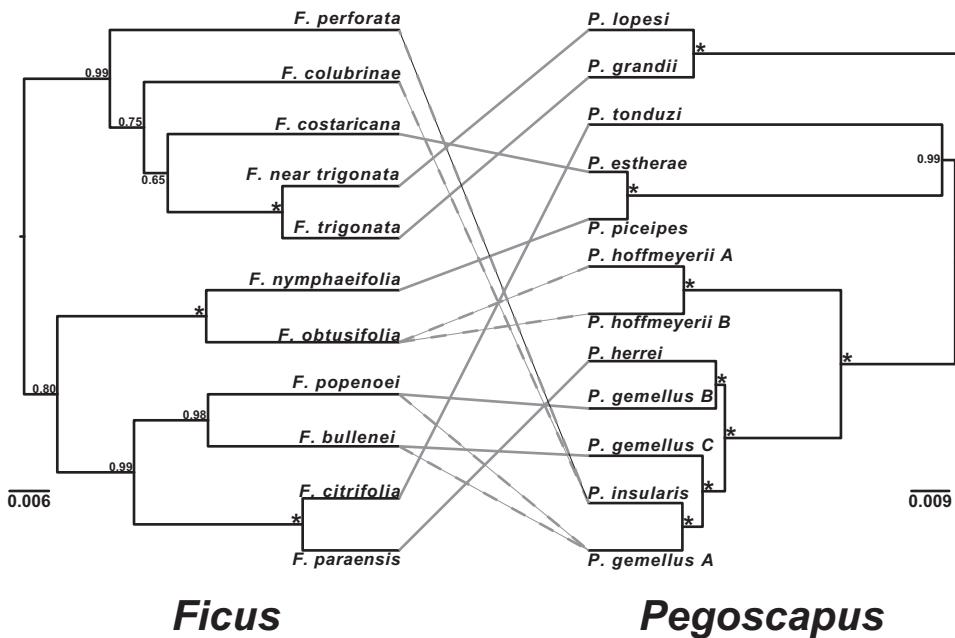


Figure 1. Tanglegram representing evolutionary relationships and associations of 11 species of Panamanian strangler figs (*Ficus* subgenus *Urostigma*, section *Americana*) and their respective pollinating fig wasps (*Pegoscapus* spp.). The maximum-clade credibility species tree estimates of the figs (SNAPP) and fig wasps (StarBEAST2) are shown with nodal support values; posterior probabilities of 1.0 are represented with an asterisk. Broken lines in associations represent when one-to-one relationships breaks down.

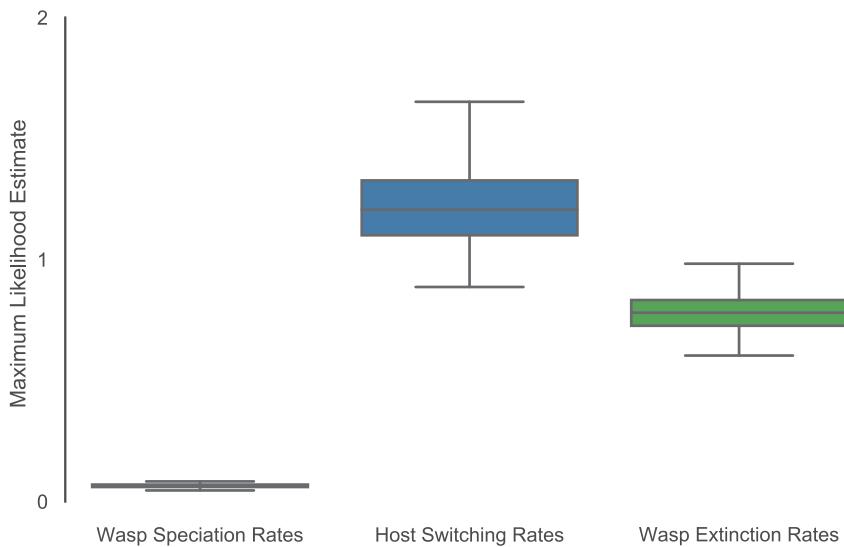


Figure 2. Maximum likelihood estimates for the rates of the three parameters estimated in ALEml for the pruned data set. Results are averaged across 1000 trees drawn from the posterior distribution of species trees sampled from the phylogenetic analysis (SNAPP) of the fig data. Boxplots show interquartile range (25%, median, 75%), with whiskers extending out up to 1.5 times the interquartile range. Outliers are not shown.

of 534 bp (± 137.62), ranging from 122 to 1,072 bp, and were present in an average of 64 individuals (± 4.84). The fig wasp species trees resulting from StarBEAST2 and SVDQst had generally congruent topologies. *Pegoscapus* wasps are grouped into three main clades, with all nodes strongly supported (Fig. 1). In the two cases where single wasp species are associated as pollinators

to two host species, the wasp species are sister in the phylogeny—*P. gemellus A* (hosts: *F. bullenei* and *F. popenoei*) sister to *P. insularis* (hosts: *F. colubrinae* and *F. perforata*). SVDQst shows the same general pattern as the StarBEAST2 trees, with one minor exception—*P. tonduzi* (host: *F. citrifolia*) is sister to *P. piceipes* (host: *F. nymphaeifolia*), with *P. estherae* (host: *F. costaricana*)

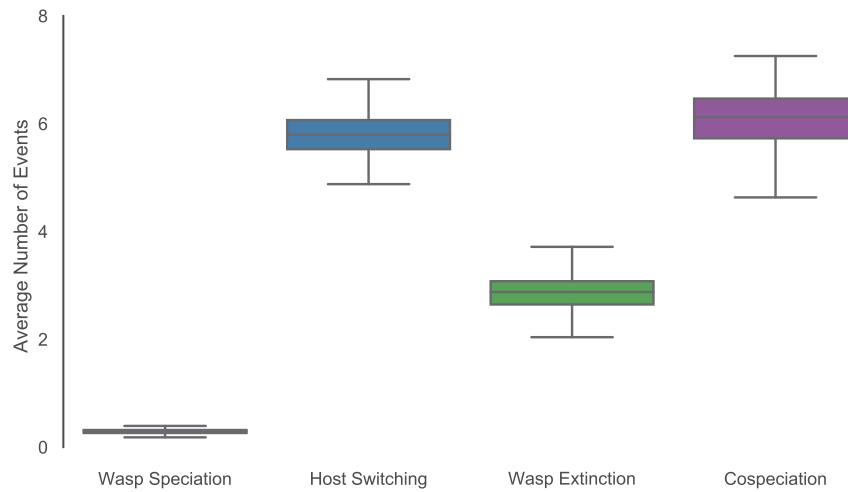


Figure 3. Average number of events estimated from ALEml for the *pruned* data set. Results are averaged across 1000 trees drawn from the posterior distribution of species trees sampled from the phylogenetic analysis (SNAPP) of the fig data. Boxplots show interquartile range (25%, median, 75%), with whiskers extending out up to 1.5 times the interquartile range. Outliers are not shown.

sister to them (Fig. S2). This node, however, has a bootstrap value of 50%, showing little support for this discordant pattern. All other nodes have 100% bootstrap support. SVDQ_{LT} shows the same branching pattern as SVDQ_{ST}, and demonstrates close genetic relatedness among wasp individuals as determined by host fig species (Fig. S3), supporting our placement of individuals within species for species tree analysis.

INFERRING COEVOLUTIONARY PROCESSES IN A MODEL-BASED FRAMEWORK

Integrating across phylogenetic uncertainty in the fig and fig wasp community, our application of the probabilistic DTL model on the *pruned* data set estimated mean maximum likelihood rates of 1.23 (± 0.20) for host switching, 0.79 (± 0.01) for wasp extinction, and 0.06 (± 0.01) for wasp speciation (Fig. 2). Given these rates and the probabilities of events along the branches of the host fig trees, the reconciled fig wasp tree set recovers a mean average of 6.07 (± 0.61) cospeciations, 5.78 (± 0.52) host switches, 2.84 (± 0.35) wasp extinctions, and 0.29 (± 0.05) wasp speciations (Fig. 3). We see generally equal amounts of cospeciation and host switching under the full DTL model with the *pruned* data set, with host switching estimated in roughly half of the tip branches within the host fig tree (Fig. 4). For the *split* data set, we estimated mean maximum likelihood rates of 2.46 (± 3.96) for host switching, 1.51 (± 3.74) for wasp extinction, and 0.07 (± 0.05) for wasp speciation (Fig. S4). Given these rates, we estimated a mean average of 11.01 (± 1.86) host switches, 5.92 (± 1.19) cospeciations, 3.88 (± 1.37) wasp extinctions, and 0.32 (± 0.09) wasp speciations (Fig. S5). Host switching is broadly distributed phylogenetically, especially on the tip branches, but is conspicuously absent from the branches leading to *F. trigonata*

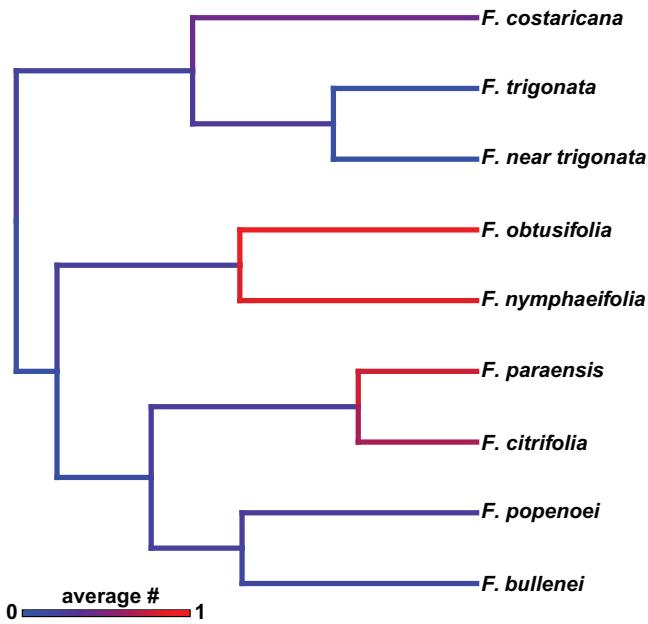


Figure 4. Average number of host switching events for the figs and fig wasps for the *pruned* data set. The maximum-clade credibility species tree representing the figs (SNAPP) was used as the host tree in the ALEml analysis. The average number of host switching events are represented along the branches of the host fig tree.

and *F. near trigonata* (Fig. S6). Collectively, these results suggest an important role for both cospeciation and host switching in producing the evolutionary patterns between this group of strangler figs and their associated pollinating wasps, and also suggests a role for wasp extinction during the evolution of this mutualism.

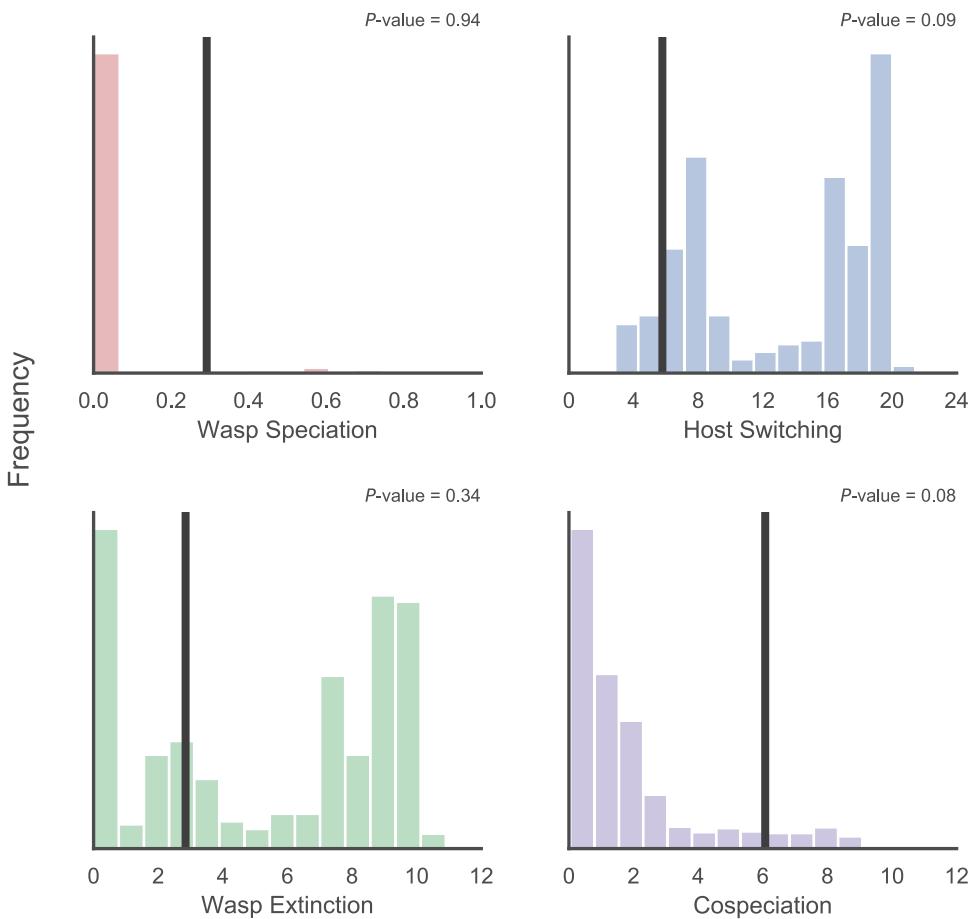


Figure 5. Null distributions of the number of events when using the fig maximum-clade credibility species tree from the *pruned* data set and a simulated species tree representing the fig wasps. Distributions are based on 1000 simulated fig wasp species trees. The vertical lines represent mean estimates from the empirical data. For wasp speciation, wasp extinction, and host switching, *P*-values represent the proportion of simulations that produced an estimate equal to or below the empirical value. For cospeciation, the *P*-value (0.08) represents the proportion of simulations that produced an estimate equal to or above the empirical value.

As there is no explicit association between the simulated trees and empirical trees, simulations provide a context for evaluating empirical parameter estimates against expectations of no cospeciation. For the *pruned* data set, the empirical estimates of wasp speciation and wasp extinction events do not significantly deviate from the null distributions, although the wasp speciations fall close to the upper limit of the distribution (Fig. 5). For the average number of host switches and cospeciations, once again, the empirical estimates do not significantly deviate from the null distributions, although both of these estimates are close to the tails of the distributions, with 9% of simulations at or below the empirical estimate for host switching, and 8% at or above the empirical estimate for cospeciation. This same pattern is seen with the maximum likelihood rates when placed in the context of the simulations (Fig. S7). Results are consistent with the *split* data, as none of the empirical estimates deviate significantly from the null distributions for number of events (Fig. S8) or maximum likelihood rates (Fig. S9). Simulations for both data sets show empirical

parameter estimates under the full DTL model to be consistent with a model that approximates free host switching, regardless of whether shared wasps are removed (*pruned*) or retained (*split*) in the analysis.

Using a model selection approach, we identified four models containing all of the model probability for the *pruned* data set (Table 2). All four models contained host switching, with models not containing this parameter receiving no support. A host switching only model contains the highest probability ($P = 0.42$), followed by a host switching-wasp extinction model ($P = 0.30$), a host switching-wasp speciation model ($P = 0.16$), and the full DTL model ($P = 0.12$). Allowing for each model to contribute to the parameter estimates given its model probability, the reconciled fig wasp tree sets recover an average of 6.83 host switches, 3.64 cospeciations, 1.20 wasp extinctions, and 0.09 wasp speciations (Table S3). Although the full DTL model of the *pruned* data set suggests equal numbers of host switches and cospeciations, accounting for model uncertainty recovers

roughly twice as many host switches as cospeciations. These results were consistent with results from the *split* data set. The same four models contain all of the model probability, with the host switching only model containing the highest probability ($P = 0.46$; Table S4). Model averaged parameter estimates for number of events were 11.50 host switches, 3.27 cospeciations, 1.44 wasp extinctions, and 0.08 wasp speciations (Table S5).

DESCRIBING COPHYLOGENETIC PATTERNS USING TRADITIONAL APPROACHES

PACo recovers a significant cophylogenetic signal between the figs and fig wasps ($m_{XY}^2 = 0.481$, P -value = 0.009, $n = 10,000$). This demonstrates the degree of congruence between the associations is greater than expected based on chance (Fig. 6). This result, however, is driven by the *F. trigonata* + *F. near trigonata* contribution (Fig. 6A). If these two fig species (and associated pollinating wasps) are removed from the analysis, the interaction is no longer statistically significant ($m_{XY}^2 = 0.553$, P -value = 0.11, $n = 10,000$), demonstrating their importance to the congruent cophylogenetic signal. Jane does not recover a significant phylogenetic association between the figs and fig wasp lineages. There were 48 equally parsimonious reconciliations with a total event cost of 20. Forty-two reconciliations contained five cospeciations, three fig wasp speciations, three host switches, nine losses, and two failures to diverge (Fig. 7). The other six reconciliations contained four cospeciations, two fig wasp speciations, five host switches, six losses, and two failures to diverge. When assessing significance of this total cost compared to a null distribution, 25.2% of random trees produced a cost equal to or below this value, with a mean cost number of 23.33 (± 4.31).

Discussion

Determining the ecological and evolutionary processes that contribute to diversification in interacting lineages is a fundamental goal of evolutionary biology. Here, we gathered genome-wide sequence data for a sympatric community of Panamanian strangler figs and pollinating wasps, and using a model-based framework estimated the relative frequencies of multiple processes shaping cophylogenetic patterns in this mutualism. The results indicate host switching to be the dominant process ongoing at this spatial and phylogenetic level, and within the Panamanian community provides support for an evolutionary history in which fig wasps have shifted to different host figs through time.

PANAMANIAN STRANGLER FIGS AND FIG WASPS HAVE A HISTORY OF HOST SWITCHING

At broad phylogenetic scales, sections of figs are primarily pollinated by corresponding genera of fig wasps, with the two groups showing a high degree of topological congruence suggesting co-

cladogenesis (Rønsted et al. 2005; Cruaud et al. 2012). Within these finer phylogenetic levels (sections of fig and genera of pollinators), results are often mixed as to whether cospeciation or other processes are most important for the associations (e.g., Weiblen and Bush 2002; Jousselin et al. 2008). In the Panamanian community of strangler figs and associated wasps, previous molecular work has identified several breakdowns of the one-to-one fig-to-fig wasp association and suggest potentially high rates of host switching as a process contributing to highly discordant phylogenetic patterns at this more fine phylogenetic scale (Molbo et al. 2003; Machado et al. 2005; Jackson et al. 2008). For example, while Cruaud et al. (2012) infer strong signals of cospeciation between figs and pollinators globally, they estimate a conspicuously high amount of host switching among Neotropical strangler figs and their pollinators (see fig. S10 of Cruaud et al. 2012). Machado et al. (2005) argue the more appropriate model for the Panamanian community is one in which genetically well-defined fig wasp species coevolve with groups of genetically less well-defined groups of figs. This model argues for a history in which pollinator host switching has led to introgression among host fig species. Essentially, host switching is common and potentially leads to introgression among fig species.

Previous cophylogenetic studies of the Panamanian community have based inferences of host switching on limited molecular data, with only a few loci sampled from the host figs and their pollinating wasps. Therefore, one of our goals was to make more precise estimates of the phylogenetic relationships among both the figs and the wasps by using thousands of RAD loci for the figs and hundreds of UCE loci for the wasps. This provides much stronger evidence that the history of fig–pollinator association has been punctuated by numerous host switch events throughout evolutionary time. Host switching is modeled to have played an important role in shaping the evolutionary association of fig wasps with several of the fig species sampled here (Fig. 4). More recent exchanges are also likely to be ongoing and detectable in ecological time (Cornille et al. 2012, E.A. Herre, unpubl. data). It is now clear that host switching has occurred at a frequency capable of potentially generating recurrent introgression among fig hosts, which could blur fig species boundaries and decrease host-specific differences in the floral volatile chemical signals used to attract pollinating wasps. Increased overlap in volatile profiles between fig species could further lower barriers to host switching in fig pollinators, leading to a positive feedback loop in which distinct wasp species are associated with introgressed sets of fig species.

Despite our inference of an evolutionary history punctuated by host switch events, contemporary ecological and mitochondrial barcoding data indicate high host specificity between Neotropical fig wasps and their hosts at local scales, with wasps doing a nearly perfect job of locating and pollinating the correct fig

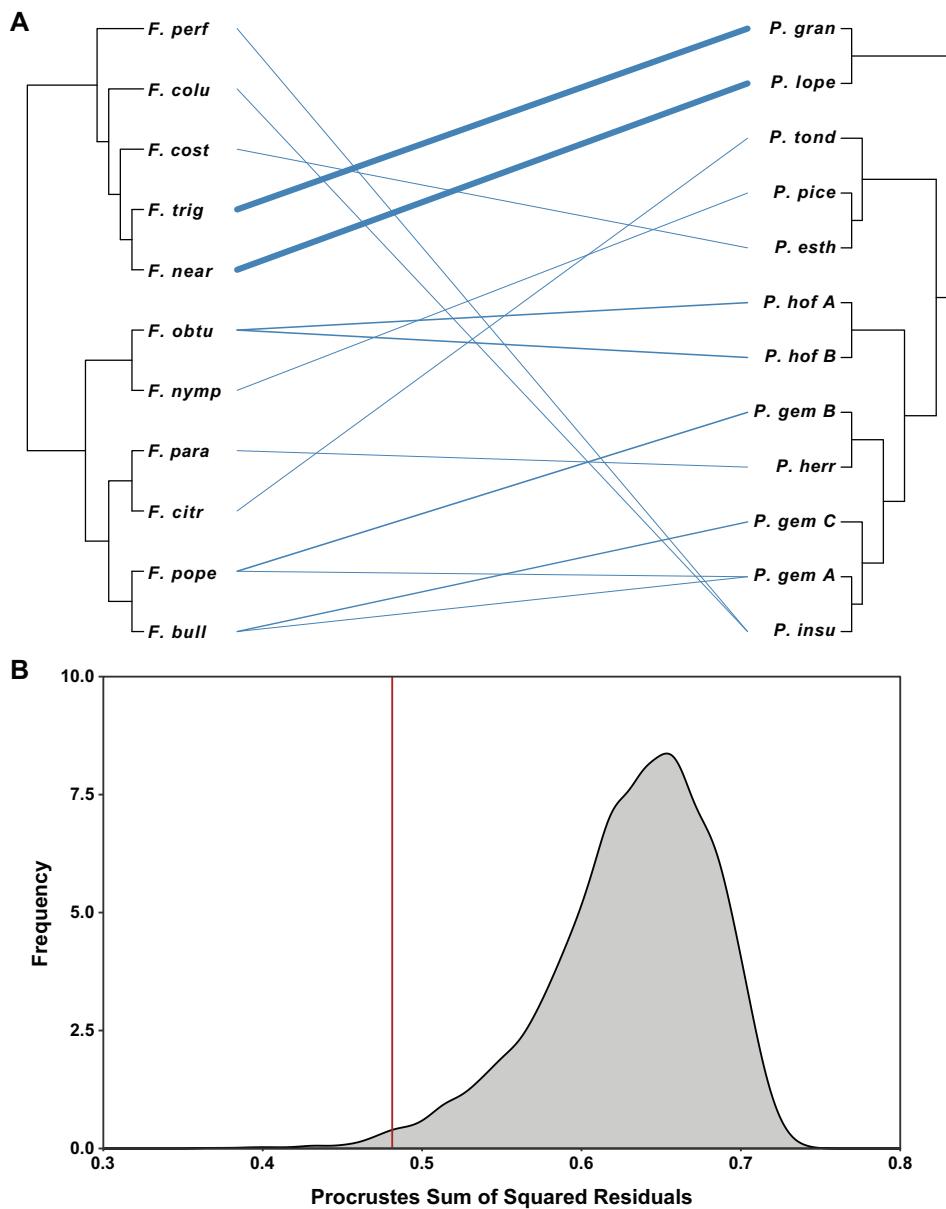


Figure 6. Results from PACo. (A) Associations between host figs and pollinating wasps, with line weight indicating the contribution of each interaction to the global-fit score. (B) A significant association is recovered for this mutualism (P -value = 0.01). As the *F. trigonata* + *F. near trigonata* host–wasp relationship contributes most to this interaction, removal of these taxa results in a global association that is no longer significant (P -value = 0.55). Taxon names have been truncated.

species (Bronstein 1987, E.A. Herre and A. Oldenbeuving unpubl. data). Our UCE data strongly support high levels of contemporary species specificity, as wasps reared from the same fig species—from independent fruit on the same tree and from different trees—cluster in phylogenetic space into well-delineated lineages (Fig. S3). These results are consistent with and expand upon previous phylogenetic inferences based on mitochondrial DNA in this community (Molbo et al. 2003; Machado et al. 2005). Strong evidence for host specificity in ecological time combined with strong evidence for host switching in evolutionary time directs attention to potential combinations of ecological and evolutionary

processes that underlie these seemingly incongruent features of this mutualism.

Floral volatiles released by receptive figs promote species specificity by attracting pollinating wasps carrying conspecific pollen. For example, Proffit et al. (2009) presented the volatile blends of three sympatric fig species to the pollinator of one of those species (*Ficus hispida*). Behavioral assays showed the pollinator is only attracted to its host species, demonstrating high levels of host affinity for this pollinating wasp. Other attributes of the figs can also exert selective pressures on the wasps contributing to the maintenance of host specificity observed in this

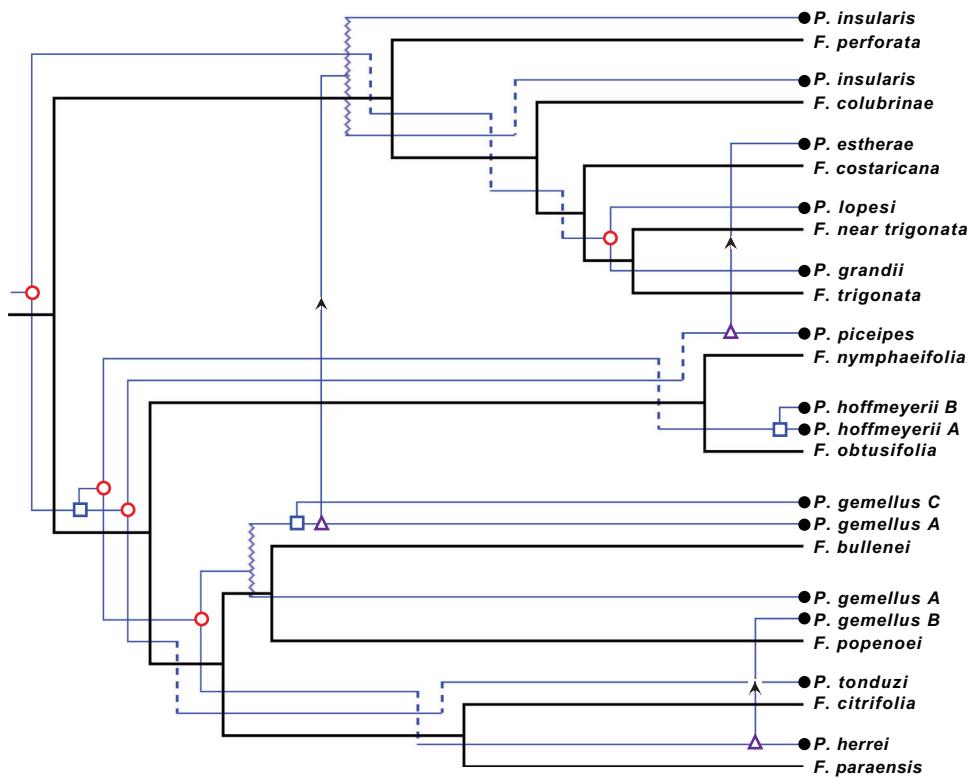


Figure 7. One of the equally most parsimonious reconciliations inferred by Jane. The black tree represents the host fig phylogeny and the blue tree the fig wasp phylogeny, onto which are mapped events that produce the best reconciliation between the two trees. Red circles represent cospeciation, blue squares represent fig wasp speciation, purple triangles represent wasp speciation associated with a host switch, broken lines represent wasp species loss, and jagged lines represent failure of wasps to diverge. The number of events representing the best cost score is not statistically significant (P -value = 0.25).

interaction. Moe and Weiblen (2012) conducted experimental studies in sympatric dioecious New Guinean figs showing that, while seed development and seedling growth were comparable for figs pollinated with conspecific and heterospecific pollen, the successful development of pollinator wasp larvae was reduced in a nonspecific host. In addition, the correlated evolution of traits important for the fig and wasp interaction, including style and ovipositor lengths (Weiblen 2004; but see Nefdt and Compton 1996) and ostiole and pollinator head shapes (Jousselin et al. 2003), may also provide mechanisms where proper matching is favored. Pollinators, however, have been occasionally documented in nonnatal host figs, with some producing viable offspring, although inviable seeds (Ramírez 1970). So while selective pressures derived from appropriate host recognition and compatibility on both figs and pollinating wasps promote species-specificity, evidence suggests opportunities for colonizing nonnatal hosts exist. Indeed, across the tropics, analyses of fig wasp species sharing the same monoecious hosts (copollinators) show that a significant fraction (32%) are not closely related and have thus switched hosts at some point in the past (Yang et al. 2015).

Life history traits and geography provide opportunity for host shifts to occur and be maintained. Fig wasps have multiple gen-

erations per year (8–12) with a large number of pollen-carrying female wasp offspring produced per tree (Korine et al. 2000; Weiblen 2002). They are capable dispersers, traveling many kilometers in search of a receptive fig (Nason et al. 1998), with some species of fig wasps having been recorded dispersing over 160 km (Ahmed et al. 2009). Many fig species have broad distributions, and in cases where pollinators have been sampled from throughout their range, multiple pollinator species have been found, typically in different geographic areas (e.g., Haine et al. 2006; Chen et al. 2012; Bain et al. 2016; Rodriguez et al. 2017). For example, Darwell et al. (2014) explored pollinator species diversity on *Ficus rubiginosa* distributed from northern Queensland to New South Wales in Australia. They described the presence of five pollinator species with limited species overlap (although they are not geographically isolated). Even higher pollinator diversity was found along the distribution of *Ficus hirta*, where Yu et al. (2019) recovered nine pollinator species with parapatric distributions associated with this host fig. Because these studies did not also sample wasps from co-occurring host figs, it remains unclear whether the multiple species associated with a fig host are all specific to that host, or are shared locally with other hosts as yet unidentified.

What broader patterns can we infer about figs and fig wasps from results in our local Panama community? Two sections of figs (strangling and free-standing) and their pollinating wasps (*Pegoscapus* and *Tetratus*, respectively) have independently colonized the Neotropics (Craaud et al. 2012). Strangler figs represent much of the species diversity in the Neotropics, with over 120 described species compared to roughly 20 described species for the free-standing figs. Many of the strangler fig species found in Panama have widespread distributions that span biogeographic barriers, such as the Andes, and within the broader strangler fig phylogeny, the Panamanian community is not monophyletic (Machado et al. 2018). We predict that, as is the case of the Australian fig *F. rubiginosa*, many of these fig species host multiple pollinator species across their distribution. Even at our local scale, Molbo et al. (2003) discovered that four of the eight sampled Panamanian strangler fig species host pairs of pollinator species in sympatry. Both local sampling of pollinator wasps through time and geographic sampling across the distributions of their host figs will be important for understanding community assembly dynamics of the fig wasps, including where the pollinators came from and how they are related (Molbo et al. 2003; Machado et al. 2005; Jackson et al. 2008; Cornille et al. 2012). We hypothesize that cases of pollinator sharing and host switching will be mechanistically explainable by similarities of the attractant floral chemical signals that fig species produce and that pollinator wasps recognize. Finally, we note the consequences of pollinator host switching and subsequent blurring of fig species boundaries also potentially affects the evolutionary trajectories of several additional trophic levels of fig and wasp associated organisms, including a diverse community of nonpollinating fig wasp parasites (e.g., gallers, kleptoparasites, and parasitoids) and other antagonists (e.g., nematodes and lepidopteran fruit predators) (Herré 1993; West et al. 1996; Marussich and Machado 2007; Piatscheck et al. 2018; Van Goor et al. 2018).

MODEL-BASED APPROACHES FOR ADDRESSING COPHYLOGENETIC QUESTIONS

Current cophylogenetic methods measure the degree to which two phylogenies compare (global-fit) or map one tree onto another using a priori event costs and the parsimony criterion to identify the optimal reconciliation (event-based). These methods are necessarily limited because they are not based on a probabilistic model where the relative contributions of underlying mechanistic processes can be statistically evaluated. Recent advances in cophylogenetic methods use approximate Bayesian computation (ABC) to estimate the posterior probability of processes important for producing cophylogenetic patterns (Baudet et al. 2014; Alcalá et al. 2017). Although ABC is likelihood free and can be sensitive to choice of summary statistics for approximating the posterior probability (Robert et al. 2011), these methods provide

an important contribution in evaluating cophylogenetic questions in a process-generating perspective.

As our application of the DTL model in ALEml uses a likelihood function, explicit predictions can be generated and tested in a statistical framework, allowing us to estimate the evolutionary and ecological processes that have contributed to the historical association of interacting lineages. In addition to the fig and fig wasp mutualism, this approach can also be applied to other systems with strong ecological associations, like hosts and parasites. For example, Groussin et al. (2017) applied this method to mammals and their microbiomes to test whether vertical versus horizontal transmission was the dominant signal in the coevolution of bacterial communities and their hosts. They discovered that 67% of bacterial groups contained a stronger signal of cospeciation than host switching, providing an empirical example for how this model can inform transmission dynamics of microbiome communities. There are two important issues that would be useful to address moving forward. A logical extension of this approach will be to incorporate absolute divergence time information of the two groups into the model. Currently, the DTL model in ALEml accounts for the relative node positioning within the host phylogeny, but does not account for branch length information of the symbiont trees. Although appropriate when addressing gene families within species, as genes evolve within species, this assumption may be violated when dealing with host/symbiont interactions as the timing of association and diversification may not be the same. For example, if diversification times were on different time scales between host and symbiont, some evolutionary processes (e.g., host switching and cospeciation) would have zero probability if associated lineages were not present at the same time. This would be most problematic if phylogenetic patterns were similar between interacting lineages, but divergence times at those shared nodes were not overlapping. This pseudocongruence would obscure the true history that a process other than cospeciation necessarily contributed to these patterns (e.g., Donoghue and Moore 2003). Although there are challenges associated with integrating temporal information in the model-based framework, a more fundamental problem is estimating accurate divergence time information for cophylogenetic analysis from organisms with different rates of sequence divergence. Divergence time estimation is challenging, and timing estimates often contain considerable uncertainty. In addition, without appropriate fossil information (or other geological data), it is not possible to convert relative branch times to absolute time with any sort of precision. It will be important to further adapt the DTL model for cophylogenetic inference, where we can account for additional parameters (e.g., divergence times) important in systems with tight ecological interactions.

An assumption of the DTL model in ALEml is that each symbiont lineage may only be associated with a single host lineage. Violation of this assumption could lead to biased parameter

estimates depending upon how this assumption is violated. This is an issue whenever an individual symbiont species is associated with two or more host species and requires the investigator to make explicit assumptions about the processes that have generated these associations. In our data set, we have two examples of pollinator sharing, with one pollinator species associated with *F. colubrinae* and *F. perforata*, and a second associated with *F. bullenei* and *F. popenoei*. We used two approaches for dealing with these shared taxa to test how their inclusion (or exclusion) influenced parameter estimates under the DTL model. In the *split* data set, following Ronquist (2003), we associated the individual tips with the host tree species from which they were sampled. Using this approach, we allowed the fig host phylogeny to determine the processes (e.g., host shift, cospeciation) producing the current association. The explicit assumption of this approach is that the shared pollinator is composed of two species, which may be a product of cospeciation or a recent host shift. For example, Molbo et al. (2003) suggests the wasp *P. gemellus A* shared between *F. bullenei* and *F. popenoei* is the product of a recent host shift. If the shared wasps are consistently pollinating their natal fig species and are no longer part of the same gene pool, a recent host shift would be consistent with the lack of detectable genetic differences between *P. gemellus A* wasps sampled from the two host species. But as splitting shared symbionts could result in an inflation of inferred host switches, we also pruned the shared taxa to satisfy the assumptions of the model and provide a comparison between treatments. When comparing results of the full DTL model from the *split* and *pruned* data sets, the *split* data set estimates high levels of host switching as opposed to cospeciations, whereas the *pruned* data set estimates similar levels of cospeciation and host switching. The distribution of host switches for the *split* data set (Fig. S6) is concentrated along tip branches, including higher values on the branches leading to the host species sharing a pollinator. The model thus suggests that recent host shifts have resulted in the shared pollinators, if, in fact, they are incipient species. Pruning the shared pollinators, however, still resulted in a considerable number of estimated host switches. Although parameter estimates under the full DTL model for the *pruned* data set recovered roughly equal numbers of cospeciations (6.08) and host switches (5.78), the full DTL model contains the fourth highest model probability ($P = 0.12$). In contrast, a host switching only model had the highest probability ($P = 0.42$) and estimated considerably higher amounts of host switching (7.51) over cospeciations (1.90). In addition, model averaged parameter estimates recovered twice as many host switches (6.83) as cospeciations (3.64) from the *pruned* data set.

Our inference of host switching as an important process in the Panamanian strangler fig community is consistent regardless of how we treat the shared pollinators. Nevertheless, symbiont

taxa associated with two or more hosts violate the model assumption of host specificity as implemented in ALEml. This model assumption will be important to consider if using this approach for cophylogenetic questions where each symbiont lineage is not associated with a single host lineage (e.g., generalist pollinators and flowering plants). Systems where interactions are more diffuse would be better suited for methods that explicitly account for multihost taxa. For example, the one-to-one association is not a requirement of PACo or Jane, where having generalist lineages is accounted for either by replicating rows in the data matrices (PACo) or applying a new parameter (failure to diverge) as done in Jane. In addition, the ABC event-based method of Alcala et al. (2017) allows symbiont lineages to be associated with multiple host lineages, modeling symbiont taxa as either a specialist or generalist based on the observed distribution of generalists in the data set.

Conclusions

This is the first study to use genome-scale data for both figs and their pollinator wasps to address the evolutionary history of association in this obligate mutualism. We analyze these more precise data using a model-based framework to test explicit predictions about the processes most important for generating observed patterns of diversification. Focusing on a Panamanian strangler fig community in which the large majority of wasp species are locally host specific, we demonstrate that the evolutionary history of the fig-pollinator association has been punctuated by frequent host switching events. A history of host shifting by the pollinators provides a potential mechanism for genetic introgression between figs, a process that has previously been suggested but not conclusively demonstrated. By adapting the implementation of the DTL model in a probabilistic method, we provide a framework for evaluating cophylogenetic processes in interacting lineages using a process-based approach. As collecting genome-wide sequence data is becoming ubiquitous for nonmodel organisms, these data coupled with better models allows us to generate meaningful inferences into the processes influencing the diversification of interacting species.

AUTHOR CONTRIBUTIONS

JDS, TAH, JDN, and EAH designed the study; EAH and KCJ led collection of fig and fig wasp samples; DARE processed the fig sequence data; JDS processed the wasp sequence data and conducted all analyses; JDS, EAH, TAH, and JDN wrote the paper; and all authors contributed to revised versions of the manuscript and approved the final version.

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DATA ARCHIVING

Raw sequence data are available from the NCBI Sequence Read Archive (SRA) under BioProject ID: PRJNA554331 (SAMN12175184–SAMN12175265 and SAMN12175274–SAMN12175304). All data sets and custom scripts are available on Dryad (<https://doi.org/10.5061/dryad.423q544>).

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. SVDQ_{ST} of the figs.

Figure S2. SVDQ_{ST} of the fig wasps.

Figure S3. SVDQ_{LT} of the fig wasps.

Figure S4. Maximum likelihood estimates for the rates of the three parameters estimated in ALEml for the *split* data set.

Figure S5. Average number of events estimated from ALEml for the *split* data set.

Figure S6. Average number of host switching events for the figs and fig wasps for the *split* data set.

Figure S7. Null distribution of the maximum likelihood rates when using the fig maximum-clade credibility species tree from the *pruned* data set and a simulated species tree representing the fig wasps.

Figure S8. Null distribution of the number of events when using the fig maximum-clade credibility species tree from the *split* data set and a simulated species tree representing the fig wasps.

Figure S9. Null distribution of the maximum likelihood rates when using the fig maximum-clade credibility species tree from the *split* data set and a simulated species tree representing the fig wasps.

Table S1. Fig sampling and sequence information.

Table S2. Pollinator sampling and sequence information.

Table S3. Parameter estimates for all models for the *pruned* data set.

Table S4. Model set and model probabilities for the *split* data set.

Table S5. Parameter estimates for all models for the *split* data set.