SHORT COMMUNICATION
Speciation in fig pollinators and parasites

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Abstract
Here we draw on phylogenies of figs and fig wasps to suggest how modes of speciation may be affected by interspecific interactions. Mutualists appear to have cospeciated with their hosts to a greater extent than parasites, which showed evidence of host shifting. However, we also repeatedly encountered a pattern not explained by either cospeciation or host switching. Sister species of fig parasites often attack the same host in sympatry, and differences in ovipositor length suggest that parasite speciation could result from divergence in the timing of oviposition with respect to fig development. These observations on fig parasites are consistent with a neglected model of sympatric speciation.

Keywords: Agaonidae, coevolution, cospeciation, mutualism, parasitism, phylogeny

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Introduction
Life history adaptations affecting the process of species formation may explain why some groups of organisms are more diverse than others (Farrell 1998), but modes of speciation have rarely been compared in closely related groups that interact with other species in fundamentally different ways. Interactions between fig wasps (Hymenoptera, Agaonidae) and their host plants (Ficus, Moraceae) range from mutualism to parasitism, and have served to test evolutionary theories of sex allocation (Herre 1985, 1987; West et al. 2000), kin selection (Hamilton 1967; West et al. 2001) and virulence (Herre 1993). However, few data are available comparing patterns of speciation in figs, their pollinating mutualists and nonpollinating parasites (Machado et al. 1996; Lopez-Vaamonde et al. 2001). Evolutionary models of obligate mutualism predict the parallel radiation of figs and pollinators (Kiester et al. 1984), but is this also the case for parasites?

All fig species are pollinated by mutualistic fig wasps (Agaonidae, Agaoninae) that feed exclusively on the developing seeds of their hosts and phylogenetic analyses indicate that fig pollination evolved once (Herre et al. 1996). Pollinators of figs are characterized by extreme host specificity, morphological adaptations and life cycles that depend entirely on host reproduction (Fig. 1). In theory, resource conflicts between pollinators and hosts could threaten the persistence of mutualism (Pellmyr & Huth 1994), and yet the richness of Ficus (~750 species worldwide) and their pollinators suggests that the interaction is evolutionarily stable. Resembling a fruit, the fig is an enclosed inflorescence containing many unisexual flowers that are accessible to fig wasps through a narrow opening at the apex of the receptacle. Pollen-carrying females are attracted by host-specific fragrances released from receptive figs (Hossaert-McKey et al. 1994) and they push their way into the fig cavity, where they lay eggs in a fraction of the flowers they pollinate. Their offspring feed on fig endosperm as larvae and mate in the fig cavity as adults. Females complete the life cycle by transporting pollen from natal figs to receptive figs, where the fertilization of flowers provides the next generation of pollinators with seed resources.

A diverse assemblage of nonpollinating wasps, including gallers that compete with pollinators for fig resources, and parasitoids that attack pollinator larvae in figs may weaken the mutualism. Unlike the pollinators, some fig parasites attack the flowers by piercing the outside of the receptacle with long ovipositors (Fig. 1b). The genus Apocryptophagus (Agaonidae, Sycophaginae) induces abnormal development of fig ovaries into large galls (Fig. 1c), which affect the mutualism negatively by damaging flowers and by competing with pollinators. The impressive diversification of figs and pollinators is thought to be the product of cospeciation, the parallel radiation of interacting lineages.
(Kiester et al. 1984). On the other hand, host shifting can also lead to the formation of new species, especially in phytophagous parasites (Bush 1994). Whether fig pollinators and gallers differ in modes of speciation is unknown, and this situation provides a novel opportunity to compare the evolution of host associations in closely related mutualists and parasites (Kerdelhue et al. 1999).

Materials and methods

Apocryptophagus parasites specialize on figs in the subgenus Sycomorus sensu lato, which is monophyletic and pollinated by the genus Ceratosolen (Weiblen 2000, 2001). Sixteen species of Sycomorus s. l. from Melanesia were the focus of sampling, with the addition of three species from outside the region. Nineteen Ceratosolen species (Weiblen 2001) and 18 undescribed morphospecies of Apocryptophagus associated with the selected hosts were included in phylogenetic analyses of manually aligned partial sequences from the mitochondrial cytochrome oxidase I gene (COI). Apocryptophagus morphospecies (hereafter referred to as species) are distinguished on the basis of body size and ovipositor length (Table 1). Kerdelhue & Rasplus (1996) showed that multiple species of Apocryptophagus on the same host differ significantly in ovipositor length and in the timing of oviposition, which are correlated with fig diameter. A single individual from each Apocryptophagus species was sequenced for partial COI (GenBank accession numbers AF364519-AF364535 and AF200371). GenBank accession numbers for pollinating fig wasps as described by Weiblen (2001) are 200374-AF200388 and AF200390-AF200393. Accession numbers for Ficus nuclear ribosomal ITS sequences from Weiblen (2000) are AF165374, AF165376, AF165378, AF165379, AF165380, AF165383, AF165388, AF165391, AF165394, AF165395, AF165396, AF165404, AF165405, AF165406, AF165408, AF165409, AF165411, AF165412 and AF165415. Heuristic searches under parsimony were conducted with PAUP* (Swofford 1998) with 1000 random addition sequence replicates, and bootstrapping with 1000 replicates provided measures of clade support.

Tests of cospeciation were performed using TREEMAP software (Page 1996) to generate a reconciled tree that maximized cospeciation and minimized duplications and losses of associations under parsimony. A randomization test estimated the probability of observing maximum cospeciation against a null distribution obtained from 10,000 random addition sequence replicates, and bootstrapping with 1000 replicates provided measures of clade support.

Fig. 1 (a) A pollinating fig wasp belonging to the genus Ceratosolen, (b) and a nonpollinating fig wasp belonging to Apocryptophagus, (scale bars are 1 mm). (c) The interior of a fig in cross-section showing female Ceratosolen pollinating flowers and laying eggs. The enlarged flowers are galls induced by Apocryptophagus, which lays eggs by piercing the fig wall. (d) Figs in early and late phases of development differ in wall thickness (scale bars are 1 cm).
pairs of randomly generated 19-taxon trees under the
proportional-to-distinguishable model. Null (H₀) and altern-
native (H₁) hypotheses that the same or different histories
underlie pollinator mtDNA and fig nrDNA were com-
pared using a maximum likelihood (ML) test of hetero-
genetics (Huelsenbeck & Rannala 1997; Huelsenbeck et al.
1997). To determine the most appropriate model of nucleotide
substitution under ML, nested models were compared
with likelihood ratio tests (Posada & Crandall 1998). A
general time reversible model (GTR) with the addition of a
parameter for heterogeneity in the rate of substitution
Γ

was used to estimate
branch lengths and model parameters. Deviations from one-to-
one specificity in fig parasites were accommodated by
duplicating fig nrDNA sequences from hosts that had
more than one parasite species, and by removing nrDNA
sequences from hosts that lacked parasites. Most parsimo-
nous trees from combined searches of fig nrDNA plus fig
wasp mtDNA sequences were then used to estimate
nrDNA and mtDNA branch lengths in the null case (H₀). The
likelihood of identical history (H₁) was then obtained
by summing the likelihoods of the separate data sets under
the same topology but given separate model parameters
for nrDNA and mtDNA. Monte Carlo simulation com-
pared the ratio of H₀ and H₁, likelihoods, given that the test
statistic (χ²) was not χ² distributed. One hundred pairs
of nrDNA and mtDNA data sets were generated using the
program Seq-General (Rambaut & Grassly 1997). Each data
set was simulated along the combined tree assuming a
Markov process with branch length estimates and model
parameters based on the observed nrDNA and mtDNA.
Separate and combined heuristic searches under parsi-
mony with 10 random addition sequence replicates were
then performed for each pair of data sets and the ratio of H₀
and H₁ for the simulations provided a null distribution
against which to compare the observed data in a one-tailed
test of significance. Fig wasp mtDNA data sets rejected the
assumption of a molecular clock due to heterogeneity
in substitution rates, disallowing tests of cospeciation
based on Bayesian estimation (Huelsenbeck et al. 2000). Log-
likelihood tests of the molecular clock for Apocryptophagus
and Ceratosolen were χ² = 86.37 (d.f. = 1, P < 0.001) and
χ² = 116.18 (d.f. = 1, P < 0.001), respectively.

Results and discussion
Heuristic searches yielded two most parsimonious trees
for Apocryptophagus (L = 819; CI = 0.41, with uninformative
positions excluded) based on 188 informative positions
of the 402 nucleotide alignment (47% informative). For Ceratosolen,
167 positions were informative (42%) and heuristic searches

Table 1  Apocryptophagus species included in the phylogenetic analyses. The relative timing of Apocryptophagus oviposition is indicated for
host species with more than one parasite. Species pairs a–h in Figs 2 and 3 are also indicated. Length measurements are reported in mm.
Means (and standard deviations) are based on N individuals from the same fig crop.

<table>
<thead>
<tr>
<th>Host species</th>
<th>Ficus species</th>
<th>Host voucher</th>
<th>Locality</th>
<th>Timing of oviposition</th>
<th>Species pair</th>
<th>Thorax length</th>
<th>Ovipositor length</th>
<th>N</th>
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yielded six trees (L = 608; CI = 0.43). The pollinator topologies were highly similar to those from intensive analyses of pollinators based on 2 kb mtDNA (Weiblen 2001) and midpoint rooting of a simultaneous analysis supported the monophyly of the pollinating lineage. The phylogeny estimate for 19 host Sycomorus species based on the nuclear ribosomal internal transcribed spacer region (ITS) was very similar to results obtained from combined analyses of molecular and morphological data (Weiblen 2000). Fifteen trees (L = 89; CI = 0.64) were obtained from heuristic searches based on 30 informative characters out of 761 aligned positions (7%; excluding nine indels).

Phylogenies for Apocryptophagus, Ceratosolen, and Sycomorus suggest that the pollinators have cospeciated with their host plants to a greater extent than the parasites (Fig. 2). Depending on which of the 15 host topologies was analysed, between nine and 10 cases of cospeciation for mutualists were inferred from reconciled trees (Page 1994). Fewer cospeciation events were inferred for figs and their parasites, between seven or eight according to host topologies. Cospeciation between mutualists was significantly greater than expected by chance; that is, if figs and pollinators were associated at random (\( P = 0.030\)–0.009). On the other hand, chance could account for the inferred level of cospeciation between figs and parasites (\( P = 0.050\)–0.193). Although many nodes in Fig. 2 lack bootstrap support, the relationships shown are essentially in agreement with the results of our analysis.

We examined potential sources of phylogenetic incongruence between the host, pollinator and parasite data sets. Phylogenetic conflicts could be due to host switching, to unequal evolutionary rates, or to systematic error. To explore the latter possibility, we employed maximum likelihood to test whether incongruence between the interacting lineages could arise from random mutation and drift in two genes that share a common history (Huelsenbeck & Bull 1996; Huelsenbeck et al. 1997). The method compares the likelihood of gene sequences assuming separate and common histories for hosts and their associates. A distribution of log-likelihood differences is generated by simulating sequence data under the null hypothesis of shared history, but assuming separate DNA substitution models and parameters for each gene. Systematic error could not be ruled out as a cause of heterogeneity among the DNA sequences of the mutualists (\( \delta = 28.8\); NS). On the other hand, error was rejected as an explanation for conflict between the fig and parasite phylogenies (\( \delta = 79.7\); \( P < 0.01\)). These results suggest that other factors such as host switching or unequal evolutionary rates account for phylogenetic conflicts between parasites and their hosts. Mean (± SD) uncorrected distances for sister pollinator species and sister parasites were 0.16 (0.05) and 0.14 (0.05), respectively. Similar levels of mtDNA divergence in pollinators and parasites favour the mode of speciation as the most probable explanation for these patterns.

Why would fig parasites speciate differently than the mutualists? We hypothesize that nonpollinating Apocryptophagus are less constrained by the reproductive requirements of their hosts than are pollinating Ceratosolen. Floral fertilization is required to produce the endosperm in which pollinator offspring depend for survival, but parasites circumvent these steps by inducing the abnormal proliferation of the nucellus in fig ovules (Fig. 1c). Bypassing...
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Fig. 3 Sister group comparisons of ovipositor length in the fig parasite, Apocryptophagus, under three alternative modes of speciation. (a–h) refer to sister groups in Fig. 2. Sister species (b) attacking sister hosts, F. adenopersma and F. ochrochlora, represent cospeciation. In contrast (a–c, g) are sister species on non-sister hosts, indicating potential instances of host switching. Closest relatives attacking the same host are represented by (d–f). We predict less divergence in ovipositor length between sister species in cases of cospeciation and host switching than in the case of a phenological shift, where divergence results from a shift in the relative timing of oviposition. Greater ovipositor length divergence between sister species (d–f) indicates a relatively large shift in the timing of oviposition, as the thickness of the fig wall increases during development (Kerdelhue & Rasplus 1996).

fertilization may enable parasites to induce galls on new host species, while pollinators shifting between host species could encounter pollen incompatibility and competition. Thus, the exchange of pollination services for larval food that defines the mutualism may also constrain the evolution of novel associations such that figs and pollinators tend to cospeciate. Speciation in parasites, on the other hand, need not be so tightly coupled with host speciation. Need not be so tightly coupled with host speciation.

We expected speciation by host switching to account for the majority of cases in which two parasite species are associated with the same plant species. However, we identified three cases in which parasite species attacking the same host are nearest relatives (Fig. 2). These cases are illustrated in Fig. 3, where sister species using the same fig differ markedly in ovipositor length by at least 2 mm. On the other hand, ovipositor lengths are more similar (< 1 mm divergence) in cases of cospeciation and host switching than in the case of a phenological shift, where divergence results from a shift in the relative timing of oviposition. Greater ovipositor length divergence between sister species (d–f) indicates a relatively large shift in the timing of oviposition, as the thickness of the fig wall increases during development (Kerdelhue & Rasplus 1996).

and phylogenetic data suggest that divergence in the timing of oviposition with regard to fig phenology may initiate and accompany the speciation of parasites attacking the same host species.

This ecologically driven divergence is similar to the sympatric mode of speciation proposed by Gibbons (Gibbons 1979; Ramadevan & Deakin 1990) to explain the origin of three closely related sympatric Megarhyssa (Hymenoptera, Ichneumonidae) species that differ only in ovipositor length. Megarhyssa parasitize subpopulations of wood-boring Tremex columba (Hymenoptera, Siricidae) that feed at different depths in tree trunks. Fig parasites support the validity of Gibbons model of resource-partitioned speciation for the interactions of parasitic Hymenoptera, and are consistent with other recent models of sympatric speciation (Johnson et al. 1996; Dieckmann & Doebeli 1999; Kondrashov & Kondrashov 1999). More data on fig phenology and oviposition are needed to confirm these suppositions. A further step in phylogeny reconstruction would be to increase sampling of Sycomorus and associated wasps with expanded molecular data sets. The prediction of divergent selection on ovipositor lengths of sister species on the same host may also be tested in field experiments. A final integrative step would be to examine patterns of speciation in other fig-inhabiting organisms, including parasitoids and nematodes.

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References


Herre EA, Machado CA, Berningham E et al. (1996) Molecular
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