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African parasitoid fig wasp diversification is a function of *Ficus* species ranges

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ABSTRACT

Host specificity is a fundamental property implicit in obligate insect–plant associations. Rigid life history constraints exhibited by parasitoid fig wasps are believed to select for specialization directed at fig trees and this is supported by evidence of phenotypic adaptation to figs and partial co-speciation with the fig wasps they attack. Conversely, the ability to colonize such novel communities occurs under relaxed specificity, a behavior typified by more generalist groups such as parasitoids. The specificity directed towards *Ficus* species by Sycoryctinae parasitoid fig wasps is important in order to understand how this form of specialization influences their diversification and interactions with other fig wasp guilds. We use genetic distance analyses and reconstruct ancestral patterns of *Ficus* trait association with two genera of Sycoryctinae parasitoid fig wasps to identify evolutionary conservatism in *Ficus* species utilization. Ancestral state reconstructions of (i) affiliate *Ficus* subsection and (ii) syconia diameters of natal *Ficus* species indicate contrasting *Ficus* species ranges between *Arachonia* and *Sycoryctes* parasitoid genera. This work demonstrates that parasitoid speciation is not tightly constrained to *Ficus* speciation and rather a function of *Ficus* range limitations. *Ficus* evolution, ecology, and functional compatibility between parasitoid and *Ficus* traits appear to constrain parasitoid *Ficus* utilization. These results suggest that contrasting ecological settings and potential number of hosts available impose different ramifications for the evolution of parasitoid *host* specificity and so to the species interactions within the communities to which they belong.

1. Introduction

Coevolutionary interactions provide a platform for explaining ecological and physiological mechanisms underpinning species-level associations across a diverse assortment of organisms (Ehrlich and Raven, 1964; Fox, 1988; Compton and van Noort, 1992; Norton and Carpenter, 1998; Pollock et al., 1999; Hongoh et al., 2005; Dodds et al., 2006; Kissling et al., 2006). Host plant evolutionary ecology can play a dominant role in structuring a community (Futuyma and McCaferty, 1990), but this process is obscured in cases of less evolutionarily-constrained associations such as where parasitoid life history strategies imply a departure from high specificity and when primary and secondary host groups are involved (Compton and Hawkins, 1992). The extent to which the evolution of specificity displayed by parasitoid fig wasps (Hymenoptera, Chalcidoidea, Pteromalidae, Sycoryctinae) towards: (i) the *Ficus* (Moraceae) they specialize on and (ii) for the galling fig wasp species they attack, remains unclear.

The trophic structure defining fig wasp interactions with *Ficus* is expected to foster complex multiple divergent species interactions (Weiblen, 2002). Fig wasps comprise a group of galling pollinators (Agaonidae), galling non-pollinators (Sycoecinae, Otitesellinae, Sycophaginae, Epichrysomallinae), and parasitoids (Sycorcytinae, Eurytomidae, Ormyridae). Fig wasp diversity is believed to have assembled through multiple independent colonizations of *Ficus* by divergent chalcid wasp groups (Rasplus et al., 1998), but with specialization on *Ficus* presumed to have co-evolved over considerable geological timescales (Zerega et al., 2005; Silvieus et al., 2007). Fig wasps depend on *Ficus* for oviposition and reproduction, shelter, and nutrition. Most species of non-pollinating wasps have been categorized as gallers, inquilines, or parasitoids, but their life histories are likely more diverse than currently appreciated (Compton and van Noort, 1992; Weiblen, 2002).

The non-pollinating fig wasp subfamily Sycoryctinae, are all parasitoids, or klepto- or hyper-parasitoids (Tzeng et al., 2008; Compton et al., 2009). All female adults have extremely long ovipositors and oviposit from the outside of figs, and as larva feed on the developing larva of galling fig wasps or feed on endosperm. Non-pollinating fig wasps exhibit characteristics that should favor *host* fidelity including the responses to chemical cues used to

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locate figs and ovipositor length adaptation to fig size (Compton et al., 1994). Past investigations infer that parasitoid fig wasps diversify as a result of a combination of co-speciation and restricted switching among the other fig wasp species they attack, or in the absence of co-speciation altogether (Machado et al., 1996, 2005; Lopez-Vaamonde et al., 2001; Jousselin et al., 2006, 2008). The number of *Ficus* species that can be successfully exploited by parasitoid fig wasps is critical to deciphering the specificity parasitoids direct at other fig wasp guilds (Poulin and Mouillot, 2003; Proffit et al., 2007).

An estimate of host specificity is important as it indicates the broad limitations of an organism's role within the extent of its ecological niche. Parasitic organisms, in the broadest definition (Eggleton and Gaston, 1990), are believed to be less evolutionarily constrained by host-associate diversification as evidenced by pervasive host-switching (Norton and Carpenter, 1998; Cronin and Abrahamson, 2001; Roy, 2001; Charleston and Robertson, 2002; Sorenson et al., 2003; McLeish et al., 2007). The evolution of parasite specificity *per se* is largely driven by the convergent influences of microhabitat, host ecology, and coevolution (Adamson and Caira, 1994). The magnitude of specificity directed at *Ficus* by parasitoid fig wasps has consequences for ecological interactions among other fig wasp guilds and their diversification (Holt and Lawton, 1993; Machado et al., 1996, 2005; Weiblen, 2002; Molbo et al., 2003; Marussich and Machado, 2007; Dunn et al., 2008). Although parasitoid fig wasps are capable of exploiting more than one fig wasp species that specialize on a particular *Ficus* species, these relationships tend to be specific to that *Ficus* species (Compton and van Noort, 1992; Jousselin et al., 2008) though exceptions are known (Marussich and Machado, 2007). Therefore, specificity for the fig syconium microhabitat by parasitoid fig wasps is perhaps the foremost condition determining *Ficus* species fidelity.

Here we demonstrate variation in *Ficus* specificity by two African Sycoryctinae parasitoid fig wasp genera (*Arachonia* and *Sycoryctes*) that specialize on galling fig wasp larva. Genetic distance estimates are used to explore patterns of *Ficus* affiliation among Sycoryctinae taxa. Ancestral character state reconstruction of *Ficus* subsection affiliation and *Ficus* species syconia diameters are used to identify patterns of specificity directed towards *Ficus*. We tested the hypothesis of evolutionary conservatism of *Ficus* species among parasitoid genera *Arachonia* and *Sycorytes* by: (i) inferring phylogenetic relationships using two mitochondrial DNA (mtDNA) loci and one nuclear DNA (nDNA) locus; (ii) identifying negligible genetic distance estimates between parasitoid fig wasp taxa associated with multiple *Ficus* species; (iii) reconstructing ancestral character state transitions in *Ficus* subsection and syconium diameter affiliation by parasitoid lineages; and (iv) estimating parasitoid divergence times to contextualize the timing of trait associations.

2. Materials and methods

2.1. Taxon sampling

Sampling took place in 2005–2008 and fig wasp collections from 29 *Ficus* species or morpho-species of a potential 107 species are represented (Table 1). There are at least 113 Afro-tropically distributed *Ficus* species that include 78 species recognized in section *Galoglychia* (Berg and Wiebes, 1992; Burrows and Burrows, 2003; van Noort and Rasplus, 2004–2009). Sections *Sycomorus*, *Urostigma*, *Sycidium*, *Oreosycea*, and *Ficus* are most strongly represented in the Indo-Australasian region (Weiblen, 2000; Jousselin et al., 2003; Berg and Corner, 2005; Rønsted et al., 2008), but have elements in the Afro-tropical region. Sycoryctinae are associated with all these figs with the exception of the four species in section *Oreosycea* and two species in the subsection *Conosycea* of section *Urostigma*. Undescribed African Sycoryctinae parasitoid fig wasps belonging to the *Sycoryctes* Mayr (1885) and *Arachonia* Joseph (1957) genera were collected from sites in Southern, Eastern, Central, and Western Africa (Table 1).

Obtaining fresh fig wasp samples from all the Afro-tropical species is logistically extremely difficult, with many fig species being rare or restricted to inaccessible areas. As such, definitively reconciling sampling effort across time and space with the results is equivocal. The difficulty in determining species number estimates and in obtaining DNA samples for this taxon in its entirety preclude realistic statements regarding sample effort quantification. Sampling effort has been concentrated on the more common *Ficus* species sampled across the said region, which excludes the Congo Basin and much of West Africa. Therefore, we assume the sample reflects species richness differences between Sycoryctinae genera. Voucher specimens for each taxon used for sequencing have been deposited in the Iziko South African Museum collection (Cape Town). Taxa were identified to genus and delimited by both morpho-type and *Ficus* species affiliation.

Substantial morphological similarity between species within a genus is extremely common within the Sycoryctinae. Therefore, all taxa were included in the analyses to explore pair-wise relationships (collected from the same or different *Ficus* species) pertinent to interpreting *Ficus* specificity patterns especially among very closely related parasitoid taxa sampled from different *Ficus* species. Furthermore, the approach used to reconstruct ancestral character states (see below) are robust to phylogenetic uncertainty. Both morpho-type and *Ficus* affiliations were used as fig wasp taxon discriminators for the phylogenetic reconstructions, allowing for tests of degree of specificity. Pilot reconstructions included potentially duplicated exemplar samples collected from the same *Ficus* species comprising *F. natalensis graniticola*, *F. sansibarica sansibarica*, *F. burkei*, *F. natalensis natalensis*, *F. petersii*, *F. natalensis/burkei*, *F. sycomorus gnapholocarpa*, *F. modesta*, *F. stuhlmannii*, *F. glumosa*, *F. bizanae*, *F. cordata*, and *F. bubu*. Inferred duplicate exemplars from the same *Ficus* species were removed for subsequent phylogenetic inference.

2.2. DNA sequencing

Fragments of mtDNA *cytochrome oxidase I* (*COI*) and *cytochrome b* (*Cytb*), and nDNA *elongation factor-one alpha* F2 copy (*EF-1α*) gene regions were sequenced. The DNA extractions used for the sequencing data were from tissue preserved in >96% ethanol. Single whole fig wasps were used for extractions, but where increased DNA yield was required for troublesome samples, up to three individuals from the same collection (same tree) were used per extraction. A QIAGEN® QIAamp DNA Micro Kit was used for all DNA extractions. The PCR reactions included GoTaq® Flexi DNA Polymerase (100 U @ Enzyme Concentration: 5 U/ml) and 5× Colorless GoTaq® Flexi Buffer (1 ml @ pH 8.5). Amplifications of mitochondrial DNA were undertaken using the following protocol: 94 °C, 30 s denaturation; 48 °C, 1.5 min annealing; 72 °C, 2.5 min extension for 30 cycles; with a final cycle of 72 °C, 10 min extension. The polymerase enzyme required a 94 °C, 3 min incubation period for the first cycle only. The PCR mixture was a 25 μl reaction including: 2.5 μl 5× buffer, 4 μl of 5 U/ml of polymerase, 0.125 μl of $MgCl_2$ (25 mM), 0.5 μl (10 mg/ml) of dNTPs, 0.1 μl (0.2 pmol/μl) of each primer, and unknown concentrations of template DNA. Amplifications of nuclear DNA were undertaken using the following protocol: 92 °C, 1 min denaturation; 54 °C, 1 min annealing; 72 °C, 1 min extension for 45 cycles; with a final cycle of 72 °C, 4 min extension. The polymerase enzyme required a 94 °C, 3 min incubation period for the first cycle only. The PCR mixture was a 25 μl reaction including: 2.5 μl 5× buffer, 0.125 μl of 5 U/ml of