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SPECIATION IN THE FIG - FIG WASP MUTUALISM

Abstract: Fig plants (*Ficus)* are pollinated by an obligate mutualistic symbiont, the fig wasp (*Agaonidae*). What is the history of speciation and evolution in this mutualism, and what adaptive pressures affect divergence? In this review, I discuss research on speciation and divergence in the fig-fig wasp mutualism, finding that the mutualism has a complex evolutionary history not well described by strict-sense coevolution. In addition to discussing research results, I discuss the importance of genetic methodology when investigating coevolution. I also propose future areas of research exploring the role of other symbionts in the evolution and speciation of the fig-fig wasp mutualism.

How are symbiosis and speciation associated? This intriguing question is being examined from a variety of viewpoints in a variety of systems of parasitic, commensal, and mutualistic symbioses. The pollinator-plant symbiosis is a particularly well-studied species interaction. In this paper, I review past and current research on the history of speciation in figs (genus *Ficus)* and their mutualistic symbiotic pollinators, the fig wasps (family *Agaonidae*).

The fig – fig wasp mutualism is obligate, in that each organism cannot survive and reproduce without the other. Fig wasps pollinate the flowers within a fig (which is an enclosed inflorescence of monoecious flowers), and then grow and reproduce within the fig. Both genera are highly diverse, with approximately 750 extant species of *Ficus* and a similar number of fig wasps (Rønsted et al. 2008).

Female fig wasps are attracted by host-specific scents to figs that are ready for pollination (Hossaert-McKey, Gibernau, & Frey 1994; Grison-Pigé, Bessière, & Hossaert-McKey 2002). These female wasps, which are carrying pollen, enter the fig through a special opening in the top. The wasps pollinate flowers within the fig, and oviposit in some of the pollinated flowers. The eggs develop and hatch, and larvae feed on endosperm produced by the fruits in which they were laid. Male fig wasps spend their entire life within a single fig; once mature, they mate with females in the same fig, who then leave (carrying pollen and fertilized eggs) to oviposit in a new fig inflorescence. This process happens on the order of a few weeks (Kjellberg, & Maurice 1989). Female fig wasps can survive outside of figs only for a few days (Weiblen 2002); therefore, in a given area there must be a constant, year-round supply of receptive figs for the wasp (and therefore fig) population to survive (Bronstein et al. 1990; Kjellberg, & Maurice 1989).

How did this system evolve? What story can we tell about the speciation events in the history of this mutualism? What role does genetic and genomic evidence have in the investigation? Researchers have been especially interested in the question of whether figs and their pollinators co-evolved. Phylogenetic analyses indicate that fig wasp pollination evolved once, between 60-100 million years ago (Herre et al. 1996; Rønsted et al. 2005), and that since then each lineage has diversified and speciated to reach the richness we see today in figs and their pollinating wasps.

The traditional story of this mutualism is one of coevolution, in which a single wasp species associated with a single fig species together adapted and diverged. Several studies support this view, at least to an extent, based largely on the intense level of fine-tuning of traits necessary for the mutualism. For example, a review by Anstett, Hossaert-McKey, and Kjellberg (1997) found that the mutualism has diversified and been maintained across lineages through coevolution; however, they also assert that some traits that are now highly associated with the mutualism were present before the symbiosis was established. Cook and Rasplus (2003) also describe a coevolutionary history; they point out, though, that the coevolution was not absolute at all times, due to the finding that the fig wasp-fig association is not one-to-one: some fig species are pollinated by multiple wasps, and some wasps pollinate multiple fig species. Machado et al. (2005) points out that many of the early studies that assert coevolution (such as Ramirez 1974; Wiebes 1981) use morphological characters across disparate fig and wasp lineages and find that groups of closely related wasp species pollinate groups of closely related fig species. This is a very different effect than one in which a single wasp species pollinates a single fig species, even when looking within closely related species groups.

Jackson et al. (2008), as well as Machado et al. (2005), point out some of the factors that complicate our understanding of the fig-wasp mutualism as strictly coevolutionary. The primary factor is that the relationship between figs and fig wasps is not one-to-one. Rather, there is evidence for host switching by pollinators, and morphologically and genetically similar wasps pollinating multiple fig species (and vice versa). Furthermore, species in the genus *Ficus* hybridize easily, and therefore there is messiness around the species designations of figs, which corresponds to messiness with those figs' associations with wasps. *Ficus* is highly diverse and plastic, both globally and locally (Machado et al. 2005).

The problem, according to some researchers (such as Jackson et al. 2008; Machado et al. 2005) is that most genomic and phylogenetic studies have investigated aspects of the mutualism that cannot adequately distinguish between strict-sense coevolution and more generalized codivergence. Rather than look at differences between groups of fig species and groups of wasp species, in order to confidently claim cospeciation, we would need to find evidence for coevolution between individual pairs of wasp and fig species (Jackson et al. 2008). Such investigation could show us whether coadaptation is occurring independently across isolated lineages, or whether broader evolutionary forces are acting on multiple lineages of wasps and figs resulting in broader codivergence. Machado and colleagues (2005) argue that in order to study this, researchers must look at closely related species pairs of wasps and figs; at multiple individuals in each species; and at multiple genetic loci in each individual. There has not been any research that has used such methodology and concluded that figs and fig wasps have cospeciated in a strict sense.

There is ample genetic evidence for more generalized co-adaptation. Rønsted et al. 2005 looked at divergence in figs at a broad scale, across the entire genus of *Ficus*. Using pre-existing molecular phylogenies combined with fossil data, they found approximately ten interacting monophyletic fig lineages exhibiting clear codivergence (defined as parallel diversification of fig and pollinator lineages) with fig wasps. Their results supported the hypothesis that specialization among pollinators led to codivergence in the mutualism.

Jackson et al. (2008) and Machado et al. (2005) provide analyses of the degree of correspondence between closely related fig and wasp lineages. Both examine genetic data from multiple loci across multiple individuals of groups of sympatric neotropical fig and wasp species. According to their analyses, the two species' phylogenies do not correspond; rather, there is low divergence, and incongruence between gene and species trees, consistent with what would result from hybridization and introgression (as in a more traditional speciation model; Jackson et al. 2008). Machado and colleagues present a model for the diversification of the fig-wasp mutualism in which genetically well-defined groups of wasp species coevolved with frequently-hybridizing, genetically messier groups of fig species. They write with precision:

> *"Here, we propose that hybridization and introgression due to pollinator host switches and pollinator host sharing may be a major factor underlying much of the tremendous diversity of fig species. Hybridization can lead to generation of new genotypic combinations that may then diversify and lead to the evolution of additional specialized pollinators. The process of divergence in the pollinators can be reinforced by their inbred population structure, and fine-tuning of the host recognition mechanisms would promote pollinator divergence and speciation.* ***By this view, the coevolutionary history of the mutualism is that of semispecific wasps (that are good biological species) moving back and forth between figs that may not be good biological species****"* (Machado et al. 2005, pg 6564, emphasis added)

From these studies, we can see that the speciation story in figs and their pollinators is a complex one, with components of coevolution, codivergence, hybridization and introgression. In order to study this, it is necessary to do genetic and genomic research on closely related species pairs with large sample sizes (of both individuals of each species, and number of genes in each individual). More of such research is necessary in order to better understand speciation in this system.

An intriguing potential caveat in this story is: what is the role of parasites in the mutualism's evolution? The fig-fig wasp symbiosis does not exist in isolation—rather, both figs and their pollinators have other symbioses, largely parasitic, that might impact the evolution of the fig-fig wasp mutualism.

To complicate matters, one of the primary parasites in this system is a different type of wasp; but one that does not provide a service to the host plant. These parasitic wasps oviposit inside flowers, but reach them by piecing the outside of the receptacle of the enclosed inflorescence, rather than entering inside as the pollinating wasps do. The parasitic wasps do not pollinate; they instead harm the fig plant because the eggs and larvae develop within the developing fruit and destroy those flowers (some parasites, such as wasps of the genus *Apocryptophagus*, cause galls on the inside of the inflorescence; Weiblen & Bush 2002). Many researchers have investigated various aspects of these parasites' biology, and their association with figs; such as their reproductive timing and ecology (Greeff & Ferguson 1999; West & Herre 1994). Kerdelhué and Rasplus (1996) found that parasitic fig wasps harm the fig-pollinator mutualism by negatively affecting the pollinator population dynamics, thereby harming the pollen-producing (male) function of the fig plant. By negatively affecting the reproductive success of both figs and their pollinators, it seems highly likely that parasitic fig wasps have an impact on the evolution and speciation of the fig-pollinator mutualism (West & Herre 1994). Weiblen & Bush (2002) found that speciation has occurred in the parasites due to niche differentiation in timing of ovipositing: parasites that oviposit later in the fig's development have longer ovipositors than those that oviposit earlier in the maturation of the fig. Given the importance of timing of fig maturation on the fig-pollinator mutualism (Bronstein et al. 1990), I wonder: could this niche differentiation in parasites affect the speciation of the pollinators and the figs? This question is one that deserves further thought and research.

The pollinating wasps also have parasites of their own, some of which have been investigated (such as nematodes; Herre 1993), although not nearly as much research has been done in this area as on fig parasites. The same question applies to these parasites: how might they affect the evolution and speciation of figs and their pollinators? A particularly good candidate for a parasite that could have such an affect is *Wolbachia*. This vertically transmitted bacterial genus has been found to induce speciation in other systems, such as in *Nasonia* (a parasitoid wasp genus; Bordenstein, O'Hara, & Werren 2001). These studies have found that *Wolbachia* infections can induce partial reproductive isolation through hybrid inviability, thereby likely creating speciation opportunities. Xiao and colleagues (2012) investigated the role of *Wolbachia* in fig wasp populations. They found that compared to uninfected groups, infected wasp populations had much lower mitochondrial DNA diversity, suggesting a partial selective sweep of mitochondria in the fig wasp through hybrid introgression from a sister species after the introduction of *Wolbachia*. Findings such as these are fascinating, as they suggest ways that symbionts can affect the speciation and evolution of organisms. It would be intriguing and valuable to investigate the role *Wolbachia* has on the fig-pollinator mutualism: by affecting the mating structure of wasps, could *Wolbachia* affect the speciation of fig species? Further research is needed in order to answer questions such as these.

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