Brood pollination mutualisms—interactions in which specialized insects are both the pollinators (as adults) and seed predators (as larvae) of their host plants—have been influential study systems for coevolutionary biology. These mutualisms include those between figs and fig wasps, yuccas and yucca moths, leafflowers and leafflower moths, globleflowers and globleflower flies, Silene plants and Hadena and Perizoma moths, saxifrages and Greya moths, and senita cacti and senita moths. The high reciprocal diversity and species-specificity of some of these mutualisms have been cited as evidence that co-evolution between plants and pollinators drives their mutual diversification. However, the mechanisms by which these mutualisms diversify have received less attention. In this review, we review key hypotheses about how these mutualisms diversify and what role co-evolution between plants and pollinators may play in this process. We find that most species-rich brood pollination mutualisms show significant phylogenetic congruence at high taxonomic scales, but there is limited evidence for the processes of both cospeciation and duplication, and there are no unambiguous examples known of strict-sense contemporaneous cospeciation. Allopatric speciation appears important across multiple systems, particularly in the insects. Host-shifts appear to be common, and widespread host-shifts by pollinators may displace other pollinator lineages. There is relatively little evidence for a “coevolution through cospeciation” model or that coevolution promotes speciation in these systems. Although we have made great progress in understanding the mechanisms by which brood pollination mutualisms diversify, many opportunities remain to use these intriguing symbioses to understand the role of biotic interactions in generating biological diversity.

**KEY WORDS** brood pollination; coevolutionary diversification; Ficus; obligate pollination mutualism; Phyllanthus; pollinating seed parasite; seed predator; speciation; Yucca
brood pollination over other, widely used synonyms in this synthesis because of its brevity (relative to pollinating seed-predation mutualism), because fig wasp larvae technically are inflorescence gallers rather than seed predators, and also because we wish to compare processes in otherwise analogous interactions that differ in whether they are strictly obligate or include copollinators (below).

Here we define coevolutionary diversification broadly as diversification (among populations, species, or clades) in which coevolution (reciprocal natural selection sensu Janzen, 1980; Thompson, 2005) drives the diversification (sensu Hembry et al., 2014; note that this is a broader definition than that of Althoff et al., 2014). Importantly, coevolutionary diversification may not necessarily create phylogenetic congruence between coevolving clades (Thompson, 2005; Poisot, 2015). This emphasis on brood pollination mutualisms is due to four characteristics of many of these systems share: (1) the highly intertwined life cycles of both plants and pollinators, (2) the fact that the pollinators control most (if not all) pollen flow in their plant hosts (Thompson, 1994), (3) the degree of species-level specialization seen between plants and pollinators in nature (Wiebes, 1979; Weiblen, 2002; Kawakita and Kato, 2006; Althoff et al., 2012), and (4) the high species richness in some plant and pollinator clades engaged in these interactions (Janzen, 1979; Kawakita, 2010). Although the hypothesis that coevolution promotes diversification has been invoked for a wide number of organisms and types of interactions (Ehrlich and Raven, 1964; Dietl and Kelley, 2002; Thompson, 2005, 2009; Hembry et al., 2014), the above attributes of brood pollination mutualisms make them ideal test cases for this hypothesis.

Despite the fact that they have been major and influential study systems for understanding the role of coevolution (or biotic interactions more generally) in diversification, we know very little about the mechanisms by which the plants and insects engaged in brood pollination mutualisms diversify. This deficiency is largely due to the patterns of diversity across the different brood pollination mutualisms. Some of these interactions (e.g., figs, leafflowers) have hundreds of species, primarily in the tropics (Berg, 1989; Kawakita, 2010), so that taxonomic sampling and indeed, taxonomic deficits in the pollinator insects, become major impediments. Research on these systems has primarily focused on cophylogenetic patterns at macroevolutionary scales and examining patterns of species-specificity in ecological time (Weiblen and Bush, 2002; Molbo et al., 2003; Machado et al., 2005; Kawakita and Kato, 2006, 2009; Cruaud et al., 2012c; Hembry et al., 2013). Other interactions (e.g., senita, globeflowers, and saxifrages) are very species-poor, and are perhaps thus not particularly suited to studying diversification; however, these systems have served as important models in evolutionary ecology. Some interactions appear to be obligate (Cook and Rasplus, 2003; Pellmyr, 2003), whereas others vary among species and populations along the continuum between mutualism and antagonism (Thompson and Cunningham, 2002; Kephart et al., 2006). Finally, many of these interactions have only been discovered in the past several decades (Thompson and Pellmyr, 1992; Fleming and Holland, 1998; Kato et al., 2003). As a result, despite their conceptual influence on the field of coevolution, it has been difficult to use these systems to test the hypothesis that coevolution (or biotic interactions) promotes diversification. Even so, recent work in many systems has begun to reveal common patterns and reinforces the potential for brood pollination mutualisms to serve as important models for the study of biotic interactions and coevolution in diversification.

In this review, we ask how brood pollination interactions diversify, and then use this evidence to ask what the role of biotic interactions between plants and pollinators (including coevolution) is in this diversification process. Throughout, we will suggest key hypotheses that need to be tested to strengthen the link between coevolution and diversification. Approaches that focus on diversification per se have been criticized both for their unworkability (coevolution is difficult to demonstrate) and on empirical and theoretical grounds (there is little, or mixed, evidence for coevolution promoting diversification, particularly in mutualisms) (Yoder and Nuismer, 2010; Hembry et al., 2014). We note therefore that our approach offers a more nuanced perspective that is informative whether the goal is to emphasize the role of biotic interactions more generally or coevolution specifically in diversification. Specifically, we ask the following questions: how specialized (at the species level) are plants and brood pollinators to each other, do they diversify through coadaptation or some other process that generates phylogenetic congruence, do they specify in allopatry, do pollinator clades displace each other competitively, and is coevolution implicated in either plant or insect speciation?

WHAT ARE THE BROOD POLLINATION MUTUALISMS?

We define brood pollination mutualisms as mutualisms in which larvae of pollinators consume seeds or other female inflorescence tissue. Brood pollination mutualisms involve diverse taxa from many different geographic areas (Table 1). These interactions are usually obligate, but in some systems they can be more facultative at least for the plants. For instance, figs, yuccas, and leafflowers are generally described in the literature as obligately pollinated by their respective brood pollinators (obligate pollination mutualisms; e.g., Pellmyr, 2003; Kawakita, 2010). In contrast, it is well established that the senita cactus is not exclusively pollinated by senita moths (Holland and Fleming, 1999) and Silene–Hadena, Silene–Perizoma, and Lithophragma–Greya interactions both exist with copollinators and can span the continuum from mutualism to antagonism, even among populations within a pair of interacting species (Thompson and Pellmyr, 1992; Thompson and Cunningham, 2002; Kephart et al., 2006). Copollinators also exist in some globeflower populations (Suchan et al., 2015). Whether strictly obligate or more facultative, we will consider all types of brood pollination for two reasons. First, pollination interactions do not need to be exclusively reciprocally obligate for pollinators and plants to exert significant selection on each other that may fuel speciation (Thompson and Cunningham, 2002; Thompson et al., 2013b). Second, brood pollination interactions that are not strictly obligate can reveal important insights about how diversification occurs in such mutualisms (e.g., Thompson and Rich, 2011).

DIVERSIFICATION IN BROOD POLLINATION MUTUALISMS

Although the patterns of diversification for brood pollination mutualisms have been elucidated, there has been relatively little research on the mechanistic basis of diversification. As highlighted already, part of this lack is due to difficulties in manipulating the systems and part is due to the relatively recent emergence of some systems. Perhaps the best-studied system with regards to patterns of diversification is the yuccas and yucca moths, that have received
TABLE 1. Taxonomy, species diversity, and geographic distributions of brood pollination mutualisms.

<table>
<thead>
<tr>
<th>Plant taxon</th>
<th>Family</th>
<th>Common name</th>
<th>Insect taxon</th>
<th>Order, family</th>
<th>Common name</th>
<th>Number of plant species implicated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ficus</td>
<td>Moraceae</td>
<td>Figs</td>
<td>Agaonidae</td>
<td>Hymenoptera</td>
<td>Fig wasps</td>
<td>Pantropical, subtropics ~750</td>
</tr>
<tr>
<td>Phyllanthus</td>
<td>(includes Phyllanthaceae)</td>
<td>Leafflowers</td>
<td>Lepidoptera: Gracillariidae</td>
<td>Old World tropics, warm temperate zones</td>
<td>Leafflower moths</td>
<td>~500</td>
</tr>
<tr>
<td>Breynia</td>
<td>Glochidion, warm temperate zones</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yucca</td>
<td>Agavaceae</td>
<td>Yuccas</td>
<td>Hesperoyucca, Parategeticula</td>
<td>Lepidoptera: Prodoxidae</td>
<td>Yucca moths</td>
<td>Nearctic, tropical Mexico 35–40</td>
</tr>
<tr>
<td>Silene</td>
<td>Caryophyllaceae</td>
<td>Perizoma</td>
<td>Lepidoptera: Noctuidae, Geometridae</td>
<td>Holarctic,</td>
<td>Greya moths and subgenera or sections of Greya, Holarctic ≥30</td>
<td></td>
</tr>
<tr>
<td>Ranunculaceae</td>
<td>Trollius</td>
<td>Globeflowers</td>
<td>Anthomyiidae</td>
<td>Palearctic</td>
<td>Globeflower flies</td>
<td>~18</td>
</tr>
<tr>
<td>Lithophragma, Heuchera</td>
<td>Saxifragaceae</td>
<td>Woodland stars; saxifrages</td>
<td>Lepidoptera: Prodoxidae</td>
<td>Greya moths and subgenera or sections of Greya, Holarctic</td>
<td>Greya moths</td>
<td>≥6</td>
</tr>
<tr>
<td>Cactaceae</td>
<td>Senita cactus</td>
<td>Pachycereus schottii</td>
<td>Lepidoptera: Pyralidae</td>
<td>Nearctic</td>
<td>Senita moth</td>
<td>1</td>
</tr>
</tbody>
</table>

For the less species-rich brood pollination mutualisms, patterns of specialization are more varied. Within co-occurring assemblages, particularly in California, multiple species each of Greya moths and their saxifrage hosts do not appear to have restricted host breadth particularly detailed attention at multiple phylogenetic scales. Other groups that have received some research attention include globeflowers and globeflower flies in the Palearctic (Desprès et al., 2002a, b, 2003; Espíndola et al., 2012a, b, 2014; Suchan et al., 2015), figs and fig wasps in some parts of their range (particularly islands and the edges of the tropics: Molbo et al., 2003; Jackson et al., 2008; Renoult et al., 2009; Cornille et al., 2011; Chen et al., 2012; Cruaud et al., 2012b; Kusumi et al., 2012; Moe and Weiblen, 2010, 2012; Darwell et al., 2014; Tian et al., 2015), *Lithophragma*–*Greya* interactions in western North America (Brown et al., 1994; Rich et al., 2008; Thompson and Rich, 2011; Thompson et al., 2013b), and leafflowers in the clade *Glochidion* in southeastern Polynesia (hereafter, Polynesian leafflowers; Hembry et al., 2013, unpublished manuscript). Taken together, we see a number of common factors that may contribute to diversification in these systems. Below, we propose and provide answers to what we think are the key questions in understanding the role of biotic interactions and coevolution in generating diversification in brood pollination mutualisms. Bear in mind that not all brood pollination mutualisms will diversify exactly the same way, but we suggest that there are several key factors that may vary in importance depending on the system. A glossary of key terms is provided in Table 2.

(1) How specialized are the plants and pollinators to each other?—Knowing how specialized brood pollinated plants and their pollinators are to each other is essential to understanding how they diversify. Accordingly, specialization in brood pollination systems has been a contentious topic in the literature. Early research on fig wasps assumed that each species of fig has (at least locally) a unique species of pollinating fig wasp (e.g., Janzen, 1979; Ramirez, 1970); this paradigm was later challenged following the advent of molecular phylogenetic methods in delimiting fig wasp taxa (e.g., Molbo et al., 2003; Machado et al., 2005; Cornille et al., 2011). Recent work examining fig wasp diversity has demonstrated that genera of fig wasps and subgenera or sections of *Ficus* are often specialized to each other (e.g., Jackson et al., 2008; Cruaud et al., 2012c; but see Renoult et al., 2009). Within these reciprocally associated subclades, however, it is not unusual for a single *Ficus* species to be pollinated by multiple wasp species either sympatrically or allopatrically, although there are still plenty of examples of locally one-to-one interactions (see reviews by Herre et al., 2008; Cook and Segar, 2010; Yang et al., 2015). There are also some good examples of a single fig wasp species visiting multiple *Ficus* host species sympatrically (e.g., pollinator sharing; Cornille et al., 2011; Wang et al., 2016).

*Yucca* has been extensively sampled in the United States, and this work has revealed that over much of their range, a single species of *Tegeticula* is monophagous (although there are a few exceptions; Pellmyr, 2003; Althoff et al., 2012). Patterns of specialization in leafflowers and leafflower moths are less clear and may vary among major clades of *Phyllanthus* s.l. and *Epicephala*. There are examples of locally monophagous leafflower moths (Kawakita and Kato, 2006), and leafflowers, which locally only have unique pollinators (e.g., Kawakita and Kato, 2006; Kawakita et al., 2015; Li and Yang, 2015). There are, however, also more complex patterns of sympatric pollinator and host sharing (Zhang et al., 2012; Li et al., 2015; Hembry et al., unpublished manuscript).

For the less species-rich brood pollination mutualisms, patterns of specialization are more varied. Within co-occurring assemblages, particularly in California, multiple species each of *Greya* moths and their saxifrage hosts do not appear to have restricted host breadth...
TABLE 2. Glossary of terms used.

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
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<tbody>
<tr>
<td>Coevolution</td>
<td>Reciprocal natural selection between two or more taxa sensu Janzen (1980), Thompson (1994, 2005); does not require or imply cospeciation or phylogenetic congruence</td>
</tr>
<tr>
<td>Cospeciation</td>
<td>Process by which host and pollinator speciate contemporaneously. Differs from cases in which host and pollinator speciation are decoupled, or phylogenetic tracking</td>
</tr>
<tr>
<td>Phylogenetic tracking</td>
<td>Process by which pollinator (or host) diversifies onto host clade in a manner which matches the phylogenetic relationships of the host taxa, without contemporaneous cospeciation occurring</td>
</tr>
<tr>
<td>Phylogenetic congruence</td>
<td>Pattern in which host and pollinator phylogenies are topologically more similar than would be expected by chance. This congruence may be observed between species, subclades, or other operational taxonomic units and may be generated by cospeciation, phylogenetic tracking, or some other process.</td>
</tr>
<tr>
<td>Host-shift</td>
<td>Process in which a pollinator lineage colonizes a new host lineage.</td>
</tr>
<tr>
<td>Duplication</td>
<td>Process in which a pollinator (or host) speciates without the other associate speciating. This term is from the cophylogenetics literature, but may also describe cases in which host and pollinator speciation are decoupled.</td>
</tr>
<tr>
<td>“One-to-one” paradigm</td>
<td>Traditional and highly influential view of brood pollination interactions in which each host species and each pollinator species is (locally or globally) associated with a single unique partner species. Articulated clearly in early fig literature such as Ramírez (1970) and Janzen (1979).</td>
</tr>
</tbody>
</table>

(Thompson et al., 2013b; Thompson, 2013). The single species of European globeflower (Trollius europaeus) is pollinated by multiple species of monophagous globeflower flies in Europe, whereas Asian globeflower flies use multiple species of globeflowers (Després et al., 2002b; Espíndola et al., 2012a). Many Silene have only one known pollinating Hadena, and many reported Hadena are only known for 1–2 Silene species; however, generalists using up to nine partner species are known in both genera (Kephart et al., 2006). Despite limited evidence for a traditional “one-to-one” paradigm, these recent studies have shown that the most species-rich brood pollination mutualisms are still highly specialized at the species level, and this specialization is important if we want to ask what role plant–pollinator interactions play in their mutual diversification. In addition, a common theme seems to be that the insect pollinators are usually the more specialized of the interacting partners.

Why are these interactions so specialized? All else being equal, intimate mutualisms, such as these, should form ecological networks which are highly reciprocally specialized and modular in structure (Guimarães et al., 2007; Thompson et al., 2013a). All of these interactions also evolved from parasitic ones and might retain a narrow host breadth from their parasitic ancestors (Thompson, 1994). Kawakita et al. (2010) proposed that leaflower moths have a more restricted host breadth than nonpollinating herbivorous relatives because some aspect of mutualism may promote greater host-specificity. Whereas this result is intriguing, the findings, based on Glochidion-associated Epicephala in Japan, appear to differ from those subsequently reported in China (Li et al., 2015) and French Polynesia (Hembry et al., 2013, unpublished manuscript). Nevertheless, this interesting hypothesis should be more widely tested in this and other brood pollination mutualisms. Proximately, both pre- and postoviposition barriers may mediate this specificity. There is evidence not only that host-recognition cues such as floral odor may mediate specificity in leafflowers (Okamoto et al., 2007, 2013), but also that larvae may suffer reduced survivorship on the “wrong” hosts in yuccas and figs (Smith et al., 2009; Moe et al., 2011; Cornille et al., 2011; see Althoff, 2016, in this issue) suggesting that the antagonistic seed-eating part of the interaction may in part be responsible for this specificity. In the case of figs, host specialization may be proximately due to the ability to successfully initiate galls on fig flowers (Ghana et al., 2015). More research is needed to ascertain the relative roles of the mutualistic and antagonistic sides of the overall interaction in determining the high levels of specialization seen in brood pollination mutualisms.

(2) Do brood pollination mutualisms show evidence for phylogenetic congruence and for cospeciation?—Cophylogenetic analyses have been a major area of focus for the study of the evolution of brood pollination mutualisms, in part because cophylogenetic patterns can (in principle) be detected with partial extant taxon sampling. At global scales, the most diverse brood pollination mutualisms, figs (Ficus) and fig wasps (Agaonidae) (Cruaud et al., 2012c), Yucca and Tegeticula (Althoff et al., 2012), and the leafflower clade Glochidion and its reciprocally associated clade of Epicephala (Hembry et al., 2013) show evidence for significant cophylogenetic structure. For figs and fig wasps, dioecious figs show greater evidence for phylogenetic congruence globally with their fig wasps than do monoecious figs; in particular, copollinators are much more likely to be sister species on dioecious than monoecious figs (Yang et al., 2015). For leafflowers and leafflower moths in general, the pattern is less clear. Epicephala-pollinated leafflowers (Glochidion, Brevnia, and Phyllanthus) are globally a polyphyletic assemblage that does not show any phylogenetic congruence with Epicephala, which is monophyletic (Kawakita and Kato, 2009). Within the clade Glochidium (a subgenus of Phyllanthus) in New Caledonia, however, there appear to be two subclades, each of which is reciprocally associated with its own clade of Epicephala (interestingly, these Glochidium-associated Epicephala do not form a monophyletic group; Kawakita and Kato, 2009). No studies have tested whether there is significant cophylogenetic structure between any of the other Epicephala-pollinated subclades of Phyllanthus s.l. (Brevnia, Phyllanthus subgenus Glochidium, Madagascar Phyllanthus, and the P. reticulatus complex) and their Epicephala. None of the above insect–plant systems show exact phylogenetic congruence, and at smaller, within-region scales, plants and their pollinators can actually show no significant phylogenetic congruence (such as Polynesian leafflowers and their Epicephala [Hembry et al., 2013], or within a co-occurring assemblage of section Americana figs and their Pegoscapus wasps [Jackson et al., 2008]).

At even smaller phylogenetic scales such as between pairs of interacting sister-species, there are relatively few examples of phylogenetic congruence. The best-studied example is that between Joshua trees (Yucca brevifolia and Y. jaegeriana) and the sister-species pollinators Tegeticula synthetica and T. antithetica in the Mojave Desert of the United States. In this case, a cophylogenetic approach suggests cospeciation, but divergence in Joshua tree and its yucca moths may not have been contemporaneous (Smith et al., 2008a). Furthermore, the two races of Joshua tree are not
completely reproductively isolated, which suggests the pollinators are not completely host-specific (Yoder et al., 2013).

Below the species level, there is an extensive and growing literature on phylogeography and population genetics in brood pollination mutualisms. Many of these studies have examined one taxonomic group with molecular data and the other group using existing taxonomy (but see Smith et al., 2011; Espíndola et al., 2014). For instance, widely distributed fig species can show substantial spatial turnover and/or phylogenetic differentiation in their pollinating fig wasps (Kobmoo et al., 2010; Moe and Weiblen, 2010; Chen et al., 2012, Darwell et al., 2014; Tian et al., 2015), but in some cases distances of >1000 km are required for this turnover or differentiation to become apparent (Kobmoo et al., 2010; Tian et al., 2015). Trollius europaeus globeflowers and three globeflower fly species show phylogeographic structure across Europe, but among species the patterns are incongruent and idiosyncratic (Espíndola et al., 2014). One Polynesian Epicephala taxon (clade Z of Hembry et al., 2013) shows no phylogenetic differentiation across nine different (and in many cases, morphologically distinct) species of leafflower tree (Glochidion) in the Society Islands (D. Hembry et al., unpublished manuscript). Tegeticula yuccasella appears to be a generalist yucca moth species that pollinates at least seven different species of Yucca across its range in the central and eastern United States (Pellmyr, 1999). A similar pattern exists with the widely distributed Phyllanthus reticulatus and P. microcarpus complex in East Asia and their diverse fauna of Epicephala (Luo et al., 2011; Kawakita et al., 2015; Li and Yang, 2015).

In many of these cases, it may be that there is cryptic differentiation in the generalist species that is associated with the use of different partners; furthermore, phylogeographic patterns are not necessarily evidence for how speciation occurs. In one test of cryptic differentiation of a more generalist species, Leebens-Mack and Pellmyr (2004) showed only limited host-associated genetic structure in mtDNA cytochrome oxidase sequences from T. yuccasella across eastern North America. Such generalist species might represent relatively recent expansions of partner breadth; however, they may also indicate that use of different partner species across geographic space does not necessarily lead to genetic differentiation. If so, this suggests intriguingly—and in contrast to the traditional co-speciation paradigm—that speciation in plants and pollinators in brood pollination interactions may in many cases actually be decoupled. Some detailed studies of closely related fig species and their wasps at the regional scale are not incompatible with such a hypothesis (Jackson et al., 2008; Cornille et al., 2011), although to the best of our knowledge, broadly “generalist” (rather than oligophagous) fig wasps analogous to Tegeticula yuccasella have not been reported. Consequently, some cases of apparent cospeciation (phylogenetic congruence) may be the result of phylogenetic tracking of insects onto plants rather than true contemporaneous cospeciation (de Vienne et al., 2013). Alternately, phylogenetic congruence may be generated by strict-sense cospeciation, but perfect congruence is “erased” over time due to host shifts (e.g., Silvieus et al., 2008). Although generalist species are the exceptions in pollination brood mutualisms, they provide a way to test the role of the mutualism in leading to population differentiation and speciation. More tests of host-associated divergence among populations of generalist species are needed to catch “diversification in action” and complement the number of phylogenetic studies above the species level.

(3) Do plants and insects in brood pollination mutualisms speciate in allopatry?—Perhaps because of the enormous species richness of some of these systems, speciation has been examined in relatively few brood pollination mutualisms. However, those groups that have been examined closely show evidence for allopatric speciation in both plants and pollinators. Comparison of contemporary ranges of sister taxa in a phylogenetic context suggests that allopatric speciation has occurred in Yucca (Althoff et al., 2012), Tegeticula (Althoff et al., 2012), the older of two Polynesian Epicephala clades (Hembry et al., 2013), and two species of Greya in California (Rich et al., 2008; Thompson and Rich, 2011). The well-examined species pairs of Y. brevifolia—T. synthetica and Y. jaegeriana—T. antithetica (discussed in more detail later) are allopatrically distributed (except for one co-occurring population in Nevada, USA), and this allopatry is thought to be in part related to a Pliocene marine incursion in the Mojave Desert (Smith et al., 2008a). Cryptic allopatric lineages also have been demonstrated in Chinese Wiebsia fig wasps (Chen et al., 2012) and Southeast Asian and New Guinea Ceratosolen fig wasps (Moe and Weiblen, 2010). Dispersal between Europe and Asia, and concomitant host-shifts, are implicated in the diversification of globeflower flies, but there is also much within-European diversification despite only a single available globeflower host (Espíndola et al., 2012a). Finally, within-species phylogeographic structure based on geography has been demonstrated in Tegeticula (Sgreaves and Pellmyr, 2001), Trollius europaeus (Espíndola et al., 2012b), and three European globe-flower fly species (Espíndola et al., 2014). Most of these examples are from the insects; the hypothesis of allopatric speciation has not been tested for brood-pollinated plant clades other than Yucca. Van Welzen et al. (2015) suggested that dispersal out of mainland Southeast Asia and perhaps also edaphic adaptation to limestone may have promoted diversification both in Breynia and their non-Epicephala-pollinated sister clade.

The widespread evidence for allopatric speciation in insects engaged in brood pollination mutualisms is intriguing, especially given that if there is a system in which allopatry would not be necessary for speciation, it would probably be brood pollination mutualisms. Thompson (1994) suggested that systems in which partner species control the movement of each other’s gametes would be good candidates for coevolution driving speciation, and it is easy to imagine scenarios in which selection to prevent interspecific hybridization between plant species might result in restricted movement/mating of pollinators across host plant species. Even so, a number of studies have argued that hybridization between closely related sympatric figs mediated by shared pollinating fig wasps may be common (Jackson et al., 2008; Renoult et al., 2009; Cornille et al., 2011; Wang et al., 2016). The role of allopatry in speciation within pollination brood mutualisms is consistent with examples of potential coevolutionary speciation from other interactions (Parchman and Benkman, 2002; Hosoi et al., 2010; Smith and Benkman, 2007; Schluter, 2010), in which geographic isolation was always implicated in diversification (Hembry et al., 2014). Continued examination of the role of geography and biotic selection in brood pollination mutualisms is crucial for understanding the mechanisms of speciation.

(4) Is there evidence for displacement of one pollinator clade by another?—That host-shifts are common in brood pollination mutualisms seems unquestionable. Only host-shifts or duplication (speciation by pollinators on the same host without host speciation; e.g., Page, 2003) followed by differential extinction can explain the observed phylogenetic incongruence between plants and brood pollinators at fine scales (Page, 2003; Jackson et al., 2008; Cruaud et al.
speciation in brood pollination mutualisms has long attracted attention (e.g., Ramírez, 1970; Kister et al., 1984), but has been difficult to distinguish from the alternative hypotheses that diversification in pollinators simply responds to the diversification of their host plants (or vice versa). The role of coevolution has only been explicitly tested in one case, that of divergence in Joshua trees (Yucca brevifolia and Y. jaegeriana) and speciation in their pollinating yucca moths, Tegeticula synthetica and T. antithetica. These species are distributed in the Mojave Desert of the western United States. Yucca brevifolia is pollinated by T. synthetica in the western Mojave Desert, and Y. jaegeriana is pollinated by T. antithetica in the eastern Mojave Desert (Pellmyr and Segraves, 2003). These two interacting plant–pollinator pairs are allopatrically distributed, with the exception of one contact zone (Tikaboo Valley in Nevada) where all four species co-occur (Smith et al., 2009).

Extensive work on this interaction has shown a complex and interestingly nuanced view of the role and mechanisms that coevolution may play in speciation. More divergence is seen in floral traits associated with the interaction than in vegetative traits associated with the interaction (Godsoe et al., 2008), and trait matching (between flower stylar canal and moth ovipositor lengths) is seen at the species level within each species pair (Godsoe et al., 2010). These divergent phenotypes between Y. brevifolia varieties persist in the face of extensive contemporary gene flow (Starr et al., 2013; Yoder et al., 2013). Furthermore, species distribution modeling suggests that the abiotic requirements of Y. brevifolia and Y. jaegeriana do not differ (Godsoe et al., 2009). In the contact zone in Tikaboo Valley, where all four species co-occur, there are asymmetric differences in pollinator specificity, with T. antithetica visiting both Y. brevifolia and Y. jaegeriana trees but T. synthetica nearly exclusively visiting Y. brevifolia trees (Smith et al., 2009). This asymmetry in visitation may explain observed patterns of asymmetric introgression between the two Joshua tree varieties (Starr et al., 2013). However, survivorship of T. antithetica larvae is lower in Y. brevifolia fruits than in Y. jaegeriana fruits (Smith et al., 2009), this difference can be explained by trait matching between ovipositor and stylar canal length. Furthermore, in the contact zone in the Tikaboo Valley, plant–pollinator specificity is important in promoting or reinforcing this coevolution and reproductive isolation between the plants (Smith et al., 2009; Yoder et al., 2013; see Royer et al., 2016, in this issue). The divergence of T. synthetica and T. antithetica appears to predate that of Y. brevifolia and Y. jaegeriana, and the chloroplast phylogeography of Y. brevifolia and Y. jaegeriana are not congruent with phylogenetic relationships between their two pollinators (Smith et al., 2008a). These findings suggest that the initial divergence in this system may have been due to a vicariant event (such as the Miocene Bouse Embayment in today’s Colorado River Valley) that affected Tegeticula, and since this initial event, coevolution between plant and pollinators has contributed to the trait divergence and speciation that is observed today.

These findings underscore the difficulty of distinguishing diversification driven by coevolution from that in which either the pollinator is responding (through coevolution) to divergence in the plant, or the plants are diversifying in response to their pollinators (as is suggested for other pollination interactions; Hodges and Arnold, 1994; Ramírez et al., 2011). Indeed, the adoption of brood pollination did not increase diversification rates in Yucca relative to their sister clade (Smith et al., 2008b). However, they do suggest a number of interesting things about how coevolution may be implicated in speciation. First, these results suggest that coevolution acts
in concert with other factors such as geographic isolation (and is potentially in conflict with the hypothesis stated above, that speciation in plants and brood pollinators may be decoupled). This finding is consistent with those from coevolving interactions other than brood pollination mutualisms (Hembry et al., 2014). Second, they suggest that although much theoretical and empirical work has focused on whether and how mutualism might promote diversification, the antagonistic side of the overall interaction might be more important. The traits showing phenotype matching in this system (moth ovipositor length and plant ovary wall thickness) interact antagonistically, suggesting that to the extent that coevolution is implicated in speciation in brood pollination mutualisms, it may be through antagonistic coevolution as suggested by theoretical work (Yoder and Nuiser, 2010). Furthermore, Althoff (2014) suggested that, in *Tegeticula*, changes in ovipositor morphology led to correlated changes in male reproductive traits that could cause reproductive isolation among pollinator species. Thus, for yucca moths changes in antagonistic traits may have a direct link to speciation.

Finally, the Joshua tree–yucca moth example is a case of diversification through some process which produces phylogenetic congruence (although it is not necessarily contemporaneous cospeciation; Smith et al., 2008a). This finding is in contrast to the extensive evidence for host-shifts at various phylogenetic scales in brood pollination mutualisms (but see dioecious figs in Yang et al., 2015). As stated already, the consequences of host-shifts for diversification in brood pollination mutualisms are not clear; one hypothesis is that the presence of two distantly related (and therefore presumably reproductively isolated) pollinators, with different functional traits, on a single host plant might act on natural trait variation in the host and promote divergence (and speciation) in the host (Cook and Segar, 2010; Hembry et al., 2013). Such a process would be a different mechanism by which coevolution might be implicated in speciation in brood pollination interactions. Because we currently lack any analogous studies from any other pairs of jointly diverging plant and pollinator species in any brood pollination mutualisms, testing the findings from the above Joshua tree work with other species pairs in similar systems is a high priority.

**DISCUSSION AND CONCLUSIONS**

Although there is a great deal we do not know about how brood pollination mutualisms diversify, answers to the above questions demonstrate that common patterns are beginning to emerge across systems. The most species-rich systems—yuccas, figs, and leafowers—show significant phylogenetic congruence at large phylogenetic scales, but this phylogenetic congruence is far from exact. Host-shifts appear to be common in all of these systems, with the possible exception of dioecious figs. There is limited evidence for both cospeciation and for duplication, and there are no unambiguous examples known of strict-sense contemporaneous cospeciation. Much of the observed phylogenetic congruence may simply be due to conservatism in host associations, host tracking, and the isolation of different subclades in different biogeographic regions.

Geographic isolation (or more specifically, allopatry) appears to be important in driving speciation in several different brood pollination mutualisms, particularly in the insects. Some intriguing evidence suggests that widespread host-shifts by pollinator lineages may displace other pollinator lineages and that speciation in plants and insects may be decoupled; these are hypotheses that should be tested more broadly. There is relatively little evidence for a “coevolution through cospeciation” model and relatively limited evidence overall that coevolution promotes speciation in these systems. What little evidence there is suggests that antagonistic coevolution rather than mutualistic coevolution might be more important.

Coevolution may accompany or promote speciation as has been demonstrated in Joshua trees, and this hypothesis needs to be tested in many additional species pairs of plants and their pollinators for us to truly understand the role of coevolution in brood pollination mutualisms.

Although there is currently limited evidence of coevolutionary diversification, there can be no question that the persistence of new species and diversification of clades greatly depends on interactions between plants and pollinators. Speciation in brood pollinators and their host plants may not involve coevolution, but the interaction between plants and brood pollinators may play a role in their diversification at maccoevolutionary scales. Coevolution is not restricted to pairwise interactions, and it is likely that whole clades of plants and brood pollinators influence each other’s evolution in the same manner as has been argued for other mutualistic and antagonistic interactions (e.g., Ehrlich and Raven, 1964; Ricklefs, 2010).

An important consideration is what type of interaction may be structuring the diversification of brood pollinators and their host plants. Certainly, these relationships are mutualistic (except of course in cases of breakdown), but diversification could still be mediated by either antagonistic or competitive interactions (Hembry et al., 2014). In antagonistic cases, plants could be under selection to escape seed predation as much as they can, while pollinators could be under selection to obtain as much of their host’s seeds as possible (or, to switch onto new host species when available). This antagonism could also involve some form of coevolutionary alternation (sensu Thompson, 1994) in which pollinator larval feeding causes different selection on plant defenses that leads to short bouts of coevolution that prompts pollinators to shift to a less-defended plant species. Such a process could be particularly important in species-rich regional assemblages in which plants and pollinators could take advantage of new partners, and new trait combinations that permit this alternation could allow bursts of diversification in a manner similar to that which has been argued for interactions between plants and their herbivores (Ehrlich and Raven, 1964; Futuyma and Agrawal, 2009).

Alternately, competitive interactions may govern the diversification of plants and pollinators, in a manner similar to that which has been argued for other animal-pollinated plant radiations (Schluter, 2000; Armbruster and Muchhala, 2009). In such a scenario, plant species would be competing with each other for pollinators, and insect species would be competing with one another for host plants. Natural selection would thus promote character displacement in traits that mediate partner-specificity, and this character displacement would promote high specificity. Any new pollinator or plant species is therefore a resource for other species, and switches among partners would be analogous to the switches among pollination syndromes that are seen in plants such as *Aquilegia* (Hodges and Arnold, 1994).

In the past two decades, the discovery of several new brood pollination mutualisms, the molecular phylogenetics revolution, and increased interest in coevolution have led to these mutualisms being used for rigorous testing of long-standing ideas about the role of biotic interactions in generating biodiversity. Although we have made great progress, there is still much about the diversification of
plants and brood pollinators that we do not understand. Many opportunities exist to apply recently developed macroevolutionary, genomic, and network analytical methods to testing hypotheses about diversification in brood pollination mutualisms. These methods will need to be coupled with focused experimental tests on the role of mutualism and antagonism in generating trait divergence to provide a robust picture of coevolution and diversification. Brood pollination mutualisms have inspired and fascinated evolutionary biologists since the late 19th century and will continue to provide a unique window onto the origins of biological diversity.

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