

Chapter 6

Origin of Active Pollination and Mutualism

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Keywords *Breyenia* • *Epicephala* • *Flueggea* • Gracillariidae • *Glochidion* • Phyllanthaceae • *Phyllanthus* • Phylogeny

6.1 Evolution of Pollination by Seed Parasites

The mutualisms between plants and their seed-parasitic pollinators, such as the fig–fig wasp, yucca–yucca moth, and leafflower–leafflower moth mutualisms, provide textbook examples of specialized pollination systems (Weiblen 2002; Cook and Rasplus 2003; Pellmyr 2003; Kato et al. 2003). Remarkably, in all three systems, the pollinator insects actively collect and transport pollen between flowers in order to ensure food for their seed-feeding larvae. Reciprocal adaptation by plants to restrict floral access by other visitors resulted in extreme mutual dependence between plants and insects. Consequently, these mutualisms served as principal model systems for the studies of coevolution and mutualism.

Despite a wealth of documented examples of specialized pollination systems in angiosperms, however, pollination by obligate seed parasites is rare. This is because seed parasitism inflicts a heavy cost on plants, whereas abundant copollinators swamp the mutualistic effect of pollination by seed parasites (Thompson and Pellmyr 1992; Thompson and Cunningham 2002). In fact, exclusion of pollinators has not occurred in plants that were more recently found as being pollinated by seed parasites, including *Lithophragma* plants pollinated by *Greya* moths (Thompson and Pellmyr 1992), senita cactus pollinated by senita moths (Fleming and Holland 1998), and *Silene* plants pollinated by *Hadena* and *Perizoma* moths (Kephart et al. 2006). Nevertheless, there are other plant–seed parasite associations that have

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evolved into reciprocal specialization (globeflower–globeflower fly and *Rheum nobile*–fungus gnat mutualisms; Jaeger and Després 1998; Song et al. 2014). Understanding of how and under what circumstances obligate pollination mutualisms evolve would thus benefit from exploring the origin of active pollination and mutualism in the Phyllanthae–*Epicephala* association.

In this chapter, we describe the pollination systems and associations with *Epicephala* of 26 Phyllanthae species studied during 2002–2007 in Southeast Asia, New Caledonia, Australia, Madagascar, Guinea, and North America (Table 6.1). Based on this information, we explore the origin of the Phyllanthae–*Epicephala* mutualism using robust molecular phylogenies for 46 species of Phyllanthae and associated *Epicephala* moths. Although the present analysis focuses on only a small proportion of the global diversity of Phyllanthae, the sampled species cover the entire range of taxonomic diversity within the tribe (Hoffmann et al. 2006; Kathriarachchi et al. 2006), allowing an overview of broad coevolutionary history of the Phyllanthae–*Epicephala* association. Overall, the results reveal an unexpectedly complex origin of the Phyllanthae–*Epicephala* pollination mutualism and provide important general insights into how a combination of evolutionary innovation and partner shifts shapes the evolutionary dynamics of mutualism in coevolving species interactions.

6.2 Diversity of Pollination Systems in Phyllanthae

As detailed in Chaps. 3 and 4, there are five Phyllanthae lineages that are obligately pollinated by host-specific *Epicephala* moths (*Glochidion*; *Breynia*; and New Caledonian *Phyllanthus*; *Phyllanthus* section *Anisonema*; and an unclassified group of *Phyllanthus* endemic to Madagascar). The remaining species are pollinated by diurnal insects that visit flowers for nectar and pollen, and do not have associations with pollinating *Epicephala*. However, *Flueggea suffruticosa* is parasitized by *Conopomorpha flueggella*, and three herbaceous *Phyllanthus* species are parasitized by seed-parasitic *Epicephala* species that do not pollinate the flowers (Chap. 5).

The plants that are not pollinated by *Epicephala* employ a variety of insects as pollinators. Species of *Flueggea* have the broadest range of flower visitors, including bees, flies, beetles, and butterflies, which all probably contribute to pollination. Although observation is limited, dipteran insects appear to be important pollinators of many other Phyllanthae, such as *Phyllanthus flexuosus*, *P. oligospermus* (both subgenus *Kirganelia*), *P. liukiensis* (subgenus *Eriococcus*), *P. roseus* (subgenus *Phyllanthodendron*), *Breynia retusa*, and *Sauropus quadrangularis*. Notably, most herbaceous *Phyllanthus*, which are phylogenetically spread across the entire Phyllanthae phylogeny (Chap. 4), are pollinated by ants that visit flowers for nectar. In a controlled experiment where only ants were allowed to visit flowers of *P. lepidocarpus* grown in cages, plants regularly attained full fruit set (Fig. 6.1), whereas they produced no fruits when insects were fully excluded. Because

Table 6.1 List of species studied

| Species sampled ^a | Abbreviation | Study site | <i>Epicephala</i> as pollinator | Criteria for pollinator determination ^b | Style spreading |
|------------------------------|--------------|---------------------------------------|---------------------------------|--|-----------------|
| <i>Margaritaria</i> | | | | | |
| <i>M. discoidea</i> | Mdis | Guinea: Bossou | No | E, M | 7.07 |
| <i>M. indica</i> | Mind | Japan: Okinawa Is. | No | M | 5.46 |
| <i>Flueggea</i> | | | | | |
| <i>F. jullienii</i> | Fjul | Laos: Mahaxai | No | M | 4.99 |
| <i>F. suffruticosa</i> | Fsuf | Japan: Hyogo/Hiroshima/Amami Island | No | E, M | 4.82 |
| <i>F. virosa</i> | Fvir | Laos: Vieng Xai/Taiwan: Fangliao | No | E, M | 4.47 |
| <i>Phyllanthus</i> | | | | | |
| <i>P. (Mc.) ussuriensis</i> | Puss | Japan: Tokyo/Kyoto | No | E, M | 7.01 |
| <i>P. (Mc.) virgatus</i> | Pvir | Laos: Vientiane | No | E, M | 5.97 |
| <i>P. (Er.) liukiensis</i> | Pliu | Japan: Okinawa Island | No | E, M | 7.87 |
| <i>P. (Er.) pulcheroides</i> | Ppul | Laos: Mahaxai | No | E, M | 8.69 |
| <i>P. (Ki.) reticulatus</i> | Pret | Taiwan: Henchun | Yes | E, M | 0.45 |
| <i>P. (Ki.) microcarpus</i> | Psp | Laos: Laksao | Yes | E, M | 0.5 |
| <i>P. (Ki.) flexuosus</i> | Pfle | Japan: Kyoto/Hyogo/Miyazaki | No | E, M | 4.87 |
| <i>P. (Ki.) oligospermus</i> | Poli | Japan: Yonaguni Island | No | E, M | 4.96 |
| <i>P. (Tn.) tenellus</i> | Pten | Japan: Okinawa Island | No | E, M | 6.41 |
| <i>P. (Sw.) amarus</i> | Pama | Japan: Ishigaki Island/Laos: Thakhaek | No | E, M | 4.09 |
| <i>P. (Sw.) warnockii</i> | Pwar | USA: New Mexico | No | E, M | 1.87 |
| <i>P. (Af.) debilis</i> | Pdeb | Japan: Ishigaki Island | No | E, M | 4.32 |

(continued)

Table 6.1 (continued)

| Species sampled ^a | Abbreviation | Study site | <i>Epicephala</i> as pollinator | Criteria for pollinator determination ^b | Style spreading |
|-----------------------------------|--------------|--|---------------------------------|--|-----------------|
| <i>P. (Go.) aeneus</i> | Paen | New Caledonia: Cap Bocage | Yes | L, M | 1.08 |
| <i>P. (Go.) gneissicus</i> | Pgne | New Caledonia: Mt. Panié | Yes | L | n. a. |
| <i>P. (Go.) guillauminii</i> | Pgui | New Caledonia: Tiébaghi | Yes | L | n. a. |
| <i>P. (Go.) vulcani</i> | Pvul | New Caledonia: Riviere Bleue | Yes | L, M | 0.62 |
| <i>P. (Go.) bourgeoisii</i> | Pbou | New Caledonia: Cap Bocage | Yes | L, M | 0.38 |
| <i>P. (Go.) chamaecerasus</i> | Pcha | New Caledonia: Chutes de Ba | Yes | L | n. a. |
| <i>P. (Go.) caudatus</i> | Pcau | New Caledonia: Riviere Bleue | Yes | L | n. a. |
| <i>P. (Go.) cf. koniamboensis</i> | Pkon | New Caledonia: Tinip | Yes | L | n. a. |
| <i>P. (Go.) mangenotii</i> | Pman | New Caledonia: Cap Bocage | Yes | L, M | 0.49 |
| <i>P. (Ci.) acidus</i> | Paci | Laos: Vientiane (cultivated) | No | L, E, M | 2.5 |
| <i>P. (Em.) emblica</i> | Pemb | Laos: Ban Chomesy | No | L, E | n. a. |
| <i>P. (Em.) lepidocarpus</i> | Plep | Japan: Kyoto/Miyako Island/Ishigaki Island | No | E, M | 3.12 |
| <i>P. (Pd.) roseus</i> | Pros | Laos: Phialat | No | E, M | 1.99 |
| <i>P. marojejiensis</i> | Pmar | Madagascar: Mt. Marojeji | Yes | E, M | 0.18 |
| <i>P. humberitii</i> | Phum | Madagascar: Mt. Marojeji | Yes | E, M | 0.39 |
| <i>Sauropus</i> | | | | | |
| <i>S. androgynus</i> | Sand | Laos: Thakhaek | No | E, M | 2.03 |
| <i>S. brevipes</i> | Sbre | Laos: Vientiane | No | E, M | 2.14 |
| <i>S. granulatus</i> | Sgra | Laos: Vientiane | No | E, M | 2.04 |

(continued)

Table 6.1 (continued)

| Species sampled ^a | Abbreviation | Study site | <i>Epicephala</i> as pollinator | Criteria for pollinator determination ^b | Style spreading |
|------------------------------|--------------|------------------------------|---------------------------------|--|-----------------|
| <i>S. quadrangularis</i> | Squa | Laos: Vientiane | No | E, M | 2.53 |
| <i>Breyenia</i> | | | | | |
| <i>B. disticha</i> | Bdis | New Caledonia: Koumac | Yes | M | 0.25 |
| <i>B. fruticosa</i> | Bfru | Laos: Vientiane | Yes | L, M | 1.45 |
| <i>B. oblongifolia</i> | Bobl | Australia: Windsor Tableland | Yes | M | 0.2 |
| <i>B. retusa</i> | Bret | Laos: Vientiane | No | E, M | 3.02 |
| <i>B. vitis-idaea</i> | Bvit | Japan: Amami Island | Yes | L, M | 0.43 |
| <i>Glochidion</i> | | | | | |
| <i>G. acuminatum</i> | Gacu | Japan: Amami Island | Yes | L, M | 0.86 |
| <i>G. lanceolatum</i> | Glan | Japan: Ishigaki Island | Yes | L, M | 0.31 |
| <i>G. obovatum</i> | Gobo | Japan: Wakayama | Yes | L, M | 0.93 |
| <i>G. rubrum</i> | Grub | Japan: Ishigaki Island | Yes | L, M | 0.87 |
| <i>G. zeylanicum</i> | Gzey | Japan: Okinawa Island | Yes | L, M | 0.24 |

^a*Phyllanthus* sections and subgenera are abbreviated as follows: *Mc.*, *Macraea*; *Er.*, *Eriococcus*; *Ki.*, *Kirganelia*; *Tn.*, *Tenellanthus*; *Sw.*, *Swartziani*; *Af.*, *Afroschwartziani*; *Go.*, *Gomphidium*; *Ci.*, *Cicca*; *Em.*, *Embllica*; *Pd.*, *Phyllanthodendron*. Placement of *Phyllanthus marojejiensis* and *P. humbertii* is uncertain

^bEach species was judged as either *Epicephala*- or non-*Epicephala*-pollinated based on literature information (L), ecological data (E), and/or style morphology of the female flower (M)

P. lepidocarpus is self-compatible, ants are probably sufficient to pollinate this species fully in wild conditions as well.

Whether a plant species is pollinated by *Epicephala* is most clearly reflected in style morphology. In species pollinated by the moths, styles are reduced and fused to form a narrow apical cavity into which moths insert the proboscis to deposit pollen. By contrast, species diurnally pollinated by various nectar-seeking insects usually have bifid styles that are spread horizontally, which facilitates passive pollen receipt from insect bodies (Fig. 6.2). Overall, species with different

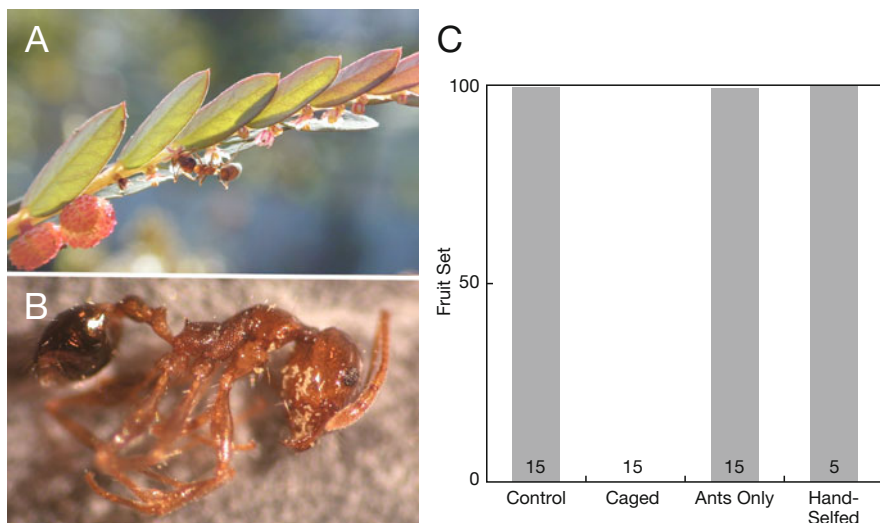


Fig. 6.1 Ant pollination of *Phyllanthus lepidocarpus*. (a) An ant, *Paratrechina flavipes*, consuming nectar on male flower of *P. lepidocarpus*. (b) *P. lepidocarpus* pollen attached to the head of *Tetramorium* sp. (c) Result of selective exclusion experiment in *Phyllanthus lepidocarpus*. Fruit set of the caged treatment is significantly lower than the fruit sets of other three treatments (Kruskal–Wallis test, $\chi^2 = 40.01$, $df = 3$, $P < 0.001$). Numbers inside bars are sample sizes. Error bars are too small to be seen

pollination syndromes have nonoverlapping degrees of style spreading (Fig. 6.3); thus, pollination systems can be reliably assigned to plant species for which sufficient ecological data are not available.

6.3 Phylogeny of Phyllanthae and *Epicephala*

To investigate the origin of the Phyllanthae–*Epicephala* mutualism, pollination systems were mapped onto the molecular phylogeny of 46 Phyllanthae species. The phylogeny is based on the combined chloroplast *matK*, *ndhF*, *atpB*, and nuclear *PHYC* gene dataset for 92 species of Phyllanthaceae including the above 46 Phyllanthae species. Maximum parsimony, likelihood, and Bayesian analyses all produced a highly resolved and well-supported phylogeny for Phyllanthae (Fig. 6.4). Similarly, the phylogeny of 26 *Epicephala* species associated with the above Phyllanthae species were reconstructed based on the combined mitochondrial *COI*, nuclear *ArgK*, *EF-1 α* , *Wg*, and the *18S rDNA* gene dataset. This produced a well-resolved phylogeny, although the phylogenetic placement of *Conopomorpha flueggella* with respect to *Epicephala* remained ambiguous (Fig. 6.5).



Fig. 6.2 Floral morphology of Phyllanthaceae plants with *Epicephala* (a–h) and non-*Epicephala* (i–p) pollinators. (a, e) *Glochidion acuminatum*. (b, f) *Breynia vitis-idaea*. (c, g) *Phyllanthus reticulatus*. (d, h) *Phyllanthus marojeiensis*. (i, m) *Flueggea suffruticosa*. (j, n) *Phyllanthus flexuosus*. The arrowheads indicate female flowers. (k, o) *Breynia retusa*. (l, p) *Sauropus quadrangularis*. For each species, male flowers are shown above female flowers (Reproduced from Kawakita 2010)

These phylogenies provide important insights into the origin of mutualism and active pollination. First, Phyllanthaceae species pollinated by *Epicephala* are not monophyletic, indicating that there have been multiple shifts in pollination systems. Reconstruction of ancestral character states for the pollination system along the Phyllanthaceae phylogeny suggest that there are five independent origins of the obligate pollination mutualism in Phyllanthaceae, with a single reversal to non-*Epicephala* pollination in *Breynia retusa* (Fig. 6.6). The pollinator *Epicephala* species are also nonmonophyletic, and ancestral character state reconstruction indicated a likely single origin of pollination behavior with a single event of secondary loss (Fig. 6.6). Major clades of *Epicephala* generally have specific

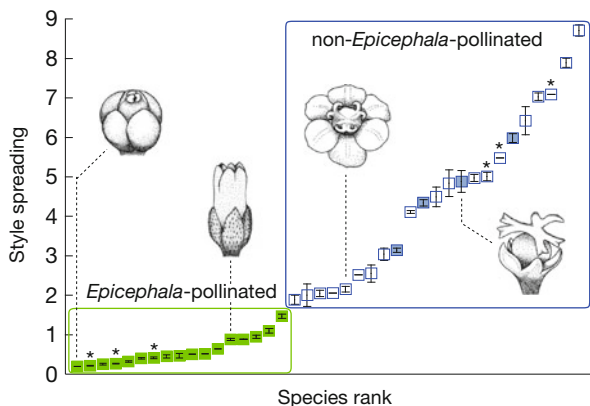


Fig. 6.3 Distribution of style spreading in Phyllanthaceae, quantified as the ratio of apical to basal style width. Species pollinated by *Epicephala* (green) have reduced styles that are medially fused, whereas non-*Epicephala*-pollinated species (blue) have horizontally spread, bifid styles. Filled and empty boxes indicate species with and without associations with *Epicephala*, respectively. Ecological data were not available for species with asterisks, but because species with different pollination syndromes had nonoverlapping distributions of style spreading, their pollination modes could be assigned reliably. Female flowers are drawn for *Phyllanthus marojejensis*, *Glochidion acuminatum*, *Sauropus brevipes*, and *Flueggea suffruticosa* (from left to right). Error bars, ± 1 SE (Reproduced from Kawakita and Kato 2009)

associations with well-defined taxonomic groups of Phyllanthaceae, but relationships at higher levels were largely incongruent, indicating that host shifts have occurred repeatedly (Fig. 6.6).

The above analysis of ancestral character state reconstruction indicates that *Epicephala*-pollinated Phyllanthaceae plants evolved multiple times independently. However, because the taxon sampling was limited to 46 species amid the global diversity of Phyllanthaceae (>1200 species), results of ancestral state reconstruction might change with the addition of more taxa. Therefore, divergence times for the Phyllanthaceae and *Epicephala* phylogenies were estimated to test whether the multiple origins hypothesis is in fact the preferred scenario. If the age of the most recent common ancestor of moth-pollinated plants is contemporary to that of *Epicephala*, a single origin of the mutualism followed by multiple losses would still be a viable hypothesis. Alternatively, evolution of pollinating behavior postdating initial host divergence would provide strong support for the multiple origins hypothesis.

A major obstacle when estimating divergence times is the scarcity of fossils, which is also the case for Phyllanthaceae and *Epicephala*. Nevertheless, there are several fossils of Phyllanthaceae and plants in other tribes of Phyllanthaceae that can be used to provide minimum age constraints on Phyllanthaceae phylogeny. The fossils used are *Bischofia*-type pollen from Bartonian, Middle Eocene (37.2 mya); *Actephila*-type pollen from Late Eocene (33.9 mya); *Phyllanthus*-type pollen from Early Eocene (48.6 mya) (Gruas-Cavagnetto and Köhler 1992); and *Glochidion* leaf



Fig. 6.4 Bayesian majority consensus cladogram of 46 Phyllanthaceae species based on sequences of combined plastid *matK*, *ndhF*, *atpB*, and nuclear *PHYC* genes. Numbers indicate maximum parsimony and likelihood bootstrap values, and Bayesian posterior probability (from top to bottom; shown only when >50). Asterisks indicate maximal nodal support (100 for all three measures)

impressions from Middle Miocene (11.6 mya; Prasad 1994; Antal and Prasad 1996). The root node (i.e., the node splitting Phyllanthaceae and Picrodendraceae) was assumed to be no older than 108 mya, which is the oldest estimate of the corresponding node in a study of Malpighiales radiation (Davis et al. 2005). Because attribution of some of the Phyllanthaceae fossils may still need refinement (Gruas-Cavagnetto and Köhler 1992), caution may be necessary when taking the precise dates resulting from this analysis. Because gracillariid moths are extremely scarce in the fossil record (Lopez-Vaamonde et al. 2006), *Epicephala* divergence times were obtained assuming a molecular clock of the COI gene. Only the COI clock was used because it is generally conserved across arthropod taxa (Gaunt and Miles 2002), has been widely used for dating in insects (Kandul et al. 2004; Quek et al. 2007; Ueda et al. 2008), and clusters at approximately $1.5\% \text{ myr}^{-1}$ in several arthropod groups (Farrell 2001; Quek et al. 2004; Sota and Hayashi 2007).

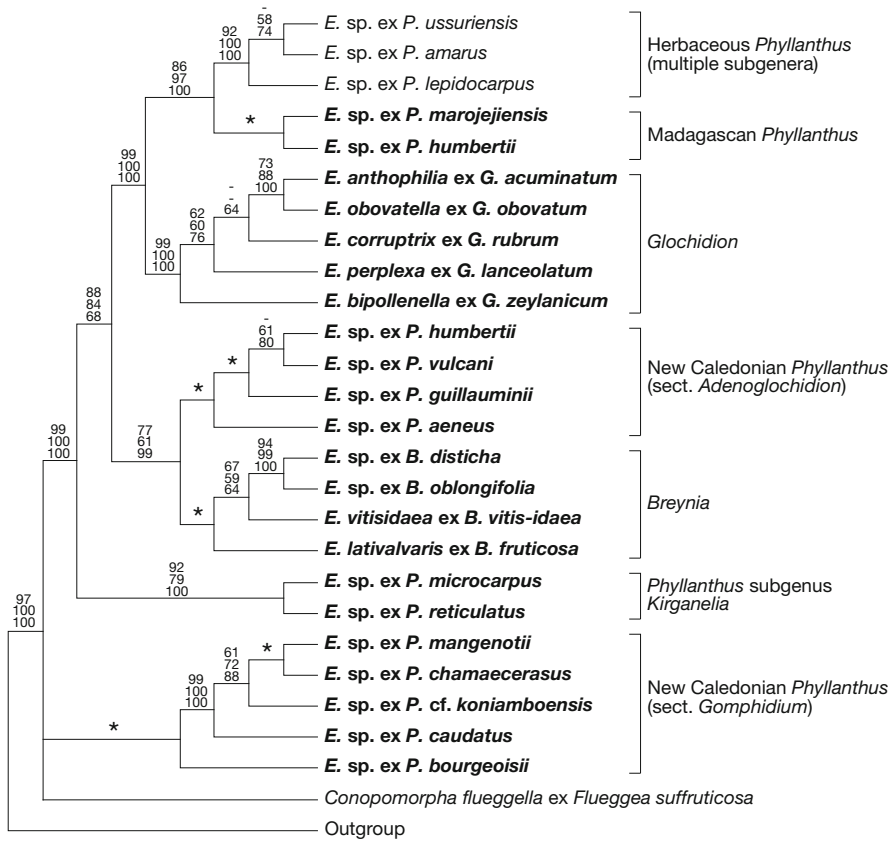


Fig. 6.5 Bayesian majority consensus cladogram of 25 *Epicephala* species and related *Conopomorpha flueggella* based on sequences of combined mitochondrial *COI* and nuclear *ArgK*, *EF-1 α* , *Wg*, and *18S rDNA* genes. Numbers indicate parsimony and likelihood bootstrap values, and Bayesian posterior probability (from top to bottom; shown only when >50). Asterisks indicate maximal nodal support (100 for all three measures). Associated host taxonomic groups are given

The analysis of divergence times indicates that the most recent common ancestor of *Epicephala*-pollinated plants occurred 41.0 mya (95% credibility interval, 39.3–48.3 mya; Fig. 6.6). In contrast, estimated ages of the split between *Conopomorpha flueggella* and *Epicephala* clustered within a timeframe between 20 and 30 mya. These estimates for the age of active pollination postdates initial host divergence by roughly 10–20 myr (Fig. 6.6), which is consistent with delayed radiation of *Epicephala* and hence multiple origins of the obligate pollination mutualism in Phyllanthaceae. Although the estimate of the timing of *Epicephala* divergence depends largely on the accuracy of the COI molecular clock, the assumed 1.5% myr⁻¹ is among the slowest of known rates for the arthropod COI gene (1.3–2.3% myr⁻¹; Brower 1994; Quek et al. 2004), and using higher rates would only give younger estimates for the age of the *Epicephala* root node; thus, the method employed is conservative with respect to providing young ages.

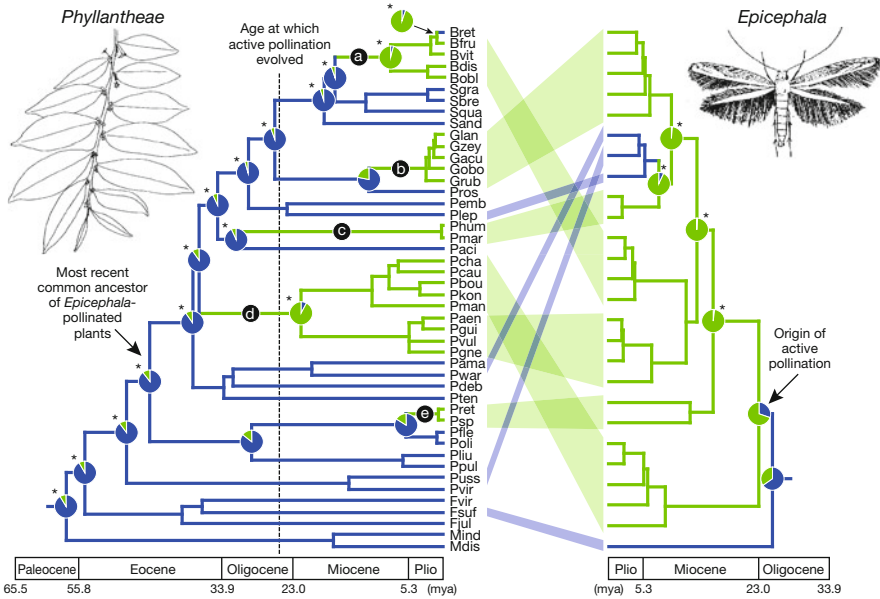


Fig. 6.6 Origin of the Phyllanthae–*Epicephala* obligate pollination mutualism. Chronograms for Phyllanthae plants (left) and associated *Epicephala* moths (right). Pie charts indicate the probabilities of *Epicephala* and non-*Epicephala* pollination systems (Phyllanthae) or the presence/absence of active pollination behavior (*Epicephala*) occurring at ancestral nodes. Asterisks indicate significant difference in likelihoods. Mutualism is represented in green, and associations with major plant and moth clades are indicated

6.4 Origin of Active Pollination and Mutualism

The above phylogenetic analyses and divergence time estimations allowed a general overview of the evolutionary history of the Phyllanthae–*Epicephala* association. Because the taxon sampling was limited to 20% of the global diversity of Phyllanthae at the section level (Kathriarachchi et al. 2006) and less than 5% at the species level, the entire picture of the evolutionary history of *Epicephala* pollination in Phyllanthae is probably much more complex than as depicted here. However, inclusion of other lineages would likely only strengthen the conclusion of repeated independent evolution because these plants generally have bifid, horizontally spread styles that are characteristic of non-*Epicephala*-pollinated plants (Fig. 6.2). Exceptions are the New World *Phyllanthus* subgenus *Xylophylla*, which consists of approximately 60 species having reduced columnar styles (Webster 1958) and section *Microglochidion*, which consists of approximately 10 species occurring on the tepuis of the Guiana Highlands (Chap. 5). Field observation and examination of herbarium specimens indicate that they are also associated with seed-feeding *Epicephala*. It is thus tempting to clarify the pollination systems of

these plants and phylogenetic positions of associated *Epicephala*, as they may represent additional origins of *Epicephala* moth pollination in Phyllanthaeae.

Our finding that the obligate pollination mutualism arose repeatedly in Phyllanthaeae is in stark contrast with the situations in the fig–fig wasp and yucca–yucca moth mutualisms. Coevolutionary analyses in the fig and yucca systems indicate that these associations arose only once in each partner lineage 40–60 mya (Pellmyr and Leebens-Mack 1999; Rønsted et al. 2005). An exception is *Hesperoyucca whipplei*, which is phylogenetically distant from the rest of the yuccas and independently established the mutualism with a yucca moth (Bogler et al. 1995; Pellmyr et al. 2007; Smith et al. 2008a). In the Phyllanthaeae–*Epicephala* system, major lineages of Phyllanthaeae had already emerged when *Epicephala* colonized these plants ~30 mya. Sequential radiation of *Epicephala* on an already diverged host lineage has likely provided opportunities for the moth pollinators to establish new mutualistic associations in distant host lineages. Thus, specialization to moth pollination occurred multiple times independently in Phyllanthaeae as *Epicephala* spread onto a broad range of the Phyllanthaeae lineage.

Our results also indicate that colonization of new host lineages by the pollinators sometimes results in a loss of mutualistic traits. A derived clade of *Epicephala* has completely lost the pollinating behavior after colonizing herbaceous species of *Phyllanthus*. These plants regularly attain full seed set through ant pollination (Fig. 6.1); thus, time and energetic costs required during pollination probably outweighed the benefit of assuring seed set in these moth lineages. At the same time, effective pollination by ants probably swamped the mutualistic effect of pollination by moths; thus, selection did not favor these *Phyllanthus* to specialize to moth pollination.

Taken together, the overall evolutionary history of Phyllanthaeae and *Epicephala* provides two general implications for the coevolutionary dynamics of mutualisms. First, although species associations are phylogenetically conserved in most coevolving interactions (Thompson 2005), rare shifts by a partner possessing the mutualistic trait can give rise to new mutualisms in phylogenetically distant partner lineages. In this sense, the active pollination behavior in *Epicephala* has been of critical importance for the establishment and maintenance of the Phyllanthaeae–*Epicephala* mutualism and thus represents a key innovation in this association. Second, the outcome of a species interaction can vary greatly depending on the community context in which it occurs (Thompson and Pellmyr 1992; Thompson and Cunningham 2002; Westerbergh 2004); thus, transitions between mutualism and antagonism can occur repeatedly within a single phylogenetic lineage. This parallels findings in other mutualisms where derived parasitic taxa are nested within ancestrally mutualistic clades (Pellmyr et al. 1996b; Machado et al. 2001; Als et al. 2004). Of particular relevance to future studies is our finding that the mutualism arose independently in several Phyllanthaeae lineages, which provides outstanding opportunities for comparative analyses of character evolution, diversification rates, and factors affecting mutualism establishment and stability.