

Chapter 13

Evolution and Diversity of Obligate Pollination Mutualisms

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Keywords Coevolution • Evolutionary stability • Fig–fig wasp mutualism • Host specificity • Leafhopper–leafhopper moth mutualism • Reciprocal diversification • Yucca–yucca moth mutualism

13.1 Diversity of Obligate Pollination Mutualisms

It is an exciting time for the study of obligate pollination mutualisms. New mutualisms continue to be discovered, and information on individual systems is rapidly growing. Presently, there are at least seven plant lineages apart from Phyllanthaceae that contain plants pollinated by seed-parasitic insects (Fig. 13.1, Table 13.1). There is little doubt that more such lineages will be discovered, inasmuch as one or two new mutualisms continue to be uncovered each decade. The abundance and heterogeneity of documented cases of obligate pollination mutualism offer an unprecedented opportunity to examine key topics of broad ecological and evolutionary relevance. In this chapter, we review the basic natural history of the seven mutualisms known outside of Phyllanthaceae (Fig. 13.1, Table 13.1) and address the following questions that are critical to our understanding of obligate pollination mutualisms.

1. Why do plants specialize to seed-parasitic pollinators despite the high cost imposed by the seed-feeding pollinator larvae?
2. How are mutualisms maintained despite the potential for selfish partners to disrupt the interaction?
3. Is pollinator specificity reinforced, and if so, why?

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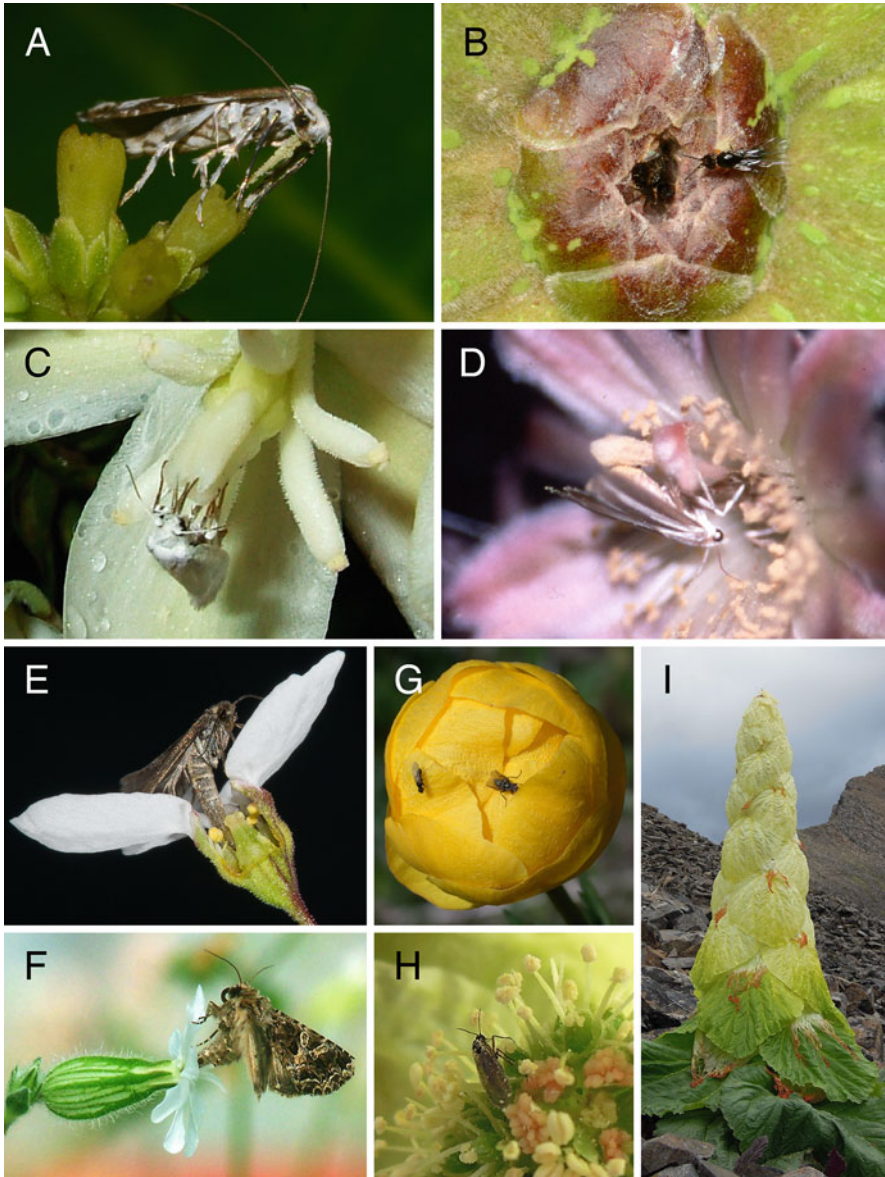


Fig. 13.1 Obligate pollination mutualisms. (a) The leafflower–leafflower moth mutualism. A female *Epicephala anthophilia* actively depositing pollen on *Glochidion acuminatum* flower in Amami-Oshima Island, Japan. (b) The fig–fig wasp mutualism. A female of an unidentified pollinating fig wasp species entering the syconium of *Ficus auriculata* in Phonsavan, Laos. (c) The yucca–yucca moth mutualism. A female *Tegeticula yuccasella* actively depositing pollen on *Yucca filamentosa* in New York, USA. (d) The senita cactus–senita moth mutualism. A female *Upiga virescens* actively pollinating the stigma of *Pachycereus schottii* with its abdomen. Photo credit: J. Nathaniel Holland. (e) The *Lithophragma*–*Greya* mutualism. *Greya politella* (Prodoxidae) ovipositing into a flower of *Lithophragma cymbalaria* in California, USA

4. Do obligate pollination mutualisms drive the reciprocal diversification of plants and pollinators?

We use the term obligate pollination mutualism to refer only to associations involving pollinators that are seed-parasitic. Although there are examples of specialized nursery pollination mutualisms in which the plants provide decaying floral parts as brood sites of pollinator larvae (Sakai 2002), these associations do not raise the type of questions listed above because decaying floral parts are not costly for the plant and because pollinator fitness is unlinked to pollination success. The above challenges posed by obligate seed-parasitic pollination mutualisms offer valuable opportunities to test and refine existing theories in ecology and evolution and thus are the focus of this chapter.

13.1.1 Fig–Fig Wasp Mutualism

Among the documented examples of obligate pollination mutualisms, those of figs and yuccas are arguably the best known (Janzen 1979; Herre et al. 2008; Pellmyr 2003). All of the >750 species in the genus *Ficus* (Moraceae), distributed throughout the tropical and subtropical areas of the world are pollinated by the fig wasps currently classified in the subfamilies Tetrapusiinae, Kradibiinae, and Agaoninae of the family Agaonidae (Heraty et al. 2013; Fig. 13.1b). That fig wasps are involved in the maturation of the cultivated fig, *Ficus carica*, was already known by Aristotle and Theophrastus as early as 350 BC (Condit 1947; Goor 1965). Ramírez (1969) was the first to recognize that many fig wasps pollinate actively; the females collect pollen into thoracic pollen pockets shortly after emergence, and later place the pollen on the stigmas of flowers in the figs where they oviposit, an observation later substantiated by Galil and Eisikowitch (1969). A smaller number of fig wasps pollinate passively, with dehiscent anthers depositing pollen on the surface of adult female wasps as they emerge from the syconia (Ramírez 1969; Kjellberg et al. 2001). Each fig wasp species is usually associated with only one *Ficus* species, although the long-presumed one-to-one relationship often breaks down, as many *Ficus* species host two or more fig wasp species sympatrically (Molbo et al. 2003; Machado et al. 2005; Haine et al. 2006; Su et al. 2008; Sun et al. 2011; Cornille et al. 2012). Two fig wasp species have lost the pollinating habit and became



Fig. 13.1 (continued) (Saxifragaceae). Photo credit: John N. Thompson. **(f)** The *Silene–Hadena* mutualism. A female *Hadena bicruris* ovipositing into a flower of *Silene latifolia*. Photo credit: Anne-Marie Labouche. **(g)** The globeflower–globeflower fly mutualism. *Chistocheta* flies at the entrance of a *Trollius europaeus* flower. Photo credit: Station Alpine Joseph Fourier. **(h, i)** The *Rheum nobile–Bradysia* mutualism. A female *Bradysia* sp. fungus gnat visiting the flowers of *Rheum nobile* **(h)** whose inflorescence axis is entirely covered with non-green bracts **(i)**. Photo credit: Bo Song

Table 13.1 Comparison of the eight known obligate pollination mutualisms

Trait	Reciprocal obligate pollination mutualism		Asymmetrical Obligate Pollination Mutualism					
	Leafflower–leafflower moth	Fig–fig wasp	Yucca–yucca moth	Senita cactus–senita moth	Saxifragaceae– <i>Greya</i>	<i>Silene–Hadena/Perizoma</i>	Globeflower–globeflower fly	<i>Rheum nobile–Bradystia</i>
Plant								
Family	Phyllanthaceae	Moraceae	Agavaceae	Cactaceae	Saxifragaceae	Caryophyllaceae	Ranunculaceae	Polygonaceae
Genera	<i>Glochidion</i> , <i>Breynia</i> , <i>Phyllanthus</i>	<i>Ficus</i>	<i>Yucca</i> , <i>Hesperoyucca</i>	<i>Pachycereus</i>	<i>Lithophragma</i> , <i>Heuchera</i>	<i>Silene</i>	<i>Trollius</i>	<i>Rheum nobile</i>
No. of species	>500	>750	ca. 40	1	2	ca. 10	1	1
Geographic range	Pantropical	Pantropical	North and Central America	North and Central America	North America	Holarctic	Europe	Himalayas
Habit	Tree or shrub	Tree or shrub	Woody succulent	Woody succulent	Perennial	Perennial	Perennial	Perennial
Sexual system	Monoecious	Monoecious, functionally dioecious	Hermaphrodite	Hermaphrodite	Hermaphrodite	Gynodioecious, dioecious	Hermaphrodite	Hermaphrodite
No. ovules per flower	6–20	1	200–300	ca. 200	150–400	ca. 40	ca. 50	1
Flowering season	Continuous or seasonal	Continuous or seasonal	June–July	April–June	April–June	Spring–autumn	June–August	June
Self compatibility	Compatible	Unknown?	Compatible	Incompatible	Incompatible	Compatible	Incompatible	Compatible
Nectar production	None or little	None	None or little	None or little	Present	Present	Present	None
Pollinator attractant	Scent	Scent	Scent	Color and scent?	Color and scent	Color and scent	Color and scent	Scent, color?

Limitation of fruit production	Variable	Unknown	Resource-limited	Resource-limited	Variable	Variable	Pollen-limited	Resource-limited
Fruit type	Capsule, berry	Fig	Dry or fleshy capsule	Berry	Capsule	Capsule	Seedhead	Samara
Floral specialization to pollination	Nocturnal flowering, reduced nectar, style fusion, stamen fusion	Urn-shaped inflorescence	Nocturnal flowering, reduced nectar	Small flower size, reduced nectar	Multivariate floral traits	None	Globular flower	Showy bracts?
Selective abortion of flowers with high egg load	Present (not all species)	Present (strength varies among species)	Present (not in all plant-pollinator combinations)	Attacked fruits invariably abscise	Absent	Absent	Absent	Infested flowers more likely retained
Defensive trait against seed parasite	Elongated gynophore, enlarged tepals	Long styles	Constricted ovary	None	None	Long corolla	Adonivermith production	None
Pollinator								
Order	Lepidoptera	Hymenoptera	Lepidoptera	Lepidoptera	Lepidoptera	Lepidoptera	Diptera	Diptera
Family	Gracillariidae	Agonidae	Prodoxidae	Pyralidae	Prodoxidae	Noctuidae, Geometridae	Anthomyiidae	Sciariidae
Genus	<i>Epicephala</i>	Various genera	<i>Tegeticula</i> , <i>Parategeticula</i>	<i>Upiga</i>	<i>Greya</i>	<i>Hadena</i> , <i>Perizoma</i>	<i>Chia스토chea</i>	<i>Bradysia</i>
No. of species	>500?	>750	ca. 20	1	2	ca. 10	6	1
No. of host per species	1-2	1-2	1-6	1	1-6	1-4	1	1

(continued)

Table 13.1 (continued)

	Reciprocal obligate pollination mutualism			Asymmetrical Obligate Pollination Mutualism				
Trait	Leafflower– leafflower moth	Fig–fig wasp	Yucca–yucca moth	Senita cactus– senita moth	Saxifragaceae– <i>Greya</i>	<i>Silene–Hadena/ Perizoma</i>	Globeflower– globeflower fly	<i>Rheum nobile– Bradystia</i>
Active pollination	Present	Present	Present	Present	Absent	Absent	Absent	Absent
Specialized pollen collecting structures	Proboscis sensilla	Pollen pockets, coxal comb	Maxillary tentacles	Abdominal scales	None	None	None	None
Co- pollinators	None	None	None	Halictid bees	Bees, flies	Various Lepidoptera	Bees, beetles	Autonomous self- pollination results in seed set
Oviposition site	Inside/outside of ovary	Ovule	Ovary tissue	Petal	Ovary	Ovary, petal	Carpel	Ovary
No. of eggs per flower	1–3	1–20 (per syconium)	one to several	1	1	1	one to several	1
No. of lar- vae per fruit	≤ 3	Many (per syconium)	≤ 6	1	1	1	≤ 10	1
Percent seed destruc- tion per fruit	Variable: 0–100%	Variable: 0–50%	Variable: 0–30%	100% (attacked fruits abscise)	Variable: 15–30%	100%	Variable: 0–50%	100%
Generations per year	Multivoltine or univoltine	Multivoltine	1	4–5	1	1?	1	1
Pupation site	On litter	In seed	In soil	In cactus branch	In soil	In soil	In soil	In seed
Mating site	On host plant	In mature fig	On host	On cactus spines	On host	Unknown	In flower	On host

Parasitoid of pollinator larva	<i>Bracon</i> (Braconidae)	Non-pollinating fig wasps (Pteromalidae)	<i>Digonogastra</i> (Braconidae)	Unknown	Unknown	Unknown	Unknown
Inquiline	<i>Bracon</i> (Braconidae); seed-parasitic moths (Tortricidae, Pyralidae, Carposimidae)	Non-pollinating fig wasps (Agaonidae, Pteromalidae, Ormyridae, Eurytomidae, Torymidae); fruit flies (Tephritidae, Lonchaenidae)	<i>Prodoxus</i> (Prodoxidae); florivore bug (Coreidae) and beetle (Tenebrionidae)	Unknown	Unknown	Unknown	Unknown
Association							
Plant dependence on seed-parasitic pollinator	Obligate	Obligate	Obligate	Facultative	Facultative	Facultative	Facultative
Pollinator dependence on plant	Obligate	Obligate	Obligate	Obligate	Obligate	Obligate	Obligate?
Synergistic diversification	Yes	Yes	Yes	No	No	No	No

Information is based on various sources

nonmutualistic: *Ceratosolen galili* on *Ficus sycomorus* in Africa (Galil and Eisikowitch 1969; Compton et al. 1991) and an undescribed *Eupristima* species on *F. altissima* in China (Peng et al. 2008). The close relatives of pollinating fig wasps are the so-called nonpollinating fig wasps that join the association by either inducing galls on fig ovules or exploiting the galls induced by other fig wasps (Weiblen 2002). They offer an excellent comparative model to test hypotheses on cospeciation and the evolution of host specificity (e.g., Weiblen and Bush 2002; Marussich and Machado 2007).

13.1.2 *Yucca–Yucca Moth Mutualism*

Engelmann (1872) was the first to observe yucca moths in the flowers of yuccas, and suggest that they are essential for yucca pollination (Pellmyr 2003). However, it was Charles Riley who unfolded the natural history of the mutualism in great detail, including the moth's ability to pollinate actively using the remarkably developed maxillary tentacles (Riley 1872, 1880, 1881, 1892; Fig. 13.1c). Yuccas, native to North and Central America, are plants of the genus *Yucca* and the monotypic *Hesperoyucca* (Agavaceae), of which the latter was formerly considered a section within *Yucca*. They are iconic components of the arid vegetation of North and Central America, although a few species occur in the rainforests of southernmost Mexico. Molecular phylogenetic analysis clearly shows that *Hesperoyucca* is distantly related to *Yucca* (McKain et al. 2016). A close relative of *Hesperoyucca* is *Hesperoe*, which has no association with yucca moths and relies instead on hummingbirds for pollination (Pellmyr and Augenstein 1997). Thus, the obligate mutualism with yucca moths is believed to have evolved independently twice: in *Hesperoyucca* and the common ancestor of *Yucca* (Bogler et al. 1995; McKain et al. 2016). Pollinating yucca moths belong to the genera *Tegeticula* and *Parategeticula*, which form a well-supported clade within the Prodoxidae family (Brown et al. 1994; Pellmyr and Leebens-Mack 1999). Two derived *Tegeticula* species, *T. intermedia* and *T. corruptrix*, independently lost the pollinating behavior and oviposit in young fruits produced by other pollinating yucca moths; they are “cheaters” in this mutualistic system (Addicott 1996; Pellmyr et al. 1996b; Pellmyr 1999). Sister to the clade of *Tegeticula* and *Parategeticula* is the genus *Prodoxus*, or bogus yucca moths, which consists of species that feed on the nonreproductive organs of yuccas and other Agavaceae (leaves, inflorescence stalks, or fruit walls; Pellmyr et al. 2006). As with nonpollinating fig wasps, they provide important comparative models for understanding the process of speciation in yucca moths (e.g., Althoff et al. 2001, 2012).

13.1.3 *Senita Cactus–Senita Moth Mutualism*

In contrast to the fig and yucca mutualisms, which have been known for more than 100 years, other examples of mutualisms between plants and pollinating seed parasites were discovered relatively recently. Fleming and Holland (1998) studied the interaction between the senita cactus (*Pachycereus schottii*; Cactaceae) and senita moth (*Upiga virescens*; Pyralidae) in the Sonoran Desert, and made the remarkable finding that the moth is an active pollinator; the female collects pollen on specialized abdominal scales, actively deposits pollen on stigmas (Fig. 13.1d), and lays a single egg on a flower petal. Fruits attacked by moth larvae abscise and produce no viable seeds, but only a fraction of the moth eggs actually survive to become seed predators, therefore the interaction is mutualistic overall (Holland and Fleming 1999). The cactus is also visited by halictid bees during the daytime, but its nocturnal flowering and temperature-dependent flower closing limit the overall contribution by bees to the fruit set (Holland and Fleming 2002). The basal lineages of *Pachycereus* produce bat-pollinated flowers that open at night (Fleming et al. 1996, 2001), therefore nocturnal flowering may not be a trait that has evolved as a result of specialization. Rather, small flowers and reduced nectar production may be adaptations associated with moth pollination (Hartmann et al. 2002).

13.1.4 *Lithophragma–Greya Mutualism*

The family Prodoxidae, to which yucca moths belong, is a basal clade of Lepidoptera characterized by a piercing ovipositor, and contains species that feed parasitically on flowers and other plant parts in the Rosaceae, Grossulariaceae, and Saxifragaceae (Pellmyr et al. 1996b). Of these, *Greya politella* and *G. enchrysa*, occurring in North America, are seed parasites of *Lithophragma* and *Heuchera* (Saxifragaceae). The *Greya* females pollinate their host plants passively as they oviposit in the flowers (Pellmyr and Thompson 1992; Pellmyr et al. 1996b; Fig. 13.1e). *G. politella* has been recorded from several *Lithophragma* and *Heuchera* species, whereas *G. enchrysa* is known only from *H. cylindrica*. It is important to note that *Lithophragma* and *Heuchera* plants used by *Greya* are also pollinated by bumblebees, solitary bees, or bombyliid flies, therefore the mutualistic effect of pollination by *Greya* is often swamped by these copollinators (Thompson and Pellmyr 1992). Nevertheless, in populations of *Lithophragma* where copollinators are rare or absent, *Greya* is the main pollinator (Thompson and Cunningham 2002; Thompson et al. 2010), and traits that enhance moth pollination have likely evolved (Thompson et al. 2013; Friberg et al. 2014).

13.1.5 *Silene*–*Hadena*/*Perizoma* Mutualism

As new mutualisms involving pollinating seed parasites were being discovered, increased attention was paid to whether moth seed parasites of *Silene* (Caryophyllaceae) contribute to the pollination of their hosts (e.g., Brantjes 1976a,b; Pettersson 1991a,b; Westerbergh 2004; reviewed in Kephart et al. 2006). Plants of *Silene* and several allied genera distributed widely in the Holarctic are attacked by seed-feeding moths in *Hadena* (Noctuidae; Fig. 13.1f) and *Perizoma* (Geometridae). Both male and female moths visit flowers nocturnally to consume nectar, and in doing so cause pollination (Labouche and Bernasconi 2010). However, because *Silene* flowers are visited by a variety of other nocturnal moths and diurnal bees and flies, *Hadena* and *Perizoma* are rarely major pollinators, and usually have only parasitic effects on their hosts. However, in populations where copollinators are rare or in years when copollinators are scarce, these seed parasites may act as mutualists of their host plants (Westerbergh 2004). In *Silene latifolia*, long corolla tubes act to constrain oviposition by *Hadena bicruris* to sites of high egg mortality (Labouche and Bernasconi 2013). If such traits shift the cost–benefit balance in favor of plants, there is the potential for this interaction to evolve toward mutualism.

13.1.6 *Globeflower*–*Globeflower Fly* Mutualism

Most of the above examples of obligate pollination mutualism involve moths as pollinators (a notable exception is the figs pollinated by fig wasps), but the pollinators of the last two examples are dipteran insects. Pellmyr (1989) studied the pollination ecology of *Trollius europaeus* in great detail and found that, of the four species of *Chiastocheta* flies (Anthomyiidae) that consume the seeds of *T. europaeus* in Sweden, three are likely mutualistic pollinators that deliver a net benefit to the plant (Fig. 13.1g). Studies of *T. europaeus* in other parts of Europe show that the plant is pollinated by *Chiastocheta* flies throughout its range, although the species composition of *Chiastocheta* varies geographically (Jaeger and Després 1998; Després et al. 2007). The flies do not pollinate actively; pollination occurs as the flies mate and eat pollen and nectar in the flowers. Notably, the tepals of *T. europaeus* form a globe with a narrow opening at the apex (Fig. 13.1g), which serves as the entrance and exit for *Chiastocheta* flies. Because related *Trollius* species pollinated by bumblebees have disc-shaped flowers (Pellmyr 1992), the globular flower of *T. europaeus* likely evolved to exclude visitors other than *Chiastocheta*. Regardless of such a specialization, *Trollius europaeus* has copollinators in many populations, and the mutualism is asymmetrically obligate wherein plants do not always entirely depend on the fly (Suchan et al. 2015).

13.1.7 *The Rheum nobile–Bradysia Mutualism*

A symbolic plant of the high Himalayas, *Rheum nobile*, attracts strong botanical interest owing to its showy nongreen bracts that conceal the entire stout, conical inflorescence axis (Fig. 13.1h). The pollinator of this remarkable plant has long remained uninvestigated, but recently, Song et al. (2014) found that the seed-feeding fungus gnat, *Bradysia* sp. (Sciaridae), is its sole pollinator; however, it is still unknown whether the plant is the only host of the insect. Fungus gnats pollinate passively as the female insects crawl on the inflorescence inside the bracts in search of suitable oviposition sites (Fig. 13.1i). Because the flowers are uniovulate, infested fruits will not have viable seeds; however, the fungus gnats pollinate more flowers than are used for oviposition, resulting in a net mutualistic outcome. During anthesis, the flowers produce an unusual floral volatile, 2-methyl butyric acid methyl ester, which is attractive to female fungus gnats (Song et al. 2014). Because the plants can set seeds by autonomous self-pollination, the degree of reciprocal dependence is also not high in this system as compared to other highly obligate interactions.

13.2 Reciprocal and Asymmetrical Obligate Pollination Mutualism

Among the eight nursery pollination mutualisms involving seed-parasitic pollinators, only three are reciprocally obligate pollination mutualisms, whereas the remaining five are asymmetrically obligate such that the plants are pollinated not only by the seed-parasitic insect partners but also by copollinators. Intriguingly enough, the three reciprocally obligate pollination mutualisms, that is, the leafflower–leafflower moth, fig–fig wasp, and yucca–yucca moth mutualisms, share the following properties (Table 13.1): (1) adult females actively pollinate the flowers; (2) the attractant released by the flowers is floral scent, which can be finely tuned into private communication signals owing to the countless combinations of volatile compounds; (3) the plants are unable to set seeds in the absence of the seed-parasitic insects; and (4) the plants and the pollinators have undergone reciprocal synergetic diversification. Thus, reciprocal obligate pollination mutualisms likely derived from asymmetrical obligate pollination mutualisms by the following three innovations: pollinator's adoption of active pollination behavior, development of volatile chemical communication between plants and pollinators, and elimination of copollinators by the plants through plant–pollinator coevolution. The resulting high reciprocal dependence and exceedingly high host-specificity have canalized gene flows and driven the reciprocal diversification of the plants and the seed-parasitic pollinators. We discuss these processes in detail in the following sections.

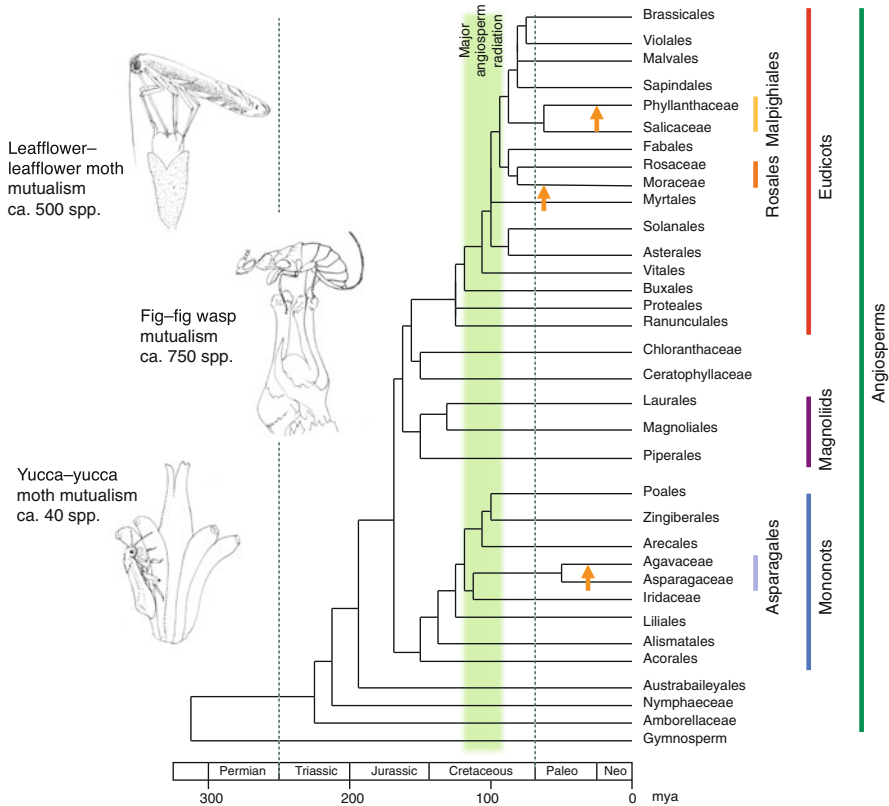


Fig. 13.2 A chronogram of angiosperm diversification indicating the origins of the three reciprocally obligate pollination mutualisms. The chronogram is based on Zeng et al. (2014)

The three reciprocally obligate pollination mutualisms are ancient and diverse (Fig. 13.2). The origin of the fig-fig wasp mutualism dates back to 60 mya, and subsequent codiversification has produced an impressive diversity of plant and pollinator species (there are more than 750 *Ficus* species; Rønsted et al. 2005). Reliable dating of the origins of the yucca-yucca moth and leafflower-leafhopper moths is hampered by the paucity of lepidopteran fossils, but the current best estimates of the ages of active pollinators are 32–40 mya for the yucca moths (Pellmyr and Leebens-Mack 1999; Gaunt and Miles 2002) and 25–30 mya for the leafflower moths (Kawakita and Kato 2009). Within Agavaceae, specialization to pollinating yucca moths evolved twice in *Hesperoyucca* and *Yucca*, the latter of which has diversified into approximately 40 spp. In Phyllanthaceae, the mutualism arose at least five times independently, and concurrent radiations in these plant lineages resulted in a total of approximately 500 plant species that are obligately pollinated by leafflower moths (Chapter 6). Intriguingly, once specialized, no fig, yucca, or leafflower species has reverted to pollination by other agents, which

strongly indicates that pollination by seed parasites is a highly successful and stable strategy for the plants.

13.3 Origin of Obligate Pollination Mutualisms

Historically, figs and yuccas have been the only groups of plants that are known to be pollinated by seed-parasitic insects. Because of their rarity among angiosperms, obligate pollination mutualisms were thought to evolve only under exceptional circumstances where plants have no means of achieving successful pollination other than specializing to seed parasites. Because seed destruction reduces plant reproductive output, copollinators, if present, are thought to provide better pollination services than seed parasites and limit plant specialization to seed-parasitic pollinators. In fact, the exclusion of copollinators has not occurred in some of the above examples, including *Lithophragma* plants pollinated by *Greya* moths (Thompson and Pellmyr 1992) and *Silene* plants pollinated by *Hadena* and *Perizoma* moths (Kephart et al. 2006). However, many others exhibit a moderate to considerable degree of specialization (Table 13.1), calling into question the view that specialization occurs under exceptional circumstances. Furthermore, detailed phylogenetic analysis of the yucca and leafflower lineages indicates that specialization to yucca moths and leafflower moths, respectively, occurred twice in yuccas and five times in leafflowers (Bogler et al. 1995; Kawakita and Kato 2009; McKain et al. 2016). Increasingly, it seems that, under some conditions, seed parasites provide superior pollination services and are preferred by plants over potential copollinators.

Seed-parasitic pollinators may be effective at transferring pollen because they usually have narrow diets, and hence a strong affinity to their host plants compared with generalist floral visitors that use various other plants as food sources. Actively pollinating seed parasites, such as leafflower moths, fig wasps, yucca moths, and senita moths, may be particularly effective pollinators that waste far less pollen than do generalist visitors. Thus, seed parasites may exert major positive effects on the male reproductive success of the plant, perhaps large enough to offset the impact of seed destruction on a plant's female reproductive success, thereby allowing plants to specialize to seed-parasitic pollinators. Increased use of seed-parasitic pollinators always decreases a plant's fitness through the female function; therefore, specialization, if it occurs at all, must occur solely via fitness gain through the male function.

Comparisons of pollen-to-ovule ratios between Phyllanthaceae plants specialized to *Epicephala* and those pollinated by other insects provide evidence of high pollen transfer efficiency by active pollinators (Mochizuki and Kawakita, unpublished data). If the pollinator of a given plant species wastes a large proportion of pollen picked up from the anthers, then plants should produce excess pollen to supplement the loss, leading to high pollen-to-ovule ratios, and vice versa. Low pollen-to-ovule ratios among *Epicephala*-pollinated species are best illustrated by differences in the relative abundance of male and female flowers (Fig. 13.3).

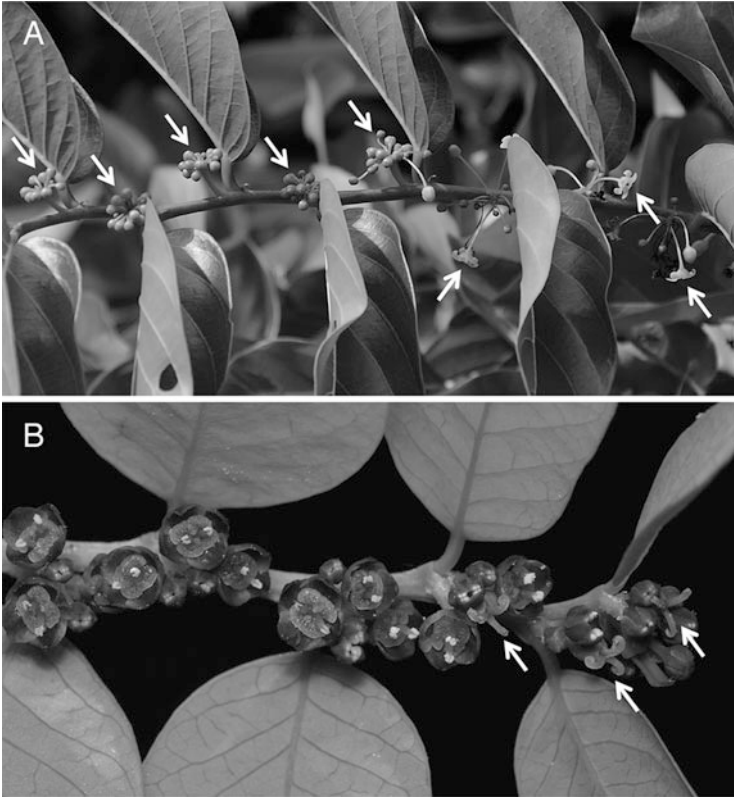


Fig. 13.3 Male-to-female flower ratios in Phyllanthaceae plants. (a) A flowering branch of *Epicephala* pollinated *Glochidion zeylanicum*, showing few male flowers (upward arrows) and several female flower clusters (downward arrows), each bearing ca. 10 flowers. (b) A flowering branch of dipteran-pollinated *Phyllanthus flexuosus*, showing few female flowers (downward arrows) and many male flowers (not indicated by arrows)

Plants specialized to *Epicephala* usually have excess female flowers relative to male flowers, whereas those pollinated by other insects have more male than female flowers. The pollen-to-ovule ratio is also much lower in figs that are pollinated by actively pollinating fig wasps than in those pollinated by passively pollinating fig wasps (Kjellberg et al. 2001; Jusselin et al. 2003), and a similarly low pollen-to-ovule ratio is suggested for yuccas (Pellmyr 2012). Although these comparisons are limited to active versus passive pollinators, active pollinators, at least, are probably remarkably effective at transferring pollen.

13.4 Evolutionary Stability of Obligate Pollination Mutualisms

Mutualisms may collapse if mutualists' costs and benefits are not balanced. As seen in Chapter 9, host sanction is one mechanism whereby hosts selectively allocate resources to the more cooperative partners, consequently reducing the fitness of selfish partners. In the case of obligate pollination mutualisms, sanction takes the form of selective abortion of flowers (or syconia, in the case of figs) with heavy egg loads. Since its first discovery in *Yucca filamentosa* (Pellmyr and Huth 1994), selective abortion has been confirmed in other yucca species (Richter and Weis 1995; Addicott and Bao 1999), in figs (Jandér and Herre 2010; Jandér et al. 2012; Wang et al. 2014), and in one *Glochidion* species (Goto et al. 2010). Host sanction has also been demonstrated in other mutualisms such as those involving plant–microbial relationships (Kiers et al. 2003, 2011), and this phenomenon provides a general explanation for the evolutionary persistence of mutualisms.

Host sanction contributes to the stability of the mutualisms in which it occurs, but it is not a universal mechanism that allows mutualisms to persist. For example, later studies in yuccas showed that selective abortion does not occur when yucca moths with short ovipositors infested the flowers, probably because yuccas have no means of detecting moth oviposition (Addicott and Bao 1999). Moreover, although host sanction can prevent the spread of an overexploiting phenotype, it does not necessarily guarantee the benefit to a host. In the case of the leafflower–moth mutualism, for example, plants do not produce any offspring, even with selective flower abscission, if moth populations increase to the point where their eggs routinely exceed numbers that cause total seed destruction. Consequently, factors that limit the costs of mutualisms, regardless of the presence of host sanctions, are also important (Segraves 2003, 2008; Althoff et al. 2005, 2013; Crabb and Pellmyr 2006; Dunn et al. 2008).

The cost of mutualism can be alleviated by the presence of a third-party partner, such as parasitoids or predators of the pollinators (Segraves 2008; Althoff et al. 2013). Dunn et al. (2008) demonstrated that in *Ficus rubiginosa*, parasitic fig wasps that oviposit from outside the syconium are more likely to attack pollinator larvae in ovules that are closer to the syconium wall. The resulting gradient in offspring viability likely poses selection on pollinating fig wasps to avoid outer ovules, thus limiting the overall intensity of seed destruction by pollinator larvae. Parasitoids also have a positive effect on seed production in the leafflower–moth mutualism; in *Breynia vitis-idaea*, the larvae of the pollinator attacked by braconid parasitoids consume fewer seeds than do those that develop normally (Kawakita and Kato 2004b). In contrast, the effect of parasitoids is obscure in the yucca–moth mutualism (Crabb and Pellmyr 2006). However, florivorous beetles sometimes consume moth eggs while feeding on style tissue, and this may have a positive effect on yucca seed production (Segraves 2008), although florivore effects on plants can vary from positive to negative depending on their annual abundance (Althoff et al. 2005, 2013). Overall, there is ample evidence showing that third-party partners

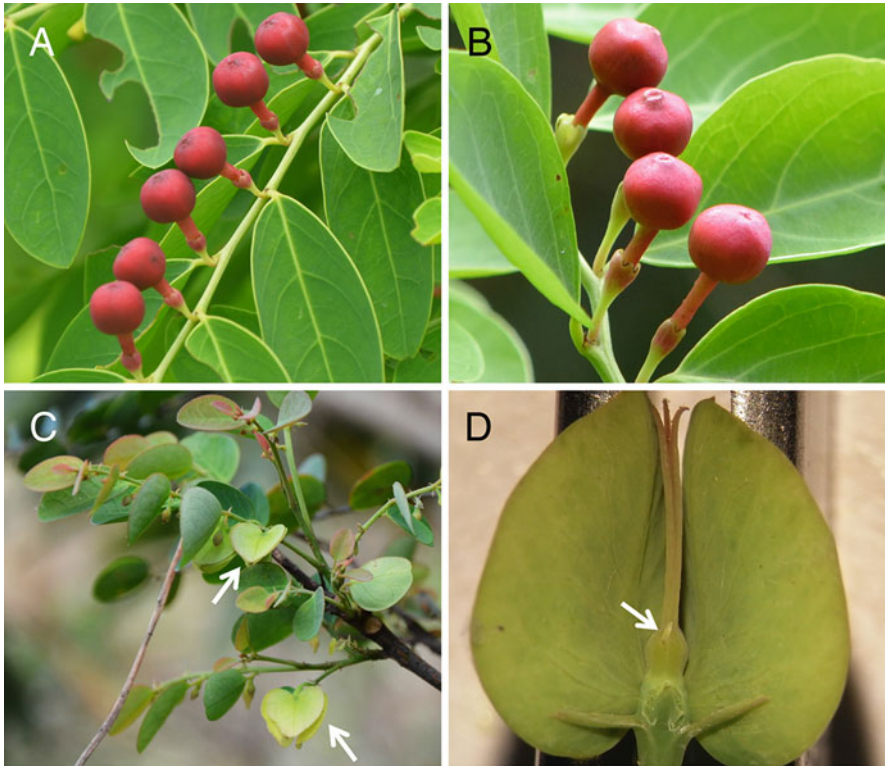


Fig. 13.4 Defensive plants traits in the Phyllanthaceae–*Epicephala* mutualism. (a, b) Elongated gynophores (stalk-like structures on fruits) of *Breynia vitis-idaea*. There is large within-population variation in gynophore length, and individuals with longer gynophores suffer less damage by seed-feeding *Epicephala* larvae. (c) Female flowers of New Caledonian *Phyllanthus nothsii* are covered with exaggerated tepals that create distance between the ovary and ovipositing *Epicephala* moth. Flowers indicated by arrows are female flowers, whereas those not indicated by arrows are male flowers. Note the size difference between female and male flowers. (d) An *Epicephala* egg laid on the ovary through the tepals. The associated *Epicephala* species has an elongate abdomen that likely coevolved with flower tepal size

contribute to limiting the costs of mutualism for plants in obligate pollination mutualisms.

Another mechanism that alleviates costs, but has thus far attracted little attention, is the evolution of traits that make host resources less exploitable (i.e., defense traits; Oliver et al. 2009). For example, in the *Silene latifolia*–*Hadena bicruris* interaction, long corolla tubes constrain moth oviposition to sites where eggs suffer high mortality without negatively affecting pollination efficiency, thus possibly preventing moth overexploitation (Labouche and Bernasconi 2013). Some individuals of *Breynia vitis-idaea* have remarkable gynophores in their fruits (Fig. 13.4a,b), whose function has been unknown. However, recent research shows that this is also a form of defense; fruits with longer gynophores suffer less

seed predation by moth larvae because hatched larvae must bore through the elongating gynophore to reach seeds, and often die in the process (Furukawa and Kawakita, unpublished data). Several *Phyllanthus* species in New Caledonia have well-developed tepals that create distance between the ovary and ovipositing moths (Fig. 13.4c,d), and associated *Epicephala* species have remarkably elongated abdomens that have likely coevolved with tepal size. Constricted ovaries in *Yucca baccata*, which reduces the number of surviving moth larvae by limiting access to ovules (Bao and Addicott 1998), are another clear example of defense. Defense traits are not always morphologically obvious; *Trollius* plants produce a C-glycosyl-flavone, adonivernith, in the carpel wall in response to infestation by *Chiastocheta* larvae, which reduces larval seed feeding ability (Ibanez et al. 2009). Note that host defense can be viewed alternatively as plant cheating (Bao and Addicott 1998), and thus itself is a source of mutualism instability; mutualisms will not persist if such a defense completely prevents the pollinator from gaining any benefit. Regardless, the prevalence of plant defenses across many mutualisms strongly indicates that the evolution of such traits is a major process by which plants limit the heavy costs imposed by seed-feeding pollinator larvae.

Interestingly, whereas the cost of mutualism for the plants (seeds) is substantially high in obligate pollination mutualisms, the cost incurred by the pollinators (pollination) is probably very small (Pellmyr 1997, 2012). As a consequence, there are no clear examples of pollinator adaptations that result directly from selection to reduce the cost of pollination. For example, two derived yucca moth species do not possess the pollinating behavior, but the loss of pollinating behavior is more likely a by-product of a phenological shift to an unexploited seed resource, after which pollination behavior became redundant (Pellmyr 2003). Loss of pollination behavior in *Epicephala* also did not happen through selection for cheating. One lineage colonized herbaceous *Phyllanthus*, which regularly attain full fruit sets through pollination by ants (Kawakita and Kato 2009), and others evolved gall-inducing ability and no longer rely on pollination (Kawakita et al. 2015). There are also two derived nonpollinating species among fig wasps, but the evolutionary forces that gave rise to these species remain unknown. It is possible that active pollination is costly in fig wasps (Jandér and Herre 2010); if so, the loss of pollination behavior may have evolved solely to eliminate the cost of pollination. Interestingly, the nonpollinating *Eupristina* sp. that coexists with the pollinator *Eupristina altissima* on *Ficus altissima* has smaller egg loads and suffers higher larval mortality than do pollinator species; the apparent rarity of derived nonpollinators among the fig wasps may thus be explained by a competitive disadvantage of nonpollinators relative to mutualists (Zhao et al. 2014). A notable pollinator adaptation is found in the *Bradysia* fungus gnat that pollinates *Rheum nobile*; this gnat appears to manipulate its host chemically so that flowers containing *Bradysia* eggs are more likely to be retained than those without eggs (Song et al. 2016).

Overall, the pollinators in obligate pollination mutualisms impose heavy costs on plants through seed destruction, and even develop strategies to exploit their hosts selfishly (as seen in the above examples of derived nonpollinators or host-manipulating *Bradysia*). Heavy costs and selfish partners are clearly threats to the

persistence of mutualisms, but multiple factors contribute to reducing their impact, such as host sanctions, the presence of third-party partners, and plant defense. It is probably useful to place obligate pollination mutualisms within a broader framework of plant–herbivore interactions, in which the roles of plant defense and trophic control are far better understood.

13.5 Evolution of Pollinator's Host Specificity

Obligate pollination mutualisms are classic examples of species-specific plant–insect associations. Although increasing evidence suggests that strict one-to-one specificity is not met in any of the mutualisms, it is striking that the majority of fig wasp, yucca moth, and leafflower moth species are monophagous. The question of whether host specificity is reinforced among pollinators is contentious. Ecological and phylogenetic studies in the yucca moth family Prodoxidae have found that close relatives of the pollinators are also highly host-specific herbivores (Pellmyr & Thompson 1992; Pellmyr 1999; Pellmyr et al. 2006), prompting the idea that high degrees of pollinator specificity are the fortuitous result of their inherently parasitic lifestyle (Thompson 1994, 2005). Conversely, the degree of host specificity among *Epicephala* is distinctly higher than that of related leaf-feeding gracillariids, pointing to the possibility that mutualism reinforces host specificity (Kawakita et al. 2010). In the fig system, nonpollinating fig wasps that are closely related to and co-occur with pollinating wasps tend to be less host-specific than the pollinators are (Weiblen & Bush 2002; Marussich & Machado 2007), although some studies report similar levels of host specificity between the two groups (Lopez-Vaamonde et al. 2001; Jusselin et al. 2006, 2008).

How and under what circumstances mutualisms reinforce host specificity is highly relevant to our general understanding of plant–insect interactions. Because shared pollinators can result in hybridization among closely related, co-occurring plants, there is a clear benefit for plants that attract specialist pollinators and thereby achieve conspecific pollination. Pollinators, on the other hand, have no constraint on their selection of host plants, as long as the plants provide suitable food for their larvae. Diet alone is unlikely to be the driver of extreme specialization in pollinating seed parasites because the two derived nonpollinating yucca moth species both utilize 4–6 yucca hosts (Pellmyr 2003; but see Moe and Weiblen 2012 for a notable example of fig wasp failing to develop on nonnatal fig host). An exciting possibility, therefore, is that plants are able to attract specific pollinator species selectively or, alternatively, repel pollinators that have high affinity to other host species. The floral odors of co-occurring *Glochidion* species are remarkably distinct, even to the human nose, which is striking, given that these plants are the product of a relatively recent radiation and are thus very closely related (<10 my; Kawakita and Kato 2009). A similar difference in floral scent is also found among co-occurring fig species. Because the fitness of the pollinators is heavily dependent on their ability to locate host plants, their chemical perception ability may be maximally tuned to the

odor of the host plants with which they are normally associated, perhaps at the expense of their ability to perceive the odor of other species. If so, these insects may not be able to detect the scents of more than one host species, provided that the host odors are sufficiently divergent. Whether the observed levels of interspecific difference in floral scent are the result of divergent selection requires further study. However, the presence of these differences is itself a strong indication that plants are selected to attract specific pollinators; otherwise, the scents of co-occurring *Glochidion* should converge to attract the whole local *Epicephala* community.

A study using *Breynia vitis-idaea* suggests that a mixture of two conventional volatile compounds is sufficient to attract its specialist pollinator, *Epicephala vitisidaea*, at the same rate as do real flowers (Svensson et al. 2010). However, many plants pollinated by *Epicephala*, including *B. vitis-idaea*, produce 20–30 volatile compounds whose functions are not yet fully understood (Okamoto et al. 2013). Studies aimed at clarifying the roles of seemingly redundant compounds should lead to improved understanding of plant–pollinator specificity in obligate pollination mutualisms. Plants and pollinators may also be coadapted phenologically and/or morphologically; thus, pollinators are more constrained in their selection of host plants than are their close relatives that feed on vegetative tissues.

Although the evolutionary processes leading to high plant–pollinator specificity remain unknown, the resulting specificity has important ecological consequences. In the southern islands of Japan, four *Glochidion* species regularly co-occur and flower at the same time during May–June. Although the species differ slightly in their microhabitat preferences, any pair of species can grow side by side (see Fig. 7.4 in Chapter 7), apparently without producing hybrids (note that hybrids do occur at extremely low frequencies). Because fertile hybrid seeds can be easily produced by artificial heterospecific pollination, pollinator isolation is likely the primary mechanism by which co-occurring plants maintain reproductive isolation. The importance of pollinator specificity in maintaining species integrity is well documented in figs (Moe and Weiblen 2012; Wang et al. 2016) and is also true for yuccas. In a lowland tropical rainforest of Malaysia, as many as nine *Glochidion* species co-occur without sharing pollinators, and dozens of *Ficus* species can be found in a single forest. Pollinator specialization thus allows multiple closely related plants to co-occur in a single area, and contributes to the maintenance of overall species richness of plants pollinated by seed parasites.

13.6 Mutualism and Reciprocal Diversification

Perhaps the most outstanding feature of the fig–wasp, yucca–moth, and leafflower–moth mutualisms is their high species richness (Table 13.1). High reciprocal dependence and specialization in these mutualisms have fueled predictions that plants and pollinators may undergo accelerated reciprocal diversification through cospeciation (Kiestler et al. 1984; Sanderson and Donoghue 1996; Schluter 2000). Specifically, because pollinating seed parasites are highly dependent on host

flowers for their own reproduction, changes in key floral traits, such as floral volatiles, are likely to be tracked by adaptation in the pollinators; similarly, changes in pollinator traits may also cause modifications in floral traits. Such a process may accelerate the divergence between populations if the plants and pollinators in each population evolve unique sets of coadapted traits (Thompson 1994, 2005). In addition, because pollinators are solely responsible for the movement of gametes among plants, reproductive isolation in plants can arise as a direct result of population differentiation in seed-parasitic pollination mutualisms.

Although the idea of coevolution-driven diversification has received theoretical support (Kiestler et al. 1984; Thompson 1994, 2005), empirical demonstration of the hypothesis has been difficult. The best documented example is that of *Yucca brevifolia* and *Y. jaegeriana*, which are pollinated by *Tegeticula synthetic* and *T. antithetica*, respectively (Pellmyr and Seraves 2003; Lenz 2007; Smith et al. 2008b, 2009; Godsoe et al. 2008, 2009). These two plant–pollinator pairs are distributed on different sides of the Mojave Desert in the Western United States, and members of each pair evolved coadapted morphological traits (stylar morphology and ovipositor length) that effectively maintain species specificity and reproductive isolation in a narrow contact zone in Southern Nevada. Although other studies suggest that coevolution may not have been important in shaping the current diversity of yuccas and yucca moths (Smith et al. 2008a; Althoff et al. 2012), the above example illustrates the power of coevolution in generating and maintaining new plant and pollinator species. Demonstration of cospeciation in figs is difficult because fig species typically have broad geographic ranges, making it necessary to sample across a vast geographic area to find any pattern. A notable example is the study of three fig–fig wasp pairs that diverged in situ on the oceanic Ogasawara Islands of Japan (Yokoyama 2003). The three fig species (*Ficus nishimurae*, *F. boninshimae*, and “Higashidaira type”) are distributed allopatrically on Chichijima Island, and each is associated with a genetically distinct fig wasp with an olfactory preference for the scent produced by their host figs. This suggests that chemical coadaptation, in addition to geographic isolation, may have promoted species divergence. These findings in yuccas and figs provide strong evidence that cospeciation driven by coevolution is a powerful driver of diversification in obligate pollination mutualisms.

Population-level analysis of cospeciation is still lacking in the leafhopper–moth mutualism, but observations indicate that cospeciation may also be common in this system. Many species of New Caledonian *Phyllanthus* are locally restricted and have very narrow distributions that may span less than 50 km. Pairs of allopatric, closely related species are usually adapted to different edaphic and/or climatic conditions, thus abiotic factors are the primary driver of species divergence. However, there are cases in which plants in such pairs are each associated with distinct moth species that are closely related to one another, indicating that cospeciation, whether or not accompanied by coevolution, is occurring (Fig. 13.5). There are many spectacular plant radiations in New Caledonia, but notably, *Phyllanthus* is the largest plant genus on the islands, with >110 species (Morat 1993). Although there are numerous explanations for variation in species

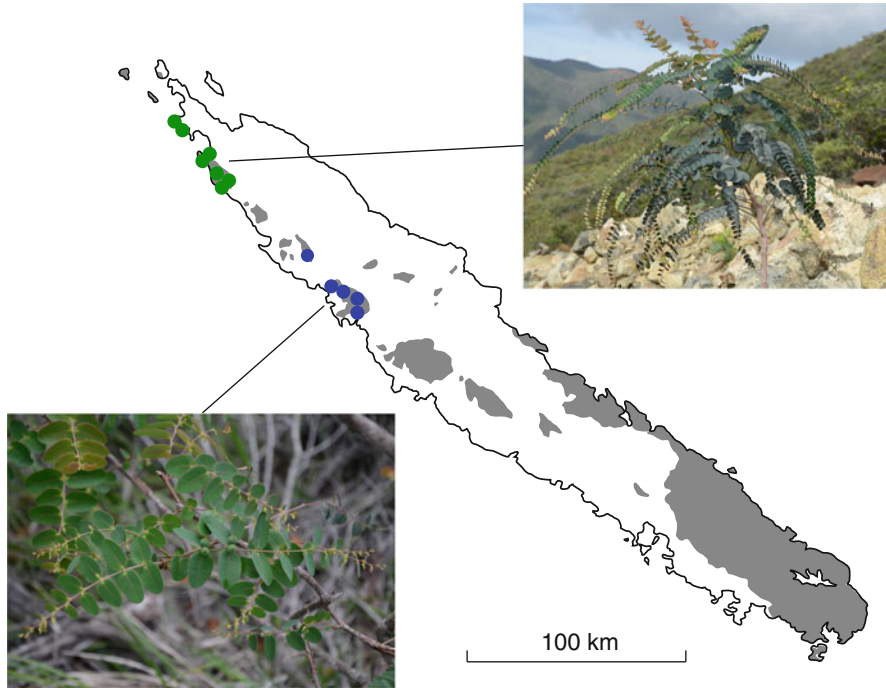


Fig. 13.5 Closely related, allopatric species pair of *Phyllanthus* on Grand Terre Island, New Caledonia. Blue and green dots indicate populations of *P. cf. koniamboensis* and *P. poumensis*, respectively. They are associated with species-specific *Epicephala* species that are also closely related

richness among plant clades, it is tempting to speculate that coevolution has driven the remarkable diversification of *Phyllanthus* and *Epicephala* in New Caledonia.

The above view of cospeciation-driven diversification, however, is not well supported by broad phylogenetic patterns. Tests of cospeciation in figs, yuccas, and leafflowers have all found clear cases of host switch and species specificity breakdown, and there are many cases in which plant and pollinator distribution ranges do not match. However, inferring the mode of speciation from current distributions or association data can be misleading because range expansions and subsequent contact with other species may obscure the pattern at the time of speciation. This may explain why some of the clearest examples of cospeciation come from recently diverged species. Estimates of divergence dates in *Yucca* and *Tegeticula* suggest that the derived *Tegeticula* clade currently associated with capsular- and fleshy-fruited yuccas diversified much later than did their host plants, which is interpreted as an indication that the plants diverged first, followed by displacement of the original pollinators (perhaps *Parategeticula*) as *Tegeticula* radiated on these yuccas (Althoff et al. 2012). Similarly, across Southeast Asia, *Phyllanthus reticulatus* is pollinated by >10 *Epicephala* species that are well diverged genetically

and distributed allopatrically. This suggests that, in this case, the plant *P. reticulatus* may have displaced other closely related plants that were once associated with these *Epicephala* species. In any case, the original process that generated species diversification may be cospeciation, with subsequent processes overriding the original pattern. Such a view is consistent with the results of phylogenetic studies showing that, although they are not strictly identical, there is a significant level of similarity between plant and pollinator phylogenies, suggesting that the association is characterized by background cospeciation.

Finally, intimate plant–pollinator associations may contribute to overall species richness, not only because they promote speciation but also because they allow recently diverged species to coexist stably in a given area. In many parts of the tropics, figs and leafflowers both flower throughout the year, and heterospecific crosses easily result in fertile hybrid seeds (e.g., Moe and Weiblen 2012). Thus, in the absence of species-specific pollinators, closely related fig or leafflower species would not coexist stably regardless of whether they have different habitat preferences. Assuming a situation in which all leafflower species have the same range size, overall species richness would be much higher when multiple leafflower species can co-occur in one area than when only one species can occur and all species are allopatrically distributed. Thus, specialized pollinators allow more species to be packed within an entire range of the clade, thereby contributing to species richness independent of the speciation process. This may explain, at least in part, why figs and leafflowers have attained diversity an order of magnitude greater than that in yuccas; up to 10 leafflower species or dozens of fig species can co-occur locally, whereas the number of yucca species that coexist in any location is usually only two.

13.7 Conclusions

Obligate pollination mutualisms are important model systems in the study of ecology and evolution. Over the years, significant findings have greatly enhanced our understanding of mutualisms and the coevolutionary process. Whereas we once considered obligate pollination mutualisms to be extremely rare associations resulting from evolutionary contingency, they now appear to be more widely occurring relationships that are logical outcomes of evolution. Rather than being stable associations locked by sanction mechanisms, they seem to be more evolutionarily flexible and prone to mutualism reversals. Moreover, we now view these mutualisms as dynamic associations mixed with host shift, partner displacement, and species-specificity breakdown, rather than strictly cospeciating associations. Undoubtedly, further study of obligate pollination mutualisms will bring major improvements to our understanding of ecology and evolution and further revisions to our views of these remarkable associations. The Phyllanthaceae–*Epicephala* association is distributed globally, and unexpected natural history findings are still being made every year. Obligate pollination mutualism in Phyllanthaceae thus holds promise as a prime model in the next generation of research on obligate pollination mutualisms.