Diversity of plants pollinated by fungus gnats and associated floral syndrome

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Summary

Dipteran insects receive much less attention as angiosperm pollinators compared to bees, moths, and birds. Although an increasing number of recent studies report plants that have evolved unique floral features to attract limited groups of dipteran insects, however, knowledge on the diversity and evolution of plants pollinated by flies is still limited. In particular, flies of the suborder Nematocera, which includes fungus gnats and gall midges, are sometimes reported as pollinators but generally overlooked because they are mostly small insects; hence, very little is known on how plants adapt to nematoceran pollinators.

This thesis aimed to clarify the diversity of plants pollinated by fungus gnats (Mycetophilidae and Sciaridae) and to gain knowledge on floral adaptations associated with evolution of fungus gnat pollination through analysis of floral color, morphology, and scent. In Chapter 2 following the general introduction, I focused on dark red floral display and short stamens, which are shared by several of the known fungus gnat-pollinated plants, and studied the pollinators of plants possessing similar floral characteristics. Consequently, I found that the following plants from five genera of five families in the Japanese flora to be predominantly pollinated by fungus gnats: *Aucuba japonica* (Garryaceae), three *Euonymus* species (Celastraceae), *Disanthus cercidifolius* (Hamamelidaceae), *Micranthes fusca* (Saxifragaceae), and *Streptopus streptopoides* (Liliaceae). This finding emphasizes the importance of fungus gnats as pollinators of angiosperms and suggests the possibility that a certain set of floral traits is linked to pollination by fungus gnats.

In the subsequent Chapter 3, I tested if the dark red floral display and floral morphology, such as the short stamens, which I focused on in Chapter 2, are convergent traits (floral syndrome) associated with evolution of fungus gnat pollination. For this purpose, I studied the genus *Euonymus* and found that the species with greenish white flowers are pollinated by bees, beetles, and non-fungus gnat flies. Phylogenetic analysis revealed that the dark red floral color alone was the trait acquired in association with the evolution of fungus gnat pollination, which has occurred at least twice in the genus *Euonymus*.

Based on these results, I performed a bioassay in Chapter 4 using the intraspecific flower color polymorphism (dark red or greenish white) of *A. japonica* to examine the difference in attractiveness of flower color to fungus gnat pollinators. Contrary to the expectation that dark red coloring is more attractive than greenish white, dark red coloring did not improve attraction of fungus gnats, suggesting that the dark red flower color has an alternative role than pollinator attraction. Analysis of floral scent revealed that acetoin, which has rarely been reported as a floral scent compound, was the dominant compound in *A. japonica* and two species of fungus

gnat-pollinated *Euonymus*. This suggests that a convergent evolution has occurred in floral scent in order to attract fungus gnats when fungus gnat pollination has evolved in the two genera.

This thesis underscores a largely overlooked importance of fungus gnats as pollinators by discovering fungus gnat pollination in five plant families in flora of Japan alone. Because many of the plants pollinated by fungus gnats grow in moist forest floor, fungus gnats are considered to play particularly important roles in these environments. The results also show that fungus gnat-pollinated plants have evolved several distinctive floral traits, such as dark red floral display and the floral scent compound acetoin, which has been considered rare in angiosperms. As this thesis demonstrates, by focusing on floral traits that have rarely been paid attention, future studies may reveal additional dipteran lineages that act as important pollinators of angiosperms and uncover the presence of hitherto overlooked floral syndromes associated with such pollinators.