Sexual differences in compositions and effects of flower microbes on a dioecious plant, *Mallotus japonicus* (Euphorbiaceae)

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Chapter 1.

Flowers play a central role in plant reproduction by dispersing and receiving pollen grains delivered by animal vectors or air. They are rich in various nutrients, and therefore provide an ideal habitat for many microbes. Recent studies have revealed that flower microbial communities can be highly variable among species, individuals, or even floral parts. However, sexual differences in flower microbial communities have rarely been investigated. In this study, I investigated flower microbes and their effects on the host plant in a dioecious plant, *Mallotus japonicus* (Euphorbiaceae), in its natural habitat in Japan.

Chapter 2.

While many studies have been conducted on particular pathogens infecting the flowers of cultivated plants, studies focusing on the diversity of microbes on flowers have started only recently. Progress of amplicon sequencing techniques has made it possible to examine the compositions of microbial populations without the need for culturing. In this chapter, I analyzed the flower prokaryotic communities of *M. japonicus* sampled in 2018 using 16S rRNA amplicon sequencing. I found a substantial differentiation in prokaryotic communities between male and female flowers. Procaryotes on male flowers included several dominant ASVs (amplicon sequence variants), mainly from the Gammaproteobacteria. These ASVs were also found on the body surface of flower visitor insects, suggesting that the visitors dispersed these microbes when they visited the flowers. On the other hand, female flower samples were overwhelmingly dominated by Alphaproteobacteria, which showed a peak of relative abundance at the middle of the flowering season. The bacterium had already been present at anthesis, and its relative abundance on flower visitors was low. Thus, flower visitors may have little effect on the microbial composition on female flowers. These differences may be associated with different reproductive strategies of male and female flowers.

Chapter 3.

Among all the different organs of a plant, flowers might have one of the most dynamic microbial communities, since many microbes are transmitted during flowering by insects and pollen while the flower is an ephemeral organ. Though previous studies have shown significant temporal changes in microbial communities during a flowering season, few studies have evaluated how stable flower microbial communities are across years. In this chapter, I analyzed microbial communities on *M.*

japonicus flowers sampled in 2019, and compared them with those in 2018 reported in Chapter 2. I found the compositions of bacterial communities surprisingly differed between the flowering seasons of 2018 and 2019. Only one of the 10 most abundant ASVs overlapped between the years. On the other hand, substantial differentiation in prokaryotic communities between the sexes, decrease of Shannon diversity index at the peak of flowering in female trees, and the dominant phyla on flowers of each sex were consistent between the years.

Chapter 4.

Among the microbes transmitted to flowers, pathogens may have highly negative effects on plants' fitness. In this chapter, I investigated whether a bacterial pathogen, *E. mallotivora*, occurs on flowers of the host plant *M. japonicus*, and whether the transmission of the pathogen to flowers can result in systemic infection and/or reduction of fruit production. The pathogen has been reported to infect through leaves, while its ecology on flowers is unknown. I first confirmed the presence of the pathogen on flowers, indicating possible transmission by visitors or pollen. Then, I showed that the bacteria can infect the plant through flowers by inoculating the pathogen to both male and female flowers. Interestingly, the symptoms on leaves appeared earlier on the female plants than on the males. Moreover, the inoculation significantly decreased the fruit set of the female plants. The results suggest a higher cost of infection in a female than in a male once the pathogen infected flowers. Although the effects of pathogen infection of flowers have rarely been investigated in wild plants, it would be an interesting topic for future study if such sexual differences in the infection cost can cause sexual conflict and intraspecific adaptation load.

Chapter 5.

In chapters 2–3, I reported contrasting microbial composition and different susceptibility to the pathogen between male and female flowers in *M. japonicus*. The differences may stem from sexually dimorphic colonization and filtering process related to contrasting floral characteristics and reproductive strategies between the sexes. Male flowers are far more frequently visited by diverse flower visitors than female flowers are, and thus male flowers may be more frequently colonized than female flowers are. Nevertheless, the much shorter lifespan of male flowers than that of female flowers may contribute to protecting the flowers from antagonist microbes. Such sex-specific interactions have been little explored in plant-flower microbe interactions. This study shows that dioecious plants provide unique opportunities to study roles of microbes in the evolution of floral traits that have mostly been overlooked in pollination ecology.