

(続紙 1)

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論文題目	Sexual differences in compositions and effects of flower microbes on a dioecious plant, <i>Mallotus japonicus</i> (Euphorbiaceae) (アカメガシワ(トウダイグサ科)における花圏微生物叢とその影響の性差)		
(論文内容の要旨)			
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, <i>Mallotus japonicus</i> (Euphorbiaceae), in its natural habitat in Japan.			
Chapter 2. While many studies have been conducted on particular pathogens infecting the flowers of the 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 <i>M. japonicus</i> sampled in 2018 using 16S rRNA amplicon sequencing. I found a substantial differentiation in prokaryotic communities between male and female flowers. Prokaryotes on male flowers included several dominant ASVs, 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. 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, little studies have evaluated how stable flower microbial communities are across years. In this chapter, I analyzed microbial communities on <i>M. Japonicus</i> flowers sampled in 2019, and compare 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, we investigated whether a bacterial pathogen *Erwinia 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. We first confirmed the presence of the pathogen on flowers, indicating possible transmission by visitors or pollen. Then, we 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. Besides, the inoculation significantly decreased 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 to flowers have rarely 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 the 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, thus they 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 indicates 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.

(続紙 2)

(論文審査の結果の要旨)

本研究では、これまで知見がなかった雌雄異株植物アカメガシワ (*Mallotus japonicus*、トウダイグサ科) の花上の微生物(細菌)について分析・実験を行った。2・3章では、アンプリコンシーケンシングを用いて網羅的な解析をおこなっている。とくに、先行研究とは異なり、雌雄異株植物に注目することで、花の雄機能・雌機能と細菌叢の関係を別々に評価しようとしたのは、ユニークな着眼点だといえる。

もっとも重要な結果の一つとして、細菌叢には大きな雌雄差が存在することを複数年の調査によって示したことが挙げられる。花粉や送粉者によって微生物に交流があることを考えると、大きな性差が維持されていることは、何らかの花形質が微生物フィルターとなっていることを示唆している。

一方、細菌叢の組成は年によって大きく変動した。葉や根と異なり、花は短い期間しか植物上で維持されないことが、大きな変動の要因の一つとなっている可能性がある。環境変動に加え、先住者効果などが働いた結果だと考えられ、植物上の微生物叢における送粉過程の役割という新しい課題に光を当てた。

4章では、病原細菌 *Erwinia mallotivora* を分離し、雄花、雌花に接種実験を行っている。葉から感染することが確認されていた本病原体は、花からも感染しうることを、雄と雌でその感染のスピードが異なることが示された。これらの結果は、相利共生的な視点から解釈されることの多い雄花と雌花、あるいは植物と花粉を運ぶ送粉者の関係について、微生物を伝播することで負の影響をもたらす対立する関係ともなりうることを意味していて、植物繁殖生態学に新しい視点をもたらした。

よって、本論文は博士(理学)の学位論文として価値あるものと認める。また、平成4年2月2日、論文内容とそれに関連した事項について試問を行った結果、合格と認めた。

要旨公表可能日： 年 月 日以降