（ 続紙 １ ）

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論文題目	Effects of flower characters on interactions with diverse flower visitors （花形質が多様な訪花者との相互作用に与える影響）		

（論文内容の要旨）

Chapter 1 General Introduction

Flower ecology has long been focusing on the interaction between the plant and a single pollinator species or group. Those studies have assumed that diverse flower characters have evolved in response to selective pressure from their specific pollinators. Only recently, intensive research on plant-pollinator networks, which describe the whole plant-flower visitor interaction of the community, critically pointed out that flower-pollinator interactions include a substantial part of generalist-generalist interactions. Furthermore, other studies have reported significant effects of antagonistic non-pollinators on plant reproduction. These findings indicate the importance of considering multiple biological interactions, including pollinators and non-pollinators, to fully understand the evolution of flowers. However, our knowledge of how flower characters affect interactions with non-pollinators is still limited. One reason is that, while knowledge for a few non-pollinators has recently been accumulated, we still know little about the interactions of flowers with diverse other organisms, such as flower microbes. Therefore, in Chapters 2 and 3, I will conduct a DNA metabarcoding of flower microbes to examine the pattern of flower-microbe interactions. The other reason is the scarcity of experimental studies on the function of flower characters in interactions with non-pollinators. Chapter 4 fills this gap by experimentally examining the effect of petal surface structure on plants' interaction with nectar-thieving ants.

Chapter 2 Comparison of flower microbes between plant species in a single plant community

Microbial communities on plants significantly affect plant phenotypes and fitness. Interactions with microbes on flower organs have critical importance since interactions on flower organs directly affect plant reproduction. However, interaction patterns of flower microbes have rarely been examined at a plant-community level. I examined the interaction pattern of flower bacteria with 29 plant species in a plant community by amplicon sequence of 16SrDNA. PERMANOVA showed that microbial community compositions are greatly different among plant species. In addition to significant contributions of habitat, flowering phenology, and plant life form on microbial communities, still the effect of plant species is large, suggesting the strong effect of other flower characters. Furthermore, the specificity of bacterial ASVs for plant species was examined based on specificity index d'. There is a large variation in plant specificity among bacteria, part of which is explained by the bacterial family. Based on d', generalist and specialist ASVs were selected as candidates for future experimental study to examine the mechanisms for the observed specificity. These results suggest that the flower-bacterial interaction is affected by species-specific variation in flower characters.

Chapter 3 Temporal variation in flower microbes within a flower season of *Solidago altissima*

Since flowering is a highly ephemeral event, the timing of flowering substantially affects the compositions of the visitors. Understanding how diverse biological interactions around flowers are affected by flowering phenology is essential to predict the effects of climate changes on the interaction of the plant with diverse flower visitors. I report that flower microbes showed dramatic seasonal changes both qualitatively and quantitatively, during the two months of the flower season of *Solidago altissima* (Asteraceae). Amplicon sequence and quantitative PCR of bacterial 16SrDNA demonstrated that flower bacterial communities of *S. altissima* changed within the two months of the flowering season. The temporal variations were observed in various aspects of microbial communities, such as composition, alpha-diversity, and total bacterial loads. These temporal changes were correlated with the replacement of the dominant bacterial class from Gammaproteobacteria to Alphaproteobacteria, suggesting that the decrease in Gammaproteobacteria mainly characterized the temporal change. The seasonal comparison of Gammaproteobacteria ASVs suggested the substantial contribution of seasonally different dispersals on the seasonal variation of flower bacterial communities. These results reinforce the notion that flowers are an ephemeral habitat where microbial communities have strong dispersal limitations.

Chapter 4 Slippery flowers as a mechanism of defense against nectar-thieving ants

The great diversity of floral characteristics among animal-pollinated plants is commonly understood to be the result of coevolutionary interactions between plants and pollinators. Floral antagonists, such as nectar thieves, also have the potential to exert an influence upon the selection of floral characteristics, but adaptation against floral antagonists has attracted comparatively little attention. I found that the corollas of *Codonopsis lanceolata* (Campanulaceae) and the tepals of *Fritillaria koidzumiana* (Liliaceae) are slippery to nectar-thieving ants living in the plant's habitat; because the flowers of both species have exposed nectaries, slippery perianths may function as a defense against nectar-thieving ants. I conducted a behavioral experiment and observed perianth surface microstructure by scanning electron microscopy to investigate the mechanism of slipperiness. Field experiments were conducted to test whether slippery perianths prevent floral entry by ants, and whether ant presence inside flowers affects pollination. Scanning electron microscopy observations indicated that the slippery surfaces were coated with epicuticular wax crystals. The perianths lost their slipperiness when wiped with hexane. Artificial bridging of the slippery surfaces using non-slippery materials allowed ants to enter flowers more frequently. Experimental introduction of live ants to the *Codonopsis* flowers evicted hornet pollinators and shortened the duration of pollinator visits. However, no statistically significant differences were found between the fruit or seed sets of flowers with and without ants. Slippery perianths, most probably based on epicuticular wax crystals, prevent floral entry by ants that negatively affect pollinator behavior. Experimental evidence of floral defense based on slippery surfaces is rare, but such a mode of defense may be widespread amongst flowering plants.

Chapter 5 General discussion

Flowers interact with far more diverse flower visitors than pollinators, which may pose a selective pressure on the plant characters and, in turn, produce the enormous diversity of flowers. Therefore, it is essential to look at the interactions with non-pollinators and how flower characters control these interactions. One approach is to identify flower characters that strongly control the interaction of flowers with each flower visitor. In Chapters 2 and 3, DNA metabarcoding of flower microbes revealed the significant contribution of flower characters, in particular flower phenology, in shaping flower-microbe interactions. Further filtering of bacteria by specificity index or hierarchical clustering extracted potential candidates for future experiments examining the mechanisms of the observed interaction patterns. Combined with recent developments in DNA sequencing and image analysis, which foster the accumulation of observations on what organisms visit what flowers, including pollinators, non-pollinating animals, and microorganisms, these approaches may elucidate unexpected relationships between flower characters and interactions. Furthermore, these observed associations should be confirmed experimentally. In Chapter 4, I conducted multiple experiments confirming the field observation that the perianth's surface of *Codonopsis lanceolata* and *Fritillaria koidzumiana* affects the behaviors of nectar-thieving ants. Combining the comprehensive observation of interactions and the experimental approach may lead to a more comprehensive picture of how flower characters affect biological interactions around flowers.

（続紙　２　）

（論文審査の結果の要旨）

　本花形質は、これまで主に花粉を運ぶ送粉者との関係において説明されてきた。しかし、本研究では盗蜜者や微生物など花に訪れる多様な生物を含め解釈する必要があると問題提起をしている。

　２章では、花形質がどの程度花上の細菌叢を左右しているか検討するため、細菌叢を網羅的に明らかにした。その上で群集間の細菌組成の距離行列に基づいた解析により、細菌叢が種間で異なっていること、開花時期やハビタットだけでは説明できない違いがあることを統計的に示した。さらに細菌種（論文の中ではASV（Amplicon Sequence Variants））の間で植物種に対する特異性に大きなばらつきがあることを、植物−送粉者相互作用で使われてきた指標を導入することで明らかにしている。単純な種間、個体間比較にとどまっていた植物と花上微生物の相互作用関係の理解を大きく進め、高く評価できる。

　３章では、２章で開花時期が細菌群集を説明する要因となっていたのを受けて、開花時期が種内でどれくらいの細菌群集の組成を説明しうるのかについて検討を行った。花上の微生物群集の研究ではあまり行われてこなかった細菌の定量を、細菌特異的なプライマーと定量PCRを使って行い、細菌の量が季節を通して1,000倍以上変動しうることを示した。またこの量の変動が質（組成）の変動と連動しておこっていることを示した。種内の時間的変異についての知見がほとんどなかったこと、また定量を試みた点で、独創性の高い研究であるといえる。

　４章では、花形質と相互作用の関連を明確にするために、野外操作実験を行った。材料としたのは、花に盗蜜に訪れるアリである。アリの盗蜜については、現象そのものは古くから知られていたものの、実証的に花形質の盗蜜への影響を検討した研究は数少ない。本研究では花表面のワックスに着目し、これを取り除くことでアリの盗蜜が増えることを明確に示した。

以上の成果は、送粉者以外の訪花者に広く目を向け、相互作用の検出からその影響の評価までの展望を示し、歴史の長い送粉生態学の新しい道筋を示したといえる。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