学位論文内容の要旨

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学位論文題名

Evolutionary ecology in gall-forming aphids: extreme polyphenism and biased sex ratios
（虫こぶ形成アブラムシの進化生態学：極端な表現型可塑性と偏った性比）

Aphids of the subfamily Eriosomatinae are characterized by complex life cycle, gall formation, host alternation, and seasonal polyphenism. The eriosomatine aphids induce galls on the leaves of *Ulmus* trees in spring, and migrate to the roots of secondary host plants, such as grasses and shrubs, where they reproduce asexually underground. Winged aphids emerge in autumn and return to *Ulmus* trees to reproduce sexual-generation offspring. The aphids in gall generation, the secondary-host generation, and the sexual generation are morphologically and physiologically distinct, even among aphids in that a variety of phenotypes (morphs) emerge within a clonal lineage. Galls induced by eriosomatine aphids are species-specific in order to meet different biological characters and this mechanism remains largely unknown. Moreover, as in other aphids, the aphids and their obligate intracellular endosymbiont *Buchnera aphidicola* maintain an extremely close symbiosis, for example, *Buchnera* can provide aphids with essential amino acids that are usually limited in phloem sap. In my research, I study eriosomatine aphids with conspicuous polyphenism and galling behavior, to elucidate the specificity of this group from various perspectives in genetics, physiology, and evolutionary ecology.

In Chapter 1, I investigated the roles of aphid endosymbionts in the three-way symbioses among plants, aphids, and aphid endosymbionts in galling system by applying high-quality genome sequencing on *Buchnera* from three gall-forming aphids *Tetraneura nigriabdominalis*, *Tetraneura sorini*, and *Eriosoma harunire*, which share same host plant *Ulmus davidiana* var. *japonica* to form galls on. Here, I generated genome assembly using a combination of Nanopore long reads and Illumina shotgun sequencing, and *Buchnera* genome sizes of *T. sorini*, *T. nigriabdominalis* and *E. harunire* were obtained as 533,871 bp, 530,863 bp and 627,315 bp, respectively, and the numbers of the protein-coding regions were 485, 474 and 489, respectively. *Buchnera* is found to be one single circle chromosome without any plasmids in three eriosomatine aphids. Genome annotation analysis also revealed that *leuABCD* genes that normally locate on plasmids of *Buchnera* (e.g. in *Acyrthosiphon pisum*) are found on the chromosomes. In addition, the *Buchnera* of *E. harunire* lacks the genes for biosynthesis of ornithine, which is an important precursor for the biosynthesis of the essential amino acid arginine. *E. harunire* may consume ornithine/arginine from the galls, or from other pathways (e.g. to synthesize ornithine by aphids) in which there is close tripartite symbiosis in galling system on nutritional compensation.

In Chapter 2, I explored the relationship between seasonal polyphenism and cuticular hydrocarbon profiles in the eriosomatine aphid, *Prociphilus oriens*. This species undergoes host alternation and exhibits five major morphs. These morphs are related to the differences between asexual and sexual generations, the difference in host plants, and the presence or absence of symbiotic ants. In *P. oriens*, linear alkanes constituted the major proportion of cuticular hydrocarbons, but there were differences in their composition among morphs. In the morphs attended by ants, the ratio of *n*-C25 was higher than that of *n*-C27. On the other hand, the ratios of longer-chain alkanes, *n*-C27 and *n*-C29, was higher in the sexual generation without ant attendance. In this species, sexual females do not have organs that secrete sex pheromones to attract males. Thus, it is likely that the hydrocarbon components of the female body surface play a major role in mate recognition by males. The proportion of branched alkanes in the long and flocculent waxy substances of the winged autumn morphs was higher than in other morphs, indicating that this component may be used for recognition of the same species. These results indicate that the profiles and function of cuticular hydrocarbons differ among morphs, and that the cuticular hydrocarbons of sexual females in particular have differed significantly from those of asexually reproducing females and males for mate recognition as sexual attractants.

In Chapter 3, parasites of the Eriosomatinae were reported, and mermithid nematodes (Nematoda: Mermithidae) were found in the abdomens of aphids sucking on the secondary hosts underground. It is the first time found in Eriosomatinae that the root-generation aphids are parasitized by nematodes. I extracted genome DNA from the isolated nematode from *E. harunire*, sequenced the 18S rDNA and 28S rDNA regions, and performed phylogenetic analysis. The phylogeny suggested that the nematode belongs to the Mermithidae and created a new genus in Mermithidae. The parasitism by the nematodes negatively affected the aphid performance, resulting in the autumnal winged females producing a small number of small sized sexual females. Based on these results, using nematodes for the control of subterranean aphids is to be promising.

In Chapter 4, I tested a hypothesis that sex investment ratio will always be biased toward females in fatal-fighting aphids with cyclic parthenogenesis. In general organisms, the investment in males and females is stable at 1:1, but in animals with cyclic parthenogenesis (e.g., rotifers, daphnia, aphids), it has been reported that the sexual investment ratio is often biased toward females. Using gall-forming aphids *T. sorini*, I tested the Trivers-Willard hypothesis and proposed a novel hypothesis. The first instar nymphs hatching from overwintering eggs are all females, and fight fiercely for possession of the gall. The larger nymphs had an overwhelming advantage in the fight. In contrast, males rarely fought. Under these conditions, mothers were expected to have advantages if they produced more females of larger size with a sex investment ratio biased toward females. This is because larger daughters would produce larger granddaughters to be born in the spring. In *T. sorini*, mothers invested to individual females 3.0-3.2 times larger than to individual males, and the ratio of sexual investment in females was 68%-72% at the population level. I confirmed that mothers with larger body size tended to produce more large females to have more fitness return.

In my study, I am trying to elucidate the evolutionary ecology in gall-forming aphids especially how a gall-forming aphid is involved in various symbioses interacting with other individuals or organisms, and surrounding environment by performing genomic, physiological, and ecological analyses. These results not only point out important mechanisms in evolutionary biology but also provide novel approaches for potential pest control.