7.5　学位論文要旨（別紙様式博 5）

学位論文要旨

学位授与申請者

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題目：Evolutionary origin and molecular bases of the gall-inducing life-style in the Gracillariidae

（ホソガ科におけるゴール形成能の進化的起源と遺伝基盤）

Scientific context

Galls induced by insects represent an advanced form of manipulation of plant development and physiology. The ability to induce galls is encountered in most orders of herbivorous insects, and is found in some of the worst agronomic pests. However, the molecular mechanisms involved in the gall-induction remain poorly known, as well as the evolutionary processes leading to the gall-inducing life-style are still discussed. This scientific context is summarized in the **chapter 1**.

Chapter 1 is a general introduction of this thesis and summarized objectives of my PhD project and study systems as well as the scientific context. The overall aim was to investigate the evolution (**Objective 1**) and molecular bases (**Objective 2**) of gall induction in insects. To achieve this, this thesis focused on Gracillariidae, an important family of insects in the order Lepidoptera that exhibits a great variety of feeding strategies. More specifically, I focused on two Japanese species, *Borboryctis euryae* Kumata et Kuroko and *Calopitlia cecidophora* Kumata, that were selected for their interesting larval feeding behaviors.

Objective 1: Inferring the evolution of gall induction in insects. This objective aims at determining where and when did the gall-inducing habit evolved in a group of insects largely dominated by leaf-miners and leaf-rollers.

Objective 2. Identifying mechanisms of gall induction in insects. This objective aims at identifying key histological alterations in plant tissues and secreted salivary effectors of insects in biological models with contrasting, and sometimes transitional feeding strategies.

I used two micro-moth **study systems**, and the first one is ***Borboryctis euryae*** that grows on two evergreen plants of the family Pentaphylacaceae, *Eurya emarginata* Makino and *E. japonica* Thunberg. *Borboryctis euryae* belongs to the subfamily Acrocercopinae and, in several aspects, its larval feeding behavior corresponds to the standard type in this subfamily. First and second instars make a sub-epidermal serpentine mine and the third instar creates a blotch mine. Then the larval hypermetamorphosis, which leads to a transition from fluid-feeding to tissue-feeding mouthparts, occurs between third and fourth instars. The larvae spend their fourth and fifth instars in this blotch mine, feeding on parenchyma tissues. *Borboryctis euryae* is distinguished from other Acrocercopinae at the fourth instar where the blotch mine is inflated. When opened, the larval chamber of the fourth instar shows traces of callus proliferation that some authors consider as a gall.

The second system is ***Caloptilia cecidophora*** Kumata, and this species is associated with *Glochidion acuminatum* Müller Argoviensis, *G. obovatum* Siebold & Zucc. and *G. rubrum* Blume (Phyllanthaceae). The life-style of *C. cecidophora* largely differs from other species of the genus. In the great majority of *Caloptilia*, the first instar larva forms a serpentine mine, the second instar forms a blotch mine, the third instar forms a tentiform mine, and remaining larval instars leave the mine and form a leaf-roll and pupate within the leaf-roll or outside. On the contrary, *C. cecidophora* induces the formation of a gall on the mined leaf, along the vein, that looks similar to some sawflies leaf-galls. All the larval development takes place inside the gall. The gall initiation process was unclear and it has been suggested that the larva firstly make a leaf-mine before inducing the gall.

These two species have in common that they potentially represent a transition from leaf-mining to gall-inducing during larval development. This characteristic is rare, if not unique, among gall-inducing insects that generally start inducing their gall from the beginning of their interaction with their host plants. If confirmed, these mixed feeding behaviors would be suitable to address questions about the evolutionary pathway of gall induction and to develop comparative approaches to decipher molecular mechanisms of gall induction.

Chapter 2 investigates the histological nature of the swelling leaf-mine induced by *B. euryae*, the nutritive value of the neoformed tissue for the larvae and the role of the insect for the induction of this abnormal tissue development. For this purpose, I combined experiments on field and laboratory-reared insects. The study on *B. euryae* reopened the old debate about the significance of callus formation in leaf-mines. Situated in the "no-man's land between leaf-mine and galls", the proliferation of undifferentiated cells in insect galleries on leaves was often considered as the result of physical conditions in the mine rather than the consequences of an active manipulation of plant development.

Chapter 3 shows a histological study of early steps of *C. cecidophora* gall induction in order to test whether early instars are able to induce gall symptoms or not. For this purpose, I reared insects on potted plants in a greenhouse and described the early steps of larval development. Using histological techniques, I investigated the morphogenetic processes leading to gall formation. I compared this life-style to that of a closely related non-gall-inducing species, *C. ryukyuensis*, associating with *G. lanceolatum* Hayata and *G. zeylanicum* (Gaertner). With a comparison of the larval development of the two species, paralleling *C. cecidophora* and *C. ryukyuensis* provided arguments to discuss the adaptive nature of galls.

Chapter 4 presents a study that aimed to explore more deeply the evolutionary process that led *Caloptilia* species to induce a leaf-gall. Using field-collected specimens I reconstructed a phylogeny of Phyllanthaceae-associated *Caloptilia*. Thanks to field sampling and collaborations, I obtained several undescribed gall-inducing species as well as other undescribed leaf-rolling *Caloptilia* associated with Phyllanthaceae. The descriptions of gall morphologies and the inferred phylogenies allowed to explore the origin of the gall-inducers as well as phylogenetic constraints on gall evolution. Phylogenetic reconstructions using ultraconserved elements (UCEs) showed that gall-inducers form a well-supported monophyletic group. The UCEs phylogeny strongly supported the monophyly of Phyllanthaceae- and *Glochidion*-associated taxa. The gall-inducer clade was placed at the most derived position of the *Glochidion*-associated clade, demonstrating that gall-induction had a single origin and most likely evolved from non-galling species on *Glochidion*. This would suggest that gall induction could be a key innovation which would have opened up a new ecological niche for *Glochidion*-associated *Caloptilia*.

Chapter 5 presents a study that aimed to identify genes possibly involved in gall induction in *C. cecidophora*. I sequenced the transcriptomes of each larval instar and applied a double comparative approach. First, I compared gene expression of leaf-mining with gall-inducing instars in order to select genes that are upregulated at the occurrence of gall induction. I also compared genes that are over-expressed in the gall-inducing *C. cecidophora* with genes expressed in the non-gall-inducing sister species *C. ryukyuensis*. Three independent comparative approaches were used to analyze the expression patterns and these screening processes provided a list of 58 genes coding possible effectors of gall formation. These candidates include genes related to the production of anti-oxidant enzymes and chitinases, and the results suggest the implication of secreted auxin for the gall formation.

The **general discussion (Chapter 6)** presents the contribution of this thesis to the overall understanding of interactions between plant-manipulating insects and their host plants. It summarizes our main findings and addresses the question of the existence of a continuum of plant manipulators and the consequences and perspectives that are provided by this conceptual framework.