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学位論文内容の要旨

博士の専攻分野名称：博士（農学）　　氏名：**LIRETTE Antoine Olivier**

学位論文題名

Elucidation of a unique vertical symbiont transmission system and mechanisms of host behavior modulation in stinkbug-*Caballeronia* symbiosis

（カメムシ-*Caballeronia* 腸内共生系における垂直伝播と宿主の行動変容の解明）

Many insects form close mutualistic interactions with microorganisms which they harbour in specialized organs. Most insect symbionts are transmitted vertically, from mother to offsprings, through diverse mechanisms. By contrast, Stinkbug of superfamilies Coreoidea and Lygaeoidea, acquire *Caballeronia* symbionts from environmental soil every generation, which they host within the crypts of their posterior midgut (i.e. horizontal transmission or environmental acquisition). In this study, two topics of the stinkbug-*Caballeronia* symbiosis were investigated. Firstly, I discovered a stinkbug-*Caballeronia* symbiosis with vertical transmission in the previously unexamined stinkbug family, Heterogastridae. In this family, females cover their egg masses with a bubble-like substrate containing the symbiont, which the hatchlings consume to acquire *Caballeronia*. Secondly, in addition to the finding of a novel symbiotic system, I revealed that colonization by their *Caballeronia* symbiont leads to host behavioral alteration in the *Riptortus pedestris*-*Caballeronia* symbiotic model system. Behavioral analysis using machine learning model with manual annotation demonstrated how gut symbiont modulates host behavior. Furthermore, transcriptomics of the nervous system revealed molecular mechanisms involved in symbiont-mediated behavior alternations.

(i). A vertically acquired *Caballeronia* symbiont in the stinkbug family Heterogastridae

To investigate the symbiotic system of family Heterogastridae of superfamily Lygaeoidea, I used two species of stinkbugs; *Heterogaster urticae* and *Sadoletus valdezi*, collected in Japan. Host phylogeny based on the mitochondrial cytochrome C oxidase subunit 1 (COI) gene confirmed the basal position of Heterogastridae in Lygaeoidea. Symbiont identity was determined through 16S ribosomal RNA (rRNA) gene sequencing of the gut symbiotic organ, confirming *Caballeronia* as the symbiotic partner of Heterogastridae stinkbugs. Microscopic observations of the gut symbiotic organ revealed that the symbiont was hosted intracellularly in *H. urticae* in the 4th region of the midgut. Furthermore, rearing experiments determined that *H. urticae* nymphs acquire the symbiont vertically by feeding on their egg mass upon hatching which is a very unique mechanism in stinkbug-*Caballeronia* symbiosis, in which vertical transmission is not known to occur. The

discovery of this unique symbiotic system in family Heterogastridae may provide clues as to how symbiotic intimacy may evolve.

(ii)-a. Behavioral alteration by *Caballeronia* in the bean bug *Riptortus pedestris*

Previous studies reported behavioral differences between non-symbiotic/aposymbiotic (APO) and symbiotic (SYM) adults of the bean bug *Riptortus pedestris*, however the impact on nymph behavior remains unknown. Therefore, I performed behavioral analyses on 4th instar nymphs over a 26-hour recording period. After 6 hours of starvation, behavior was recorded for 2 hours without food source (before feeding), followed by 24 hours with an available food source. Behavior assessment was conducted using DeepLabCut software, which applies neural network-based pose estimation to track tested nymphs. Behavioral analysis revealed that SYM nymphs spend most of their time on stationary behavior, whereas APO individuals spend longer time walking and showed increased behavioral transitions compared with SYM individuals. Manual annotation of stationary behavior comprising water intake, feeding, and resting further showed that SYM insects exhibited reduced feeding attempts, but prolonged feeding duration compared to APO individuals. Based on these findings, I revealed that symbiosis impact host *R. pedestris* to spend more time feeding, while APO individuals display frequent walking with intermittent feeding behavior.

(ii)-b. Modulation of nervous system gene expression by *Caballeronia* in the bean bug *Riptortus pedestris*

In animals, behavior is controlled by the coordinated actions of neuronal cells within the nervous system. To determine the metabolic process controlling the observed behavioral differences between SYM and APO nymphs, I performed transcriptomics analysis of their nervous systems. The nymphs' brains and ventral nerve cords were separated into 4 regions, the optic lobes, brain, first thoracic ganglion (G1), and second thoracic ganglion (G2), from which RNA was extracted. Transcriptomics analysis revealed thousands of differentially expressed genes (DEGs) across the *R. pedestris* nervous system upon *Caballeronia* infection. From Gene Ontology (GO) term enrichment analysis, enriched biological processes included amide biosynthetic processes in the optic lobes, regulation of hormone levels in the brain, amide biosynthetic and metabolic processes a in the G1, and regulation of signal transduction in the G2. Furthermore, many DEGs were involved in regulatory pathways critical for the control of locomotion, feeding, and the processing of information that leads decision making in locomotion and feeding. Indeed, genes involved in neurotransmitter signaling pathways such as dopamine receptor 2-like and tyrosine hydroxylase were upregulated in SYM G1. Meanwhile, downregulation of the γ-aminobutyric acid (GABA) pathway in the SYM G1, and Serotonin receptors 2A and 2B in the SYM brain was observed.

Overall, my results highlight how how symbiosis with horizontal symbiont transmission could evolve into symbiosis with vertical transmission and demonstrate how the acquisition of *Caballeronia* alters the feeding and walking behavior as well as the expression of gene pathways involved in behavioral regulations such as biogenic amines in *R. pedestris* nymphs.