

Science and Technology Group Annual Report FY2020

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1 Introduction

My research focuses on parasitic and mycoheterotrophic plants, plants that acquire at least part of their essential resources from their hosts, either other plant species or fungi, respectively. I study their diversity, ecophysiology, and genomics. I combine traditional ecophysiological methods with genomics to study how is the ecophysiology of parasitic and mycoheterotrophic plants related to their genomes. I mainly focus on Japanese, including Okinawan, species of various trophic strategies from partially (green) to fully heterotrophic (non-green) species.

2 Activities and Findings

Project 1: Diversity of parasitic and mycoheterotrophic plants

I visited multiple sites around Okinawa for which I had received coordinates from my collaborator, Dr. Kenji Suetsugu, and verified the presence of focal species there. In addition to that, I have found additional sites with the presence of focal species myself. I took photos of all species found (Fig 1). Unfortunately, I was not able to sample most of these plants or carry out any physiological fieldwork as the plants grow in protected areas (mainly the Yambaru National Park) where research permits are needed. Therefore, I applied for appropriate permits with the help of Okinawa Environmental Research Support Section (OERSS), but the application is still in review.



Fig 1: Selected parasitic and mycoheterotrophic plants from Okinawa. From top left to right: *Korthalsella japonica*, *Sciaphila yakushimensis* var. *okinawensis*, *Balanophora tobiracola*, *B. fungosa*, *Cuscuta* sp., *Mitrostemon yamamotoi*, *Scurrula yadoriki*, and *Cassytha filiformis*.

Project 2: Ecophysiology of parasitic and mycoheterotrophic plants

This spring, I plan to start with ecophysiological field measurements and sampling. First project (2a) will focus on the effects of parasitism by *Cassytha filiformis* on its shrub host. I have already found 6 beach sites suitable for this project along the west coast of Okinawa. Second project (2b) will focus on the ecophysiology of *Korthalsella japonica*, a mistletoe species from the Yambaru National Park. I have already obtained a permit for this research. Besides finding suitable sites for conducting these fieldwork projects, I have already purchased and prepared all equipment needed.

Project 3: Genomics of parasitic and mycoheterotrophic plants

I extracted high molecular weight DNA from 4 plant samples received from my collaborators using anion exchange columns (NucleoBond HMW kit; Takara). PCR free DNA libraries were prepared with the NEBNext Ultra II kit for Illumina (NEB) and sequenced on the NovaSeq6000 sequencer at OIST. After receiving the sequencing data, I assembled and annotated the plastid genomes of these species.

The plastid genome of *Burmannia cryptopetala* (Fig 2) is not much reduced in size compared to non-parasitic plants but shows signs of an ongoing genome shrinkage. We have detected 70 putatively functional protein coding genes, 6 rRNA genes, and 28 tRNAs. At least ten of all the protein coding genes are fragments of original genes (and likely pseudogenes).

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The plastid genomes of *Balanophora subcupularis* and *B. yakushimensis* are highly reduced in size and with other two known *Balanophora* plastomes (Su et al. 2019, PNAS), they have the most AT-rich genomes to date (> 87%; Fig 2). We have found 18 and 16 putatively functional genes in the plastid genomes, including 2 rRNA genes, 13 and 11 genes coding ribosomal proteins, and 3 protein-coding genes of varying or unknown function (*ycf1*, *accD*, *clpP*). Our *Balanophora* species also appear to utilize the same novel genetic code reported for their two relatives, i.e. the code with the TAG reassignment from stop codon to tryptophan (Su et al. 2019, PNAS).

The plastid genome of *Mitrastemon yamamotoi* is also highly reduced in size and gene content (26 genes), but not that extremely as the *Balanophora* species (Fig 2). Its genome is very similar to that described for its relative *M. kanehirai* from Taiwan (NCBI: MF372930; unpublished).

In addition to already assembled plastid genomes, I have started with the analyses of mitochondrial and nuclear genomes of above-mentioned species. Currently, I also continue with high molecular weight DNA isolation from other plant samples provided by colleagues. I plan to assemble 6 more plastid genomes of focal plants in the first half of this year. After receiving appropriate research permits for sampling (should be soon), I will proceed with the genomics on Okinawan parasites and mycoheterotrophic plants.

Taking into account that the genomes of heterotrophic plants might be huge in size (10-100Gbp), I estimated the genome size of selected species using flow cytometry before starting with their sequencing. In collaboration with Dr. Petr Koutecky and Instrumental Analysis Section at OIST, I conducted flow cytometric measurements in two parasitic species, *Aeginetia indica* and *Korthalsella japonica*. The estimates of their genomes are 8 and 43Gbp, respectively. Therefore, I decided to focus only on their plastid genomes as their whole genomes will be very hard to sequence and analyze.

3 Collaborations

Filip Husnik, OIST, Okinawa, Japan

Kenji Suetsugu, Kobe University, Kobe, Japan

Shota Sakaguchi, Kyoto University, Kyoto, Japan

Petr Koutecky, University of South Bohemia, Ceske Budejovice, Czech Republic

4 Publications and other output

- OIST-RIKEN Joint Symposium, Series 1. OIST, April 6-7 2021, poster presentation: On the way to heterotrophy: Ecophysiology and genomics of Okinawan parasitic and mycoheterotrophic plants. My poster was selected for an additional workshop discussion on April 7.
- I also prepared an information booklet focused on Okinawan parasitic and mycoheterotrophic species and distributed these booklets among staff of the Environmental Research Support Section at OIST and OIST community members. This way people who are interested in heterotrophic plants and their protection can contribute to my research by e.g. finding new locations of heterotrophic plants on Okinawa.

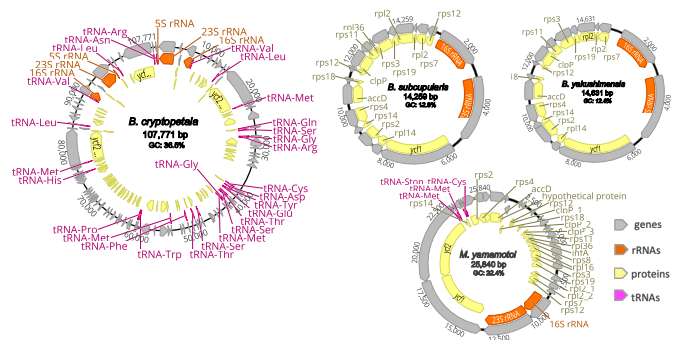


Fig 2: Plastid genomes of mycoheterotrophic *Burmattia cryptopetalae* and parasitic *Balanophora subcupularis*, *B. yakushimensis*, and *Mitrastemon yamamotoi*.