Relationship of *Caenorhabditis inopinata* with the wasp and bacteria in the fig ecosystem

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Abstract Fig trees (*Ficus* spp.) have an obligate mutualism with tiny fig-pollinating wasps. *Caenorhabditis inopinata* is a *Caenorhabditis* species discovered from syconia of *Ficus septica* in Ishigaki island Japan. DNA sequence analyses revealed that *C. inopinata* is the most-closely-related sister species of *C. elegans*, but the new nematode shows ecological features distinct from *C. elegans*. For example, the main proliferating niche of *C. inopinata* is inside of the fresh fig syconia and the nematodes are vectored by fig wasps from old to new syconia, whereas *C. elegans* is mainly found in organic-enrich environments like leaf litter, rotten fruits and only occasionally found from insects. In this study, we aimed to clarify *C. inopinata* lifecycles in the relation of the fig and wasp developments. We also investigated bacteria community structure in the fig syconia and the wasps using 16S rRNA-based microbiota analysis to understand the complex ecosystem. We found that *C. inopinata* proliferates mainly in the early stages of fig syconia. Ratio of the dauer stage increased in the late stage of syconia when the wasps develop to adults. Significant changes in the microbiota was observed among different fig stages. Moreover, overgrowth of specific bacterial taxa such as *Rhodobacter* sp. are found in the late stage of syconia, which may affect the nematode development and population structure. The PCoA analysis separated immature fig syconium microbiota from that of other stages of fig syconium. The wasp microbiota was more similar to that in the late stage of syconia. These results taken together suggest that fig-wasp is not only a pollinator of the fig but also the vector of *C. inopinata* and bacteria.

Introduction

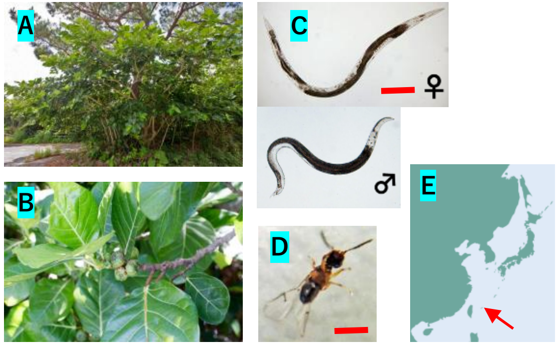

Figure 1 *C. inopinata* habitats.
A: *F. septica* (host fig tree). B: Syconia of *F. septica*.
C: *C. inopinata* (adult female and male). Red bar indicates 200 µm.
D: Pollinator wasp *Ceratosolen* sp.(vector of *C. inopinata*). Red bar indicates 1mm.
E: The sampling points. Arrow shows Ishigaki island (sampling point).

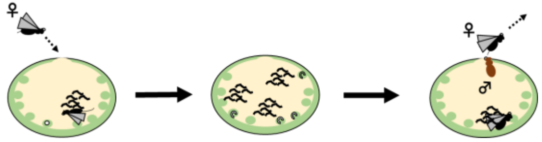

Figure 2 Lifecycle of *C. inopinata*, fig syconium and pollinator wasp.
C. inopinata is vectored to a new syconium from old one by female pollinator wasps. In the syconium, *C. inopinata* propagate by eating bacteria. In the mature stage of the syconium, *C. inopinata* dauer enters the pollinator wasp body and are vectored to newly developed syconium.

Results

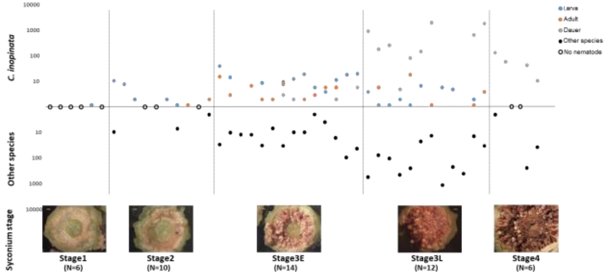

Figure 3 Dynamics of *C. inopinata* population in the fig syconium.
Fig syconia were collected from Ishigaki islands. The syconia were classified as stage1, 2, 3E, 3L and 4 based on the pollinator wasp developments and syconium morphologies. Stage1 is the initial stage which is not acceptable for the pollinator wasp. Stage2 is the pollinator wasp-acceptable stage. In the stage2 syconium, mother wasp lays eggs in the syconium. Stage3E is the stage where the pollinator wasp larvae is growing. Stage3L is the stage where the pollinator wasp progenies have grown up to pre-adults stage. Stgae4 is the stage where the grown-up pollinator wasps escape from the syconium.

Nematodes were collected from each stages of the fig syconia and counted, then species were identified by 18S rRNA. Dots of the same vertical row in the figure indicate from the same syconium. We identified a total of 5 nematode species from in the syconia. *C. inopinata* was most frequently found followed by *Acrostichus* sp., *Aphelcnchoides* sp., *Caenorhabditis* spp.(*C. briggsae* and an unknown species). The nematode numbers in the figs start increasing after wasp entered (stage2), peaked at stage 3L and then decreased in stage4. The All *C. inopinata* nematodes detected in stage 1-3E were propagative stages, but dauer form nameatodes were dominant in stage3L and stage 4.

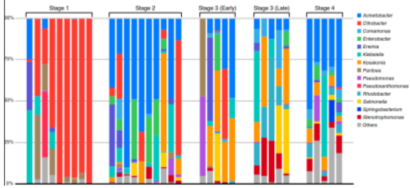

Figure 4 Bar charts of the fig syconium bacterial community.
Bacterial composition in fig syconia analyzed by 16S rRNA. We detected large amount of *Citrobacter* in stage1, while other stages contain a higher variety of bacteria like *Acinetobacter*. In stage3L fig syconia, *Rhodobacter* become a major bacteria.

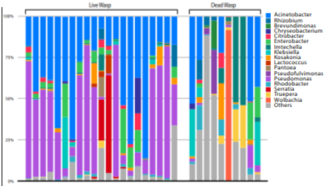

Figure 5 Bar charts of the pollinator wasp bacteria community. Bacterial community of live or dead pollinator wasp was analyzed by 16S rRNA. Live wasps contained *Acinetobacter* and *Pseudomonas* mainly. Bacterial community of the dead wasps showed different pattern in each sample.

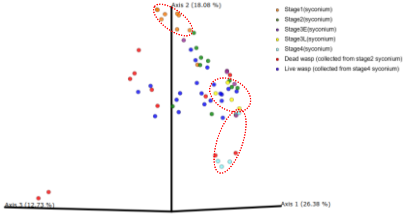

Figure 6 Beta diversity of the fig syconia and the wasp bacterial communities.
PCoA analyises of the bacterial composition showed that Stage1 syconia were independent except one sample from other stages. Live wasps and dead wasps were diverged and overlapped with stage4 or stage2 syconia.

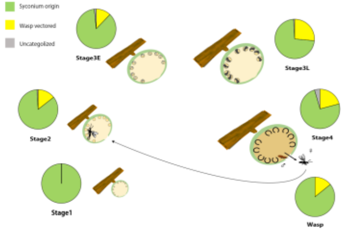

Figure 7 Bacteria in the fig-ecosystem.
Bacteria detected from fig syconia or wasps were categorized into 3 groups (Syconium-origin, Wasp-vectored and Others). Bacteria in Stage1 syconium bacteria were categorized as Syconium-origin. `Wasp-vectored` were those detected from wasps but not from stage1 syconium. `Others' were the bacteria not detected from stage1 syconium or wasps but detected from stage2-4 syconium.

Conclusion

- *C. inopinata* population development in a fig syconium seems synchronized with wasp/fig syconium maturity.
- Bacterial community in the fig syconium changed with fig syconium maturity.
- Pollinator wasp has a important role not only as a nematode vector but also as the mediator of bacteria community.
- *C. inopinata* seems well-adapted to the well known fig-wasp mutualistic life system.