Molecular assessment of wild *Achatinella mustelina* diet

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Status of the Project and Personnel

Dr. Richard O'Rorke left the project in June to pursue an opportunity in Australia. Dr. Geoff Zahn joined us in August to oversee the experiments.

Food Similarity Between Proposed Donor and Enclosure Snail Sites

If populations of *Achatinella mustelina* in difficult-to-access areas are to be successfully relocated to enclosures at sites more amenable to conservation efforts, it must be assured that conditions at the proposed sites are similar to those where the snails currently reside. One factor that may be important is the availability of preferred snail food sources. We plan to determine whether epiphytic microbial communities are similar between donor and proposed enclosure sites by sequencing DNA amplicons of material swabbed from the surface of leaves at each location.

At each current snail site, leaves from at least 10 plants containing snails were recorded, collected and returned to the lab. In the lab, leaf surfaces were swabbed and these swabs will be subjected to DNA sequencing to determine species composition. The same sampling strategy is being carried out for plants (same species as current snail plants) at the proposed enclosure sites. If leaf-surface microbial communities are similar between current and proposed sites, it is an indication that food source and availability will not be limiting factors in snail health at proposed sites following translocation. If microbial communities are dissimilar, further work will be done to determine whether these differences are functionally meaningful and/or whether it is possible to inoculate plant surfaces at the proposed sites with microbial food sources from the current sites to ease any potential snail relocation shock.

Donor Site	Proposed Enclosure Site
Skeeter Pass	Ka'ala Bog
Culvert 69	Three Points/ Makaleha
Ekahanui	Palikea Area

Snail Food "Farming" for Improved Captive Breeding

Previous work (see Annual Report 2014 and attached manuscript draft) demonstrated that the diet of captive snails differs from that of snails in the wild.

We propose an experiment to test whether and how to cultivate preferred snail food on more accessible and convenient host plants and locations. Using a controlled experiment in a growth chamber we plan to assess the extent to which plant identity, community membership, environment and leaf surface pre-treatments enable cultivation of target snail food fungi. Results from this experiment should provide insight into captive breeding programs proposed by SEP and other stake-holders, as well as potential site remediation for future translocations in the field.

Snail Transplant Studies

In preparation for potential snail transplant to novel enclosures, we assessed whether snail introductions impact microbial community composition. *Auriculella ambusta* snails, serving as a proxy for *Achatinella* were transplanted from ginger and jasmine onto *Metrosideros polymorpha* at a restoration site on Mt. Tantalus. Snail enclosures were maintained using window-screen bags, and non-snail controls were established on the same trees as blocked experimental replicates. Using DNA sequencing as in previous studies, we determined the extent to which phyllosphere fungi and bacteria, with and without snails, resemble prior snail habitat, snail feces, snail mucus, or the contemporary environment. The experiment was sampled weekly and maintained for 6 weeks.

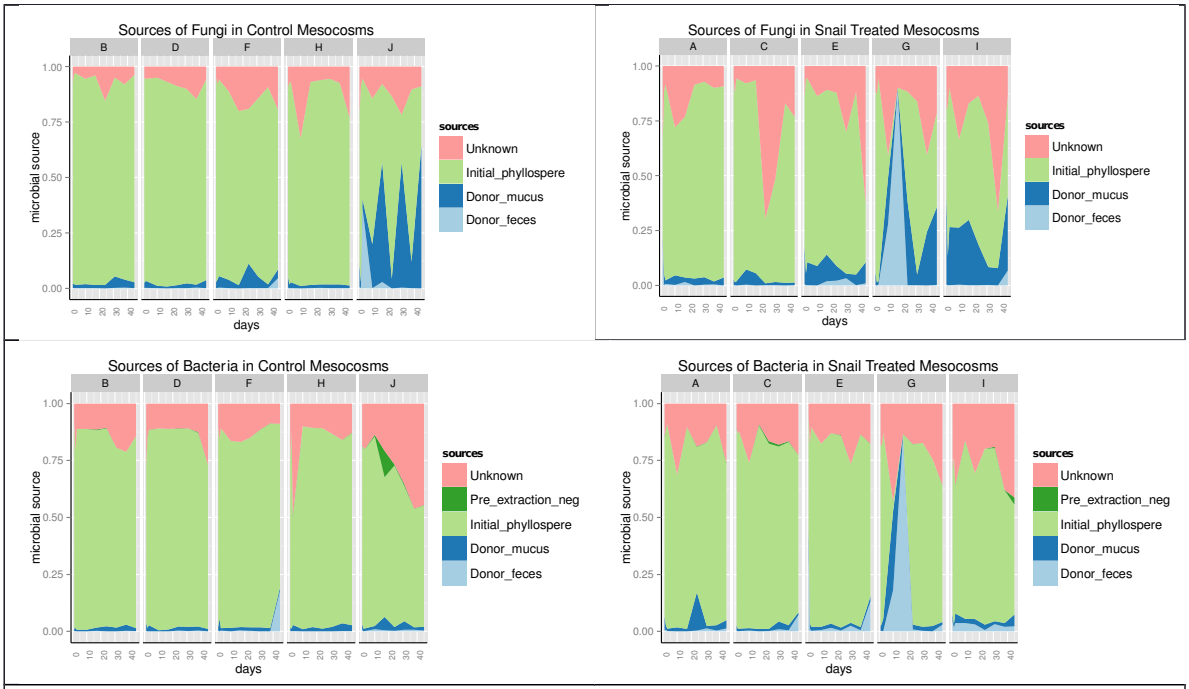

Figure 1. "Source" of microbial community composition on leaves. Clockwise from upper left: Fungal No-Snail, Fungal Snail, Bacteria No-Snail, Bacteria Snail. "Unknown" contribution to community composition is presumed to be aerial deposition from the environment.

Abundance of microbes on the leaf surface was assessed using scanning electron microscopic (SEM) imagery.

Results

Initial analysis of our data suggests that snails transport their own microbes (the farming hypothesis) only briefly, and that this influence is relatively minor and attenuates after two weeks. Instead, snails appear to disrupt intact phyllosphere communities, increasing the contribution of aerial microbes over time (Figure 1). This does not lead to a single stable snail-like community, but instead contributes to communities of microbes that differ considerably from each other and from non-snail controls, which are more homogenous (Figure 2). That is to say, the presence of snails appears to tip the balance between a deterministic and stochastic microbial community assembly process.

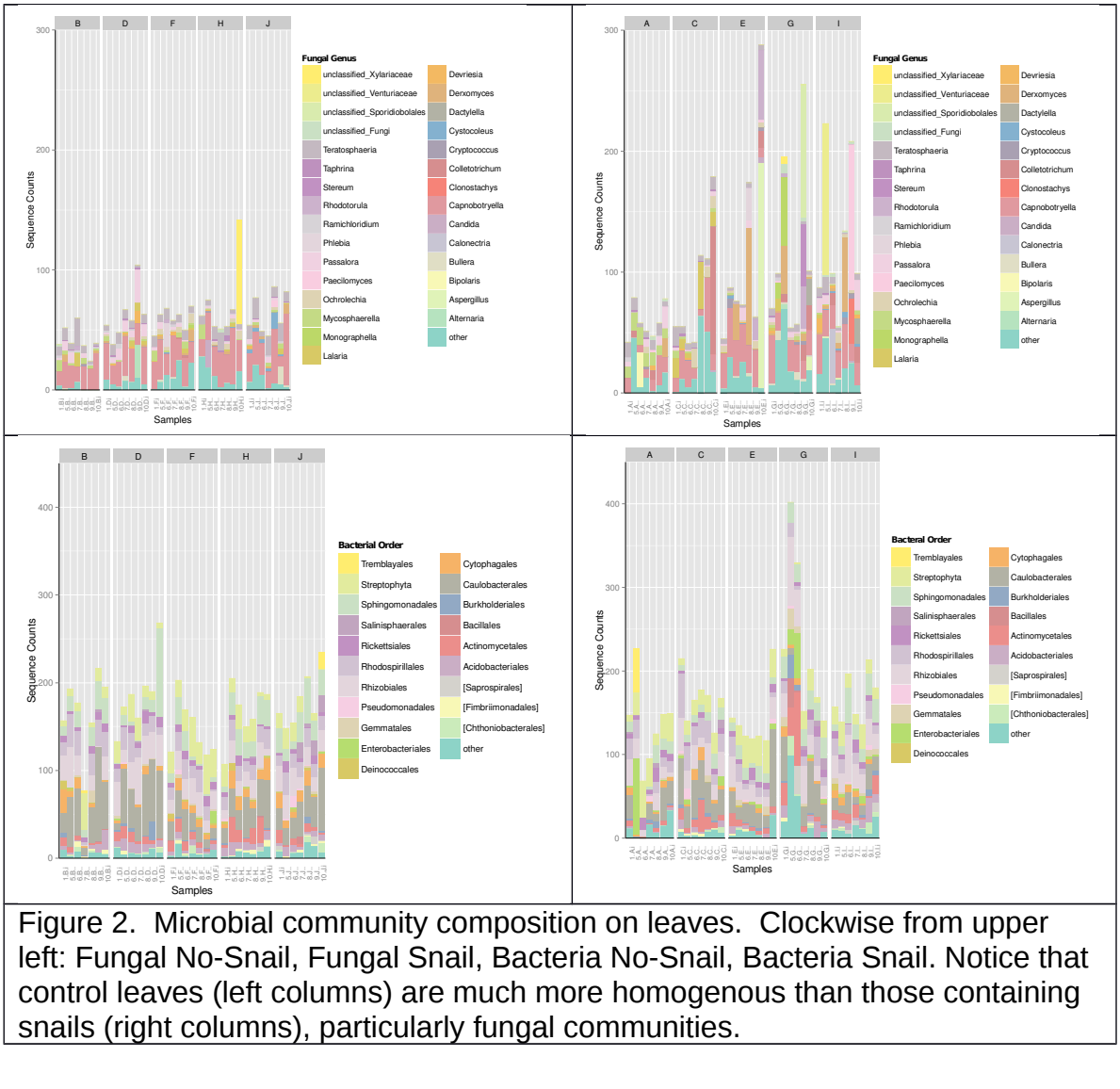

Figure 2. Microbial community composition on leaves. Clockwise from upper left: Fungal No-Snail, Fungal Snail, Bacteria No-Snail, Bacteria Snail. Notice that control leaves (left columns) are much more homogenous than those containing snails (right columns), particularly fungal communities.

Analysis of SEM images demonstrated, unsurprisingly, that snails significantly reduce the abundance of surface microbes.

Significance

Experimental results suggest that, at least for transplanted *Auriculella ambusta*, microbial input from source locations is rapidly swamped by contemporary phyllosphere and aerial microbes. Transplanting, snails, therefore, is insufficient for simultaneous transplanting of snail food.

That snails significantly reduce the abundance and stability of phyllosphere microbes, however, suggests that a synergistic approach may abet transplantation of food sources. Because grazed leaves are more receptive to aerial-dispersed microbes, repeated applications of preferred food items (via spray bottle slurries for example) may enable us to grow snail food in novel habitats. This dynamic is the subject of our next experiment.

Snail Feeding Trials

The results of our feeding trials are under review in *Biological Conservation*. The draft manuscript is attached and the title page/abstract are appended below:

Escaping the captive diet: enhancing captive breeding of endangered species by determining dietary preferences

O'Rorke, Holland, Cobian, Gaughen, Amend

Abstract

Endangered species can be safeguarded against extinction by raising subpopulations in *ex situ* facilities that mimic their wild habitats. This is difficult when the endangered animal's diet is cryptic. We present a combined molecular and behavioral approach to assess the *ex situ* diet of *Achatinella*, a critically endangered genus of tree snail, to determine how diet of captive snails differs from wild snails. *Ex situ* snails are currently fed biofilms growing on the surface of leaves, as well as a cultured fungus isolated from this same habitat. Amplicon sequencing of DNA extracted from feces of cultured snails confirms that this cultured fungus is abundant in the wild, but that it dominates the diet of the *ex situ* snail diet (comprising ~38% of sequences). The diet of captive snails is significantly less diverse compared to wild snails. To test the hypothesis that snails have diet preferences, we conducted feeding trials. These used a surrogate snail species, *Auriculella diaphana*, which is a confamilial Oahu endemic, though non-federally listed. Contrary to our expectations we found that snails do have feeding preferences. Furthermore, our feeding preference trials show that over all other feeding options snails most preferred the "no-

microbe” control, which consisted only of potato dextrose agar (PDA). PDA is rich in simple carbohydrates, which is in contrast to the wild environment of tree-snails, which is oligotrophic. These results suggest further research should focus on calorie budgets of snails and on devising new approaches to supplementing their *ex situ* diet.