BIOTROPICA
THE JOURNAL OF THE ASSOCIATION FOR TROPICAL BIOLOGY AND CONSERVATION

BIOTROPICA 0(0): 1–7 2016 10.1111/btp.12339

Diet selection at three spatial scales: Implications for conservation of an endangered Hawaiian tree snail

Melissa R. Price[1,2,3,5], **Richard O'Rorke**[4], **Anthony S. Amend**[4], and **Michael G. Hadfield**[1]

[1] Pacific Biosciences Research Center, University of Hawai'i, Mānoa, HI, U.S.A.

[2] Department of Natural Resources and Environmental Management, University of Hawai'i, Mānoa, HI, U.S.A.

[3] Sherman Hall, University of Hawai'i, 1910 East West Rd., Honolulu, HI 96822, U.S.A.

[4] Botany Department, University of Hawai'i, Mānoa, HI, U.S.A.

ABSTRACT

Several recent studies suggest local adaptation in multiple taxa across Hawaii's steep environmental gradients. Restoration efforts in devastated tropical island ecosystems may be deficient if we lack an understanding of the interactions and dependencies in communities that occur along these gradients. Endangered Hawaiian tree snails are part of a snail–epiphyte–plant system where they graze fungi and other microbes on the leaf surface, a process difficult to observe using conventional techniques. Tree snails have undergone catastrophic decline due to introduced predators, removal by shell collectors, and human-influenced habitat degradation. Prior to this study, little was known about the relationship among tree-snails, their host plants, and the epiphytic microbes on which they feed. In this study, we identified scale-dependent selection of substrates in *Achatinella sowerbyana* and *Achatinella lila* across the species' ranges. We assessed: (1) within-plant diet selection using high-throughput DNA sequencing (micro-scale); (2) among-plant selection of tree host species (small-scale); (3) and the influence of climate on this system (macro-scale). Selection of substrates occurred at two scales: fungal communities in fecal samples differed in composition from those available on leaf surfaces; and at all sites, snail occurrence on *Metrosideros polymorpha*, a foundational forest plant, was significantly higher than expected based on availability. Habitat restoration efforts should focus on outplanting of *M. polymorpha*, the preferred snail host tree, in degraded habitat. Fungal differences across sites suggest relocation efforts to predator-free enclosures may be hindered by microbial shifts associated with geographic distance or differing environments.

Key words: *Achatinella lila*; *Achatinella sowerbyana*; amplicon sequencing; epiphytic fungal community; fecal analysis; habitat restoration.

The unique geologic history of the Hawaiian Islands gave rise to steep environmental gradients over very short distances (Price & Clague 2002), shaping forest ecosystem dynamics along these gradients (Idol *et al.* 2007). Recent studies have suggested that taxa that evolved along these gradients may be locally adapted (O'Rorke *et al.* 2015). Varieties of the tree 'ōhi'a lehua (*Metrosideros* spp.), which dominate Hawaiian ecosystems at multiple successional stages, are locally adapted to environmental conditions along elevational gradients (Martin & Asner 2009, Morrison and Stacy 2014). Communities associated with this dominant forest plant, such as endophytic microbes, are also structured by precipitation and temperature (Zimmerman & Vitousek 2012). Tree snails in the family Achatinellidae, that often use *Metrosideros* spp. as a host tree and feed on fungal epiphytes growing on leaf surfaces (O'Rorke *et al.* 2015), have radiated into speciose lineages that occur from 400 to 1200 m elevation (Pilsbry & Cooke 1912–1914).

The Hawaiian Islands have experienced a tremendous loss of native forests due to agricultural and urban development, introduced ungulates, the introduction of invasive plant species, and increasingly, forest fires (D'Antonio & Vitousek 1992). Other human-influenced effects, such as predation by introduced species (Hadfield 1986), and over-harvesting by collectors (Hadfield & Mountain 1980) led to the extinction of at least 30 species in the Hawaiian tree-snail genus *Achatinella*, and resulted in the declaration of all remaining species in the genus as Endangered (U.S. Fish and Wildlife Service 1981).

Restoration of degraded forest habitat is hindered when we lack knowledge of ecological requirements, particularly if an animal has a cryptic or difficult-to-observe feeding method. Hawaiian tree snails (Achatinellidae) are part of a native snail–epiphyte–plant system where they graze fungi and microbes on the leaf surfaces (Hadfield & Mountain 1980, Kobayashi & Hadfield 1996, O'Rorke *et al.* 2015), a process difficult to observe using conventional techniques. Although over 40 species have been listed as Endangered since 1981 (USFWS Recovery Plan 1992), only one detailed study of the achatinellid diet, in the tree-snail species *Achatinella mustelina*, has been published (O'Rorke *et al.* 2015). Today, novel methods such as next-generation sequencing allow identification of the foraging substrate, and will improve planning for tree-snail habitat restoration, as well as appropriate site selection and habitat improvement for predator-free enclosures.

Recent studies using high-throughput DNA sequencing of epiphytic fungi have demonstrated the highly variable nature of this

Received 5 October 2015; revision accepted 29 February 2016.

[5]Corresponding author; e-mail: melrprice@gmail.com

dietary resource (O'Rorke *et al.* 2015). Microbial communities growing in and on leaves may vary among plant species, or even within populations of the same plant (Cordier *et al.* 2012, Bâlint *et al.* 2013). Environmental variables such as precipitation and temperature structure communities, resulting in broadly differing foliar endophytic communities over very short distances in the Hawaiian Islands (Baker *et al.* 1979, Zimmerman & Vitousek 2012).

Identification of these diverse fungal communities at multiple scales is important not only for endangered animals that depend on them as a food source but also for conserving native forest ecosystem diversity (Heilmann-Calusen *et al.* 2014). Studies of marine snail–epiphyte–plant systems suggest that plant growth is enhanced in the presence of epiphyte-grazing snails (Underwood *et al.* 1992). These effects hold true for plants exposed to increased temperatures (Cao *et al.* 2014), rendering snails a particularly important part of these systems over the next century as high-elevation montane systems become warmer and drier (Safeeq *et al.* 2013).

Tree snails such as *Achatinella sowerbyana* and *A. lila* may exhibit a substrate preference in one of two ways, by selectively grazing patches of epiphytic fungi (*within* host plants) and/or by targeting tree hosts with epiphytic microbial communities reflecting their tastes (*among* host plants). In a sister species of tree snail, *A. mustelina*, a previous study found that snails did not selectively graze within a host plant (O'Rorke *et al.* 2015). Instead, they indiscriminately grazed plants on which they occurred. In this study we wished to discover at which scale diet selection may occur. To this end, we aimed to: (1) discern whether two co-occurring tree-snail species in the Ko'olau Mountain Range selectively or indiscriminately graze epiphytic fungi (micro-scale); (2) discern whether selection occurs at the level of host-tree species (small-scale); and (3) identify environmental factors that may shape substrate availability (macro-scale), such as geographic distance, precipitation, and temperature. Current tree-snail management plans include the construction of two predator-free exclosures near the two sites where *A. lila* and *A. sowerbyana* co-occur. Knowledge of vegetation preferences and dietary similarities or differences between snail species will assist in habitat restoration efforts within these two predator-free exclosures.

METHODS

Study site.—Sister species *A. sowerbyana* and *A. lila* co-occur in a small remnant of their historical ranges on the moist, windward slopes of the northern Ko'olau Mountain Range (Fig. S1—Online Supplementary Material). Remnant populations are patchily distributed (Hadfield *et al.* 1993, Holland *et al.* 2009) in forest dominated by *Metrosideros polymorpha*. The six sites where *A. sowerbyana* remains in numbers high enough for detection, which include the only two known sites where *A. lila* persist, were examined in this study. Each site is *ca* 50 m × 50 m in diam, with some sites slightly smaller or larger, occurring on very steep slopes with relatively short (<5 m) vegetation.

Micro-scale sampling: Within-host fungal diversity.—We spent *ca* 8–12 h searching each of six sites for snails, and *ca* 4–6 h collecting fecal and leaf samples at each site. Due to the low profile and relatively low density of vegetation, as well as flagging of snail trees present from repeated surveys over 30 years, this amount of time is adequate to sample tree snails present within the site. We removed fecal samples deposited onto Petri dishes by snails, or recently defecated onto leaves with a sterile swab, and placed them into a dry sterile tube. For each fecal sample, we collected a paired leaf epiphyte sample by rubbing a sterile swab on the top and bottom surfaces of three mature, healthy leaves from the same host tree. After returning to the laboratory, samples were kept at −20°C until DNA extraction.

Genomic DNA was extracted from feces and leaf swabs following the manufacturer's instructions for the PowerSoil DNA Extraction Kit (Mo-Bio, Carlsbad, CA, USA). Duplicate amplification reactions were run for each sample using primers ITS1-f (Gardes & Bruns 1993) and ITS2 (White *et al.* 1990), adapted for use on an Illumina platform following the PCR protocol of Smith and Peay (2014). PCR reactions of 25 μl volume consisted of Phusion Hot Start Flex mix (New England Biolabs, Ipswich, MA, USA), forward primer (0.2 μM), reverse primer (0.192 μM), and *ca* 5 ng gDNA. Amplification products were visualized on a 1.25 percent agarose gel, then made equimolar using SequelPrep Normalization plates (Invitrogen, Carlsbad, CA, USA), and cleaned with a Sera-Mag Speed-beads (FisherSci, Waltham, MA, USA) with a bead ratio of 1.8:1. Normalized and cleaned samples were quantified (Qubit fluorometer; Invitrogen) using the dsDNA HS assay, run on a Bioanalyzer Expert 2100 High Sensitivity chip (Agilent Technologies, Santa Clara, CA, USA), and quantified with qPCR to determine cluster density before sequencing. Sequencing was performed at the University of Hawai'i Genetics Core Facility (Hawai'i Institute of Marine Biology; HIMB) using one quarter of an Illumina MiSeq flow cell with MiSeq Reagent v3 Chemistry enabling 300 bp paired-end reads.

The pipeline for sequence processing is available in Supporting Information (Appendix S1—Online Supplementary Material), but a brief summary of this process follows. The forward and reverse FASTQ reads were merged (PEAR; Zhang *et al.* 2013). Merged reads were discarded if <75 bp or >550 bp and index sequences were used to assign paired reads to samples (Caporaso *et al.* 2010). Merged reads were dereplicated to simplify computer processing, singletons removed and then screened for chimeras (UCHIME; Edgar *et al.* 2011), and clustered at 97 percent similarity (UPARSE; Edgar 2013). Taxonomy was assigned to clustered sequences using the Wang method (MOTHUR; Schloss *et al.* 2009) with a modified UNITE fungal database augmented with ITS1 sequences from non-target out-group Eukaryotes (supplemental material), similar to O'Rorke *et al.* (2015).

Small-scale: Distribution of tree snails among host plants and vegetation surveys.—To determine if snails have preferred host trees, we conducted snail surveys at six sites between October 2012 and May 2013. We recorded the species of tree snail and host plant, latitude and longitude for all snails >12 mm shell length, corresponding to a minimum age of *ca* 2 yrs old (Severns 1981, Price & Hadfield 2014). We conducted vegetation surveys

at these same six sites between June and November of 2014 by the line-intercept method (Canfield 1941) to determine the relative abundance of each tree species ('available') and selection of host trees by snails ('selected'). All of the native trees assessed in the surveys were evergreens with relatively slow growth rates. The advantage of the line-intercept method is the ability to calculate relative abundance for each species, and the relative ease of conducting this type of survey in very steep and rugged terrain with complex vegetation, compared with other methods. The disadvantage of this method is the inability to calculate relative volumes, and the possibility that we did not detect some rare tree species. Depending on the extent of the patch where snails occurred, we conducted from five to ten 20-m line transects *ca* 5-m apart and oriented vertically to the terrain. A 20-m tape measure was laid out along each transect. For all woody-stemmed species touching the transect or an imaginary vertical wall extending overhead from the transect, we recorded the plant species and the length of the transect that was in contact with the plant. Any tree that intersected the transect and either was previously flagged as a tree-snail host or where we observed a snail at the time of the vegetation surveys was noted as a 'snail tree'.

Macro-scale: Geography and environmental data.—Using the software program ArcGIS (ESRI 2013, v. 10.2 for desktop), we extracted annual mean temperature (°C) and annual mean precipitation (mm) for each snail location from layers publicly available online through the Geography Department at the University of Hawai'i at Mānoa (http://rainfall.geography.hawaii.edu, downloaded August 15, 2013; Giambelluca *et al.* 2013).

Micro-scale: Within-plant host analysis.—Data were analyzed in R using the packages 'vegan' (Dixon 2009), MASS (Venables & Ripley 2002), and were visualized with ggplot2 (Wickham 2009). Full scripts are available (Appendix S2—Online Supplementary Material). Libraries were rarified to 1000 reads, and samples with fewer than 1000 reads were discarded. Singletons were removed. A Bray-Curtis dissimilarity matrix was constructed on square root transformed values (Bray & Curtis 1957). To identify factors that influence variance among samples, results were visualized by MDS ordinations and modeled using a two-way PERMANOVA (Anderson 2005) with the factors 'snail species' (*A. sowerbyana* or *A. lila*) and 'sample type' (leaf, fecal) using type III sums of squares under a reduced model. As communities did not differ by 'snail species', all fecal samples with paired *M. polymorpha* leaf samples were used in downstream analyses. Whether different OTUs were significantly associated with levels of factors such as leaf/feces or location was determined in R using the package 'Indicspecies' (De Cáceres & Legendre 2009).

Small-scale: Among-plant host analysis.—For each site, the arcsine-transformed proportions of each plant species ('available') and each host-tree species ('selected') were calculated. Values were compared using a paired *t*-test for each tree species that occurred at a minimum of five sites. In addition, at the two locations where *A. sowerbyana* and *A. lila* co-occur we used chi-square analyses to compare tree-snail distribution among plant species to discern if the two snail species differ in plant use.

Macro-scale: Environment factors shaping the snail–epiphyte system.—Analyses for molecular data, including distance relationships and comparisons between fecal and leaf-swab samples, were restricted to the snail host-plant *M. polymorpha*, the host tree where the majority of snails occurred. Fungal community beta diversities in both leaf and fecal samples were compared with mean annual precipitation and mean annual temperature at each sample's location. This was accomplished using partial-Mantel tests where the matrix distance between sites was the factor that community dissimilarity was controlled against.

RESULTS

A total number of 224 snails were observed across the six sites, including 7 individuals of *A. lila* at the Poamoho Summit (of a total of 34 snails) and 39 individuals of *A. lila* at Punalu'u (of a total of 53 snails). Considering the combined total of both species, the number of snails observed at each site ranged from 24 to 53 snails (mean ± SD = 37 ± 10).

Micro-scale: Within-host preferences.—A total of 27 paired fecal and leaf-swab samples ($N = 54$) were collected from four tree species among six locations (Table 1). Of these, 22 were from *M. polymorpha*, allowing comparisons among sites within the same host-plant species. The number of fecal samples collected was small due to the rarity of these endangered species and the even rarer occurrence of fecal deposition during sample collection times.

The one-quarter Miseq run yielded 1.17×10^6 reads, of which 9.1×10^5 reads passed merging, quality control, and chimera screening. Of these reads, 8.4×10^5 reads (92.3%) assigned to the Kingdom Fungi, 7.6 percent assigned to an unknown clade (*i.e.,* they had no homolog in the UNITE database), and 1169 (0.1%) assigned to non-fungal Eukaryotes. Sequence data have been deposited in the NCBI SRA under Biosample accessions: SAMN03217561–SAMN03217625. At 97 percent similarity, 1667 operational taxonomic units (OTUs) of fungi were identified. Of these, 71.5 percent of the OTUs were identified to the class level, 63.6 percent to the order level, and 40.8 percent to the genus level.

Fungal community composition was best explained by whether it was leaf or feces derived (Table 2), and did not differ significantly among host trees of co-occurring tree-snail species *A. sowerbyana* and *A. lila* (Table 2). Even though fungal community composition differed between sample types (leaf and fecal), no fungal OTU was statistically associated with either fecal- or leaf-derived samples, as the indicator species statistic values were below 0.8 for all OTUs.

Small-scale: Among-host preferences.—The most abundant host-tree species at all sites was *M. polymorpha*, covering 41–75 percent of the available habitat (Fig. 1). Snails were observed on a total of eleven host-tree species, all of which are native to Hawai'i, nine during snail habitat searches (Table 1), and an

TABLE 1. *Hawaiian tree-snail distribution among plant species, and fungal sample distribution among locations and types (fecal or leaf). Distribution among plants is for snails detected during diurnal searches of habitat. Fecal sample numbers were limited due to the rarity of these Endangered snails. Poamoho Trail, Punalu'u Summit, Opa'eula, and Opa'eula Summit only had* A. sowerbyana *present. Poamoho Summit and Punalu'u sites, where snails occurred almost entirely on* M. polymorpha*, included both* A. lila *and* A. sowerbyana.

Location	Plant host species	Snails	Feces
Poamoho Trail	*Antidesma pulvinatum*	3	0
	Melicope clusiifolia	2	0
	Metrosideros polymorpha	17	2
	Perrottetia sandwicensis	1	0
	Wikstroemia oahuensis	1	0
Poamoho Summit	*Broussaicia arguta*	1	1
	M. polymorpha	33	3
Punalu'u	*Kadua affinis*	2	0
	M. polymorpha	51	9
Punalu'u Summit	*B. arguta*	4	0
	Ilex anomala	3	1
	K. affinis	2	0
	M. clusiifolia	7	3
	M. polymorpha	25	4
Opae'ula	*Dubautia* sp.	4	0
	M. polymorpha	35	3
Opae'ula Summit	*B. arguta*	4	0
	Dubautia sp.	1	0
	K. affinis	1	0
	M. clusiifolia	5	0
	M. polymorpha	22	1

TABLE 2. *Sample source, whether tree-snail feces or the corresponding leaf epiphytes, but not the snail species (*Achatinella sowerbyana, A. lila*), significantly explained the variation in fungal communities among samples, based on a permanova test.*

Source	df	SS	MS	Pseudo-F	*P*(perm)
Snail species	1	3865	3865	1.06	0.293
Fecal or leaf	1	11854	11854	3.25	0.0001
Residual	35	1.28E+05	3652		
Total	37	1.44E+05			

additional two during vegetation surveys (*Freycinetia arborea, Metrosideros tremuloides*). Of the seven species present at a minimum of five sites, snails occurred more often on *M. polymorpha* than expected based on tree species availability ($t = 5.94$, $P < 0.01$), less often than expected on *Chamaesyce rockii* ($t = 5.78$, $P < 0.01$), *Broussaicia arguta* ($t = 2.79$, $P = 0.039$), and *Dubautia* sp. ($t = 4.08$, $P < 0.01$), and in the same proportion as expected for *Freycinetia arborea* ($t = 1.09$, $P = 0.33$), *Kadua affinis* ($t = 1.27$, $P = 0.26$), and *Melicope clusiifolia* ($t = 2.27$, $P = 0.086$). Among-host occurrence patterns for *A. sowerbyana* and *A. lila* did not differ between the two snail species in the two locations where they co-occurred (Pearson $\chi^2 = 0.373$, $P = 0.54$).

Macro-scale: Geographic distance, precipitation, and temperature.—Community dissimilarity was significantly correlated with geographic distance for both leaf fungal communities ($r = 0.229$, $P < 0.001$) and fecal fungal communities ($r = 0.267$, $P = 0.017$). That is, fungal communities that came from trees close to one another were more similar. Fecal fungal communities were more similar to one another than leaf fungal communities at all geographic distances examined. Similarly, in MDS plots, fungal communities in fecal samples were more tightly clustered than fungal communities derived from leaf-swabs samples (Fig. 2). When distance was held constant, fungal community beta diversities in both leaves and fecal samples were significantly correlated with both precipitation ($r_{leaf} = 0.131$, $P_{leaf} = 0.026$; $r_{fecal} = 0.362$, $P_{fecal} < 0.01$) and temperature ($r_{leaf} = 0.216$, $P_{leaf} < 0.001$; $r_{fecal} = 0.264$, $P_{fecal} = 0.025$).

DISCUSSION

The Hawaiian tree-snails *A. sowerbyana* and *A. lila* are strongly associated with the host-tree *M. polymorpha*, and by extension its epiphytic community. This interaction suggests that tree snails, their preferred host-tree *M. polymorpha,* and the epiphytic microbial community found on *M. polymorpha* leaves exist as a system that may be analogous to other well-studied marine snail–macrophyte–epiphyte systems (Underwood *et al.* 1992). Given the broad distribution of *M. polymorpha* from sea level to mountain top, and its life history as an early colonizer of lava flows (Drake 1992), this plant species was likely a dependable host and food source for the Hawaiian ancestors of these species. Hawaiian tree snails, like their marine counterparts (Boch *et al.* 2011), interact with and may even influence a surprising number of food items available in the epiphytic communities present on *M. polymorpha* leaves.

Do tree snails show micro-scale within-plant host preferences?.—The Hawaiian tree-snails *A. sowerbyana* and *A. lila* appear to be selectively consuming or digesting fungi. Fungal beta diversity showed much lower dispersion among fecal samples than among leaf samples (Fig. 2), indicating that fungal communities in fecal samples were more similar to each other than the epiphytic fungal communities from which they were grazed. Sample source identity (feces or leaf) best explained community similarity, and samples did not differ between tree-snail species. No indicator species was significantly associated with either leaf or fecal samples, but fungal community composition differed between paired fecal and leaf samples. Similar to what was observed for the tree-snail species *A. mustelina*, the same food 'items' were found in both feces and leaf samples (O'Rorke *et al.* 2015), but they differed in abundance between leaf and fecal samples. As these tree-snail species are federally listed as Endangered, we were not able to include gut analysis at multiple points in the digestive process to discriminate among stages and processes, but a previous study of mycophagous

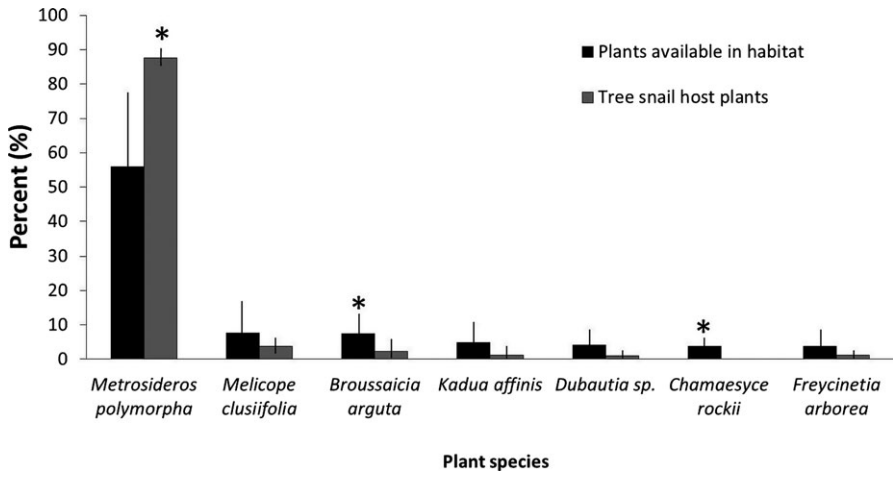

FIGURE 1. Relative abundance of the seven most common plant species among Hawaiian tree-snail habitats, compared with tree-snail occupancy of these plant species. *Metrosideros polymorpha*, commonly referred to as ʻōhiʻa lehua, was the most common plant species, and was preferentially occupied as a host tree by snails in all locations surveyed. *P<0.05

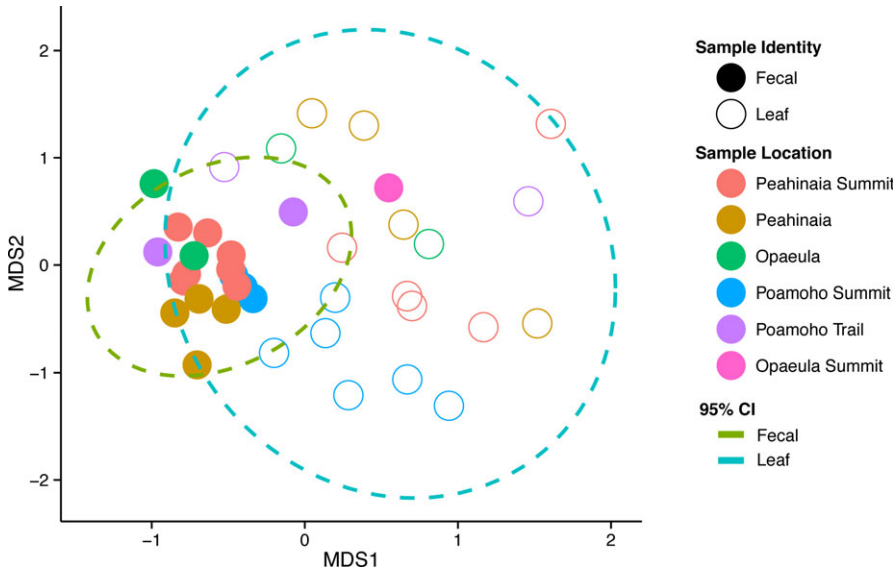

FIGURE 2. Multi-dimensional Scaling Plot for Hawaiian tree-snail fecal samples and *M. polymorpha* leaf samples identified by location. Fungal communities from fecal samples clustered more tightly than those from plant samples, suggesting selective ingestion or digestion by Hawaiian tree snails.

snails found feces to be indicative of gut contents (O'Rorke *et al.* 2015).

Does foraging substrate differ between co-occurring tree-snail species?.—Snails may currently exist at low enough abundance to avoid direct competition at the few sites where they still co-occur, as a comparison of *A. sowerbyana* with *A. lila* did not show significant differences at the among-host or within-host scales (Table 2). As radula dentition (Gwatkin & Suter 1895), diet, and host-plant selection did not differ significantly between the two species of tree snails examined in this study, species-specific out-planting of host plants may not be necessary for habitat restoration.

Do snails show small-scale among-plant host preferences?.—Our study builds on the study in the tree-snail species *A. mustelina* (O'Rorke *et al.* 2015), by demonstrating grazing habitat selection at the level of host tree. Tree snails examined in this study are preferentially selecting the most abundant tree in the ecosystem across all sites, *M. polymorpha*, as a host tree, occurring on it at all sites in even higher abundance than expected based on a random distribution among available host plants. Snails were also observed on ten other native plant species at low abundance. Some of these ten species were occupied in proportion to availability, and others were occupied less often than expected based on expected random distribution among hosts. These results highlight plant species that are important to tree-snail survival,

and will guide habitat restoration efforts and out-planting choices for improvement of existing exclosure structures.

Do macro-scale factors such as temperature and precipitation shape the snail–epiphyte system?.—The epiphytic fungal community varied by geographic region, and was strongly structured by precipitation and temperature, but the fecal samples were more homogenous across sites than expected given the variability among leaf samples. Consumption or digestion appears to be a more rigorous environmental filter than climate or geography alone, perhaps because some fungi are more easily digested than others (Wallis *et al.* 2012), or perhaps due to selective feeding on patches within hosts.

What was the effect of geographic distance on fungal communities?.—The foraging substrate available to *A. sowerbyana* differed among locations, even though only the most commonly occurring host tree, and the tree overwhelmingly selected by snails (*M. polymorpha*) was considered. This is likely due to dispersal limitation of microbes as found in other fungal community studies (Cline & Zak 2014), in combination with an environmental gradient (Zimmerman & Vitousek 2012). Surprisingly high dissimilarity was noted among some fungal epiphyte communities that were geographically close, and high similarity was noted among some epiphytic communities at relatively large distances, suggesting additional factors such as host genetic identity or phenotype may be shaping epiphytic communities (Unterseher *et al.* 2013). Relocation efforts into exclosures must take into consideration microbial shifts associated with novel locations and environmental differences, as the available diet will differ based on these factors.

Conclusions and future work.—Substrate selection is non-random in the Hawaiian tree-snail *A. sowerbyana*. At the micro-scale, snail selection of epiphytic fungi may occur either through selective ingestion, or through random ingestion and selective digestion, as the composition of fungal communities in feces differed by proportion from what was available on leaves. Further study is needed to examine if tree snails influence the structure of the epiphytic fungal community. For example, other snail species act as important vectors for dispersal of fungi that survive passage through the gut (Silliman & Newell 2003, Boch *et al.* 2011). Smaller microbes that survive grazing benefit from the removal of competitors and may be more abundant on snail-grazed plants (Underwood *et al.* 1992). At the small-scale, tree snails appear to selectively occupy the foundational Hawaiian forest tree *M. polymorpha* across the species' range. In other words, even though this was the most abundant tree in the ecosystem, snails were found even more often on this tree than expected based on a model of random distribution among available trees, and this was true at all six sites examined. In many of Hawaii's forests, invasive plant species are suffocating native plant species, including *M. polymorpha*, resulting in degraded habitat for native invertebrates. This study highlights the centrality of the foundational forest plant *M. polymorpha* and its associated epiphytes in the restoration of forest habitat, due to their necessity as both structural and dietary components for endangered tree snails. Habitat restoration efforts should concentrate on out-planting of *M. polymorpha* and removal of invasive species such as Strawberry Guava (*Psidium cattleianum*) which quickly forms dense stands, often crowding out native plants (Zimmerman *et al.* 2008, Cole *et al.* 2012). At the macro-scale, snail relocation efforts must take into account differences in the epiphytic community that occur with distance and precipitation gradients, as relocation will likely mean a shift in available diet items.

ACKNOWLEDGMENTS

Sample collection and processing were funded by the U.S. Fish and Wildlife Service under grant agreement 122007G010 (F07AP00003; MGH). Sequencing and amplicon library preparation were funded through the O'ahu Army Natural Resources Program cooperative agreement W9126G-11-2-0066 with the Pacific Cooperative Studies Unit, University of Hawai'i (ASA). We thank D. Sischo, V. Costello, J. Tanino, and S. Pillman for assistance in sample collection, and D. Drake for valuable advice regarding vegetation sampling.

DATA AVAILABILITY

DNA sequences: in NCBI SRA under Biosample accessions: SAMN03217561–SAMN03217625.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article:

FIGURE S1. Map of the study site in the Ko'olau Mountain Range.

APPENDIX S1. The pipeline for sequence processing.

APPENDIX S2. Full R-scripts.

LITERATURE CITED

Anderson, M. J. 2005. PERMANOVA. Permutational multivariate analysis of variance, a computer program. Department of Statistics, University of Auckland, New Zealand.

Baker, G. E., P. H. Dunn, and W. S. Sakai. 1979. Fungus communities associated with leaf surfaces of endemic vascular plants in Hawaii. Mycologia 71: 272–292.

Bálint, M., P. Tiffin, B. Hallström, R. B. O'Hara, M. S. Olson, J. D. Fankhauser, M. Piepenbring, and I. Schmitt. 2013. Host genotype shapes the foliar fungal microbiome of balsam poplar (*Populus balsamifera*). PLoS ONE 8: e53987.

Boch, S., D. Prati, S. Werth, J. Rÿetschi, and M. Fischer. 2011. Lichen endozoochory by snails. PLoS ONE 6: e18770.

Bray, J. R., and J. T. Curtis. 1957. An ordination of the upland forest communities of southern Wisconsin. Ecol. Monographs. 27: 325.

Canfield, R. H. 1941. Application of the line interception method in sampling range vegetation. Forestry 39: 388–394.

Cao, Y., W. Li, and E. Jeppesen. 2014. The response of two submerged macrophytes and periphyton to elevated temperatures in the presence and absence of snails: A microcosm approach. Hydrobiologia 739: 49–59.

Caporaso, J. G., J. Kuczynski, J. Stombaugh, K. Bittinger, F. D. Bushman, E. K. Costello, N., Fierer, A., Gonzalez Pena, J. K. Goodrich, J. I. Gordon, G. A. Huttley, S. T. Kelley, D. Knights, J. E. Koenig, R. E. Ley, C. A. Lozupone, D. McDonald, B. D. Muegge, M. Pirrung, J. Reeder, J. R. Sevinsky, P. J. Turnbaugh, W. A. Walters, J. Widmann, T. Yatsunenko, J. Zaneveld, and R. Knight 2010. QIIME allows analysis of high-throughput community sequencing data. Nat. Methods 7: 335–336.

Cline, L. C., and D. R. Zak. 2014. Dispersal limitation structure fungal community assembly in a long-term glacial chronosequence. Environ. Microbiol. 6: 1538–1548.

Cole, R. J., C. M. Litton, M. J. Koontz, and R. K. Loh. 2012. Vegetation recovery 16 years after feral pig removal from a wet Hawaiian forest. Biotropica 44: 463–471.

Cordier, T., C. Robin, X. Capdevielle, M. L. Desprez-Loustau, and C. Vacher. 2012. Spatial variability of Phyllosphere fungal assemblages: Genetic distance predominates over geographic distance in a European beech stand (Fagus sylvatica). Fungal Ecol. 5: 509–520.

D'Antonio, C. M., and P. M. Vitousek. 1992. Biological invasions by exotic grasses, the grass/fire cycle, and global change. Annu. Rev. Ecol. Syst. 23: 63–87.

De Cáceres, M., and P. Legendre. 2009. Associations between species and groups of sites: Indices and statistical inference. Ecology 90: 3566–3574.

Dixon, P. 2009. VEGAN, a package of R functions for community ecology. J. Veg. Sci. 14: 927–930.

Drake, D. R. 1992. Seed dispersal of Metrosideros polymorpha (Myrtaceae): A pioneer tree of Hawaiian lava flows. Am. J. Bot. 79: 1224–1228.

Edgar, R. C. 2013. UPARSE: Highly accurate OTU sequences from microbial amplicon reads. Nat. Methods 10: 996–998.

Edgar, R. C., B. J. Haas, J. C. Clemente, C. Quince, and R. Knight. 2011. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27: 2194–2200.

ESRI 2013. ArcGIS Desktop: Release 10.2. Redlands, CA: Environmental Systems Research Institute.

Gardes, M., and T. D. Bruns. 1993. ITS primers with enhanced specificity for basidiomycetes: Application to the identification of mycorrhizae and rusts. Mol. Ecol. 2: 113–118.

Giambelluca, T. W., Q. Chen, A. G. Frazier, J. P. Price, Y.-L. Chen, P.-S. Chu, J. K. Eischeid, and D. M. Delparte. 2013. Online rainfall atlas of Hawaii. Bull. Am. Meteor. Soc. 94: 313–316.

Gwatkin, H. M., and H. Suter. 1895. Observations on the dentition of Achatinellidae. Proc. Acad. Nat. Sci. Philadelphia 47: 237–240.

Hadfield, M. G. 1986. Extinction in Hawaiian Achatinelline snails. Malacologia 27: 67–81.

Hadfield, M. G., S. E. Miller, and A. H. Carwile. 1993. The decimation of endemic Hawaiian tree snails by alien predators. Am. Zoo. 33: 610–622.

Hadfield, M. G., and B. S. Mountain. 1980. A field study of a vanishing species, *Achatinella mustelina* (Gastropoda, Pulmonata) in the Waianae Mountains of Oahu. Pac. Sci. 34: 345–358.

Heilmann-Calusen, J., E. S. Barron, L. Boddy, A. Dahlberg, G. W. Griffith, J. Nordén, O. Ovaskainen, C. Perini, B. Senn.-irlet, and P. Halme. 2015. A fungal perspective on conservation biology. Conserv. Biol. 29: 61–68 doi: 10.1111/cobi.12388.

Holland, B. S., S. L. Montgomery, and V. Costello. 2009. A reptilian smoking gun: First record of invasive Jackson's chameleon (*Chamaeleo jacksonii*) predation on native Hawaiian species. Biodivers. Conserv 19: 1437–1441. doi: 10.1007/s10531-009-9773-5.

Idol, T., P. J. Baker, and D. Meason. 2007. Indicators of forest ecosystem productivity and nutrient status across precipitation and temperature gradients in Hawai'i. Trop. Ecol. 6: 693–704.

Kobayashi, S. R., and M. G. Hadfield. 1996. An experimental study of growth and reproduction in the Hawaiian tree snails *Achatinella mustelina* and *Partulina redfieldii* (Achatinellinae). Pac. Sci. 50: 339–354.

Martin, R. E., and G. P. Asner. 2009. Leaf chemical and optical properties of *Metrosideros polymorpha* across environmental gradients in Hawai'i. Biotropica 41: 292–301.

Morrison, K. R., and E. A. Stacy. 2014. Intraspecific divergence and evolution of a life-history trade-off along a successional gradient in Hawaii's Metrosideros polymorpha. Evol. Biol. 27: 1192–1204.

O'Rorke, R., G. M. Cobian, , B. S. Holland, M. R. Price, V. Costello, and A. S. Amend. 2015. Dining local: the microbial diet of a snail that grazes microbial communities is geographically structured. App. Environ. Microb. 17: 1753–1764 doi: 10.1111/1462-2920.12630.

Pilsbry, H. A., and C. M. Cooke. 1912–1914. Achatinellidae: Manual of conchology, 2nd ser. Vol. 21 lviii + 428 pages 63 plates.

Price, J. P., and D. A. Clague. 2002. How old is the Hawaiian biota? Geology and phylogeny suggest recent divergence. Proc. R. Soc. Lond. B 269: 2429–2435 doi:10.1098/rspb.2002.2175.

Price, M. R., and M. G. Hadfield. 2014. Population genetics and the effects of a severe bottleneck in an *ex situ* population of critically endangered Hawaiian tree snails. PLoS ONE. 9(12): e114377 doi:10.1371/journal.pone.0114377.

Safeeq, M., A. Mair, and A. Fares. 2013. Temporal and spatial trends in air temperatures on the island of Oahu, Hawaii. Int. J. Climatol. 33: 2816–2835.

Schloss, P. D., S. L. Westcott, T. Ryabin, J. R. Hall, M. Hartmann, E. B. Hollister, *et al.* 2009. Introducing MOTHUR: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl. Environ. Microb. 75: 7537–7541.

Severns, R. M. 1981. Growth rate determinations of *Achatinella lila*, a Hawaiian tree snail. Nautilus. 95: 140–144.

Silliman, B. R., and S. Y. Newell. 2003. Fungal farming in a snail. Proc. Nat. Acad. Sci. 100: 15643–15648.

Smith, D. P., and K. G. Peay. 2014. Sequence depth, not PCR replication, improves ecological inference from next-generation DNA sequencing. PLoS ONE 9: e90234.

Underwood, G. J. C., J. D. Thomas, and J. H. Baker. 1992. An experimental investigation of interactions in snail-macrophyte-epiphyte systems. Oecologia 91: 587–595.

Unterseher, M., R., Gazis, P. Chaverri, C. F. García Guarniz, and D. H. Zavaleta Tenorio. 2013. Endophytic fungi from Peruvian highland and lowland habitats form distinctive and host plant-specific assemblages. Biodivers. Conserv. 22: 999–1016.

U.S. Fish, Wildlife Service. 1981. Endangered and threatened wildlife and plants; listing the Hawaiian (O'ahu) tree snails of the genus Achatinella as Endangered Species. Fed. Reg. 46: 3178–3182.

U.S. Fish and Wildlife Service. 1992. Recovery Plan for the O'ahu Tree Snails of the Genus Achatinella. U.S. Fish and Wildlife Service, Portland, OR. 64 pp.

Venables, W. N., and B. D. Ripley. 2002. Modern Applied Statistics with S. Fourth. Springer, New York.

Wallis, I. R., A. W. Claridge, and J. M. Trappe. 2012. Nitrogen content, amino acid composition and digestibility of fungi from a nutritional perspective in animal mycophagy. Fungal Biol. 116: 590–602.

White, T. J., T. D. Bruns, S. Lee, and J. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In M. A. Innis, and D. H. Gelfand (Eds.). PCR protocols: A guide to methods and applications, pp. 315–322. Academic Press, London.

Wickham, H.. 2009. ggplot2: elegant graphics for data analysis. Springer-Verlag, New York, NY, USA.

Zhang, J., K. Kobert, T. Flouri, and A. Stamatakis. 2013. PEAR: A fast and accurate Illumina Paired-End reAd mergeR. Bioinformatics 30(5): 614–620 doi:10.1093/bioinformatics/btt593.

Zimmerman, N. B., R. F. Hughes, S. Cordell, P. Hart, H. K. Chang, D. Perez, R. K. Like, and R. Ostertag. 2008. Patterns of primary succession of native and introduced plants in lowland wet forests in Eastern Hawai'i. Biotropica 40: 277–284.

Zimmerman, N. B., and P. M. Vitousek. 2012. Fungal endophyte communities reflect environmental structuring across a Hawaiian landscape. Proc. Natl Acad. Sci. 32: 13022–13027.