THE ECOLOGY AND ANTI-FUNGAL PROPERTIES OF ORCHIDS IN A PHYLOGENETIC CONTEXT ON MO'OREA, FRENCH POLYNESIA

JENNIFER YANG

Integrative Biology, University of California, Berkeley, California 94720 USA

Abstract. The Family Orchidaceae is one of the largest groups of flowering plants present today. While orchids are present throughout the island of Mo'orea, little ecological work has focused on individuals of this family. Many species of orchids have been used in ethnobotanical and medicinal practices, acting as high antioxidant reserves and as anti-cancer activating agents. This study expands on both the ecology and antifungal activity of six orchid species on Mo'orea. As such, the goals of this study are: 1) to study the ecology of orchids, 2) to determine possible anti-fungal activity by species of interest and 3) to evaluate possible trends in ecology and anti-fungal use in a phylogenetic context. This study shows that epiphytic orchids are distinguished by ecological variables more than terrestrial orchids. A maintenance of low antifungal activity existed throughout six species of interest. Possible trends in distance from stream and antimicrobial assay can be used for further analysis of ecological and antimicrobial trends.

Key words: orchids, antifungal, Vanilla, antimicrobial, epiphytes, phylogenetic relationships, ecology,

INTRODUCTION

Biodiversity is a vital component of the structure and function of ecosystems. It has a positive effect on ecosystem properties, with evidence of creating stable environments (Tilman 1996). Without diversity, ecosystems may be strongly affected—possibly even destroyed, limiting the number of organisms and resources for human use (Chapin et al. 1997). On islands, biodiversity is an even greater concern. Islands are paradoxical in nature, serving as hotspots for species diversification, while being extremely vulnerable to extinction events (Marin 2004). Since islands have lower species richness than their mainland counterparts, disturbance of island environments alters the ecosystem (Marin 2004). An analysis of extinction events show that most extinctions have been on islands as opposed to on mainlands (Sax and Gaines 2008).This susceptibility to decreasing biodiversity not only changes the relationships between organisms in nature, but also threatens the availability of plants and animals for human use. Conservation of island ecosystems is vital to the preservation of unique flora and fauna that not only foster an environment for species diversification, but also introduce possible new advancements in human culture.

Maintaining biodiversity oftentimes involves studying ecosystem dynamics through increased awareness of species. Studies on habitat and feeding preference and inter- and intraspecific relationships indicate possible solutions to disturbance events. Focusing on organisms who are threatened can prevent possible changes to present ecosystem dynamics. Past studies on an endangered freshwater pearl mussel, *Margaritifera margaritifera* indicate important measures to sustain habitat restoration or captive breeding to maintain numbers (Geist 2010). Further, human destruction of habitats also dents ecosystems, such as through destruction of forests. For the rufous treecreeper, *Climacteris rufa*, foraging and nesting resources are threatened by logging (Craig 2007).

In addition to maintaining stable environments, biodiversity also affects advances in commercial and pharmaceutical products for human consumption and use. Pharmaceutical companies use plant compounds for commercial use. For pharmaceuticals, biodiversity means more diversity in chemicals (Young 1999). Natural plant phytochemicals are vital components of prescription drugs. Nearly 25% of prescription drugs contain at least one phytochemical and many contain synthetic substitutes dependent on the chemical structures of these natural compounds (Farnsworth and Bingel 1977, Duke 1993).

In French Polynesia, use of plant phytochemicals as ailments began with Polynesian ethnobotany. Flowering plants presently found on these islands were used by Polynesians, confirmed by laboratory results. Studies on *Hibiscus rosa-sinensis* show that it was used to treat headaches, sores and inflammations, while *Physalis angulata* was used to facilitate childbirth and to treat dengue fever (WHO 1998). Previous research on the flowering plants *Thespesia populnea* and *Hibiscus tiliaceus* show antimicrobial activity, supported with previous knowledge of use in ethnobotany ailments (Cox 2008). Laboratory research on the antioxidant effects of *Hibiscus tiliaceus* show significant free radical scavenging by flower extracts equal to those of standard antioxidants (Kumar 2008).

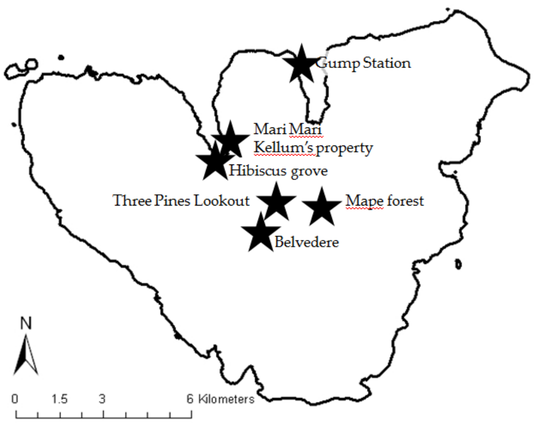
One group of flowering plants, the Family Orchidacaeae, has been used in many cultures, but not in Polynesian ethnobotany. Orchids are a diverse group of monocots with roughly 18,500 individual species (Cozzolino and Widmer 2005). They have been utilized by Tibetan and South African cultures to cure fungi, dysentery and ringworm (Bulpitt 2005). In India, orchids have been found to be used in both human and animal medicines (Kala and Senthikumar 2009). Modern uses of vanilla include its aid to apoptosis of cancer cells by TRAIL, an anticancer agent (Lirdprapamonkol et al. 2010). Vanillin, a main constituent of vanilla, also has been proven to counter hepatotoxicity in rats injected with acetaminophen (Boobalan and Deppa 2010). Vanillin has further been studied for commercial and medicinal properties. One study revealed the antimicrobial effects of vanillin against four species of bacteria, four species of fungi, and two unknown yeasts in mangos (Ngarmsak et al. 2006). Species of the genus *Bulbophyllum* have demonstrated defense against human cervical carcinoma cells (Chen et al. 2005). Chen's study further expands on possibilities that species of the genus *Bulbophyllum* may substitute *Dendrobium* species in traditional Chinese herbal remedies (Chen et al. 2005). The traditional Chinese medicine "Shi Hu" is derived from stems of various representatives of the genus *Dendrobium* (Zhang 2007). Detection and isolation of a number of chemical compounds in the species *Dendrobium nobile* supports herbal use, as antioxidant effects of many of the constituent compounds far exceeds that of Vitamin C (Zhang 2007).

This study continued with studies on orchid ecology and antifungal activity. Previous studies on distribution and ecology will serve as a basis for investigation, and the ecological study expanded (Cochrane 2001). Correlations between ecological variables were analyzed to detect possible relationships between different habitat preference variables, as well as relationships with anti-fungal activity. This study had the following goals: 1) understand the ecology of orchid species on Mo'orea 2) detect antifungal activity of selected orchid species, and 3) evaluate if antifungal activity is related to ecological variables or to phylogenetic relationships between species.

METHODS

Study sites

A number of localities were selected as study sites based on availability of specimens on Mo'orea, French Polynesia (17 º30'S, 149 º 50'S). Mo'orea is a high volcanic island located in the South Pacific, part of the Society Island archipelago. Study sites were selected based on abundance of orchid species. Study sites included both low and mid-elevation sites along the northern coastline and along the interior between Cook's Bay and Opunohu Bay (Figure 1). GPS points of each study site can be found in Figure 2.



Map outline courtesy of Elliot Chan

Figure 1. Location of study sites on Mo'orea, French Polynesia

Study Site	*GPS Coordinates*
Belvedere	17º32'25.79"S, 149 º49'36.08"W
Gump Station	17 º29'28.58"S, 149 º49'35.43"W
Hibiscus Grove	17°30'59.34"S, 149°50'57.72"W
Mape Forest	17 º32'23.55"S, 149 º49'33.08"W
Mari Mari Kellum's Property	17 º30'51.34"S, 149 º50'53.42"W
Three Pines Lookout	17°32'12.14"S, 149°49'46.33"W

Figure 2. List of study sites with corresponding GPS coordinates

Study organisms

This study features six representative species of the Family Orchidaceae on Mo'orea, French Polynesia. Individuals of the Family Orchidaceae are flowering monocots (Cozzolino and Widmer 2005). Four species of this study were epiphytic, residing on tree hosts: *Bulbophyllum longiflorum, Dendrobium biflorum, Taeniophyllum fasciola* and *Vanilla tahitensis.* Two terrestrial species were also examined: *Malaxis resupinata* and *Spathoglottis plicata*. Terrestrial species are defined as species that place their roots into the ground directly, without the aid of any host species.

Ecological survey

An intensive ecological survey was conducted from 5 October 2010 to 12 November 2010. At least one species of study orchids was present at each study site. Only plants with a distance less than 530 cm from the ground were accounted for. Plant size was measured using a transect tape or measuring tape, and data recorded to the nearest centimeter. Percent canopy was found using a densiometer constructed from a convex mirror with a mesh covering with one hundred equal sized squared. Individual squares with canopy covering more than 50% were accounted for. Elevation was recorded with the aid of topographical maps and GPS readings. Distances from streams were estimated using Google Earth Pro and a topographical map.

Additional information was recorded for epiphytic species. Circumference of branch epiphytic orchids were residing on and distances from ground were recorded to the nearest centimeter with aid of a transect tape.

Plant collection

For each species, 5 individuals were collected. Plant extracts and antimicrobial tests were run for each collected individual (n=30). Plant samples were photographed, data recorded, and ethanol extracts performed the day of collection.

Extract preparation

Plant material was extracted with 70% ethanol, as previously conducted by Cox, 2008. Plant extract was made to a concentration of 1g plant material/8ml 70% ethanol. Due to differences in sizes of individual orchid species, extract concentrations and not amount of plant material was kept constant. Leaf matter was wiped clean with a Kim Wipe dipped in ethanol. Leaf matter was cut into small pieces and blended with a mortar and pestle until a homogenous green mixture was obtained. The resulting homogenous liquid was transferred into a Falcon tube and kept in the refrigerator. Tubes were shaken by hand every day until use. Plant extracts were used in the antimicrobial assay at most four days after extraction.

Antimicrobial assay

Sugar-enriched agar medium was prepared with 14g agar powder, 100g white granulated sugar, and 1L filtered tap water in a large Erlenmeyer. The mixture was autoclaved and approximately 25ml poured into each Petri dish. After the mixture solidified overnight, each Petri dish was inverted, sealed with Parafilm, and refrigerated until use.

Commercial baking yeast, *Saccharomyces cerevisiae,* was used due to availability. An incubator was constructed from a cardboard box and a commercial desk lamp. Incubator temperature was kept constant at 28-30 º C. Three grams of *S. cerevisiae* were mixed with 50ml of filtered tap water. Each Petri dish received 0.2 ml of the yeast mixture, spread evenly with a pipette tip. Petri dishes were sealed with Parafilm, inverted, and incubated for 24 hours.

Using a hole punch, filter paper discs were created to apply plant extracts and controls. Each filter paper disc was 0.6cm in diameter. Each Petri dish received ten small circular discs that had been dipped three times each in selected treatment extract. A negative control of ethanol and positive control of commercial anti-fungal cream mixed with ethanol were used. Plates were resealed with Parafilm and incubated for 3 days. After this time, yeast inhibition of each filter paper disc was measured using a scale of 0 (no inhibition) to 3 (high inhibition).

Phylogenetic analysis

Supertree was created using Mesquite (Maddison and Maddison 2010) with data obtained from Cameron et al. 1999. All ecological variables as well as antimicrobial assay results were mapped onto tree for further analysis. An Independent Contrasts was evaluated for antimicrobial content and each of the ecological variables.

Statistical analysis

All data were analyzed using JMP statistical software. Habitat assessment variables were analyzed using discriminant analysis to assess differences between species, separated by epiphytic and terrestrial species. A one-way ANOVA was used to evaluate differences between antimicrobial content of plant species. Corresponding Tukey-Kramer looked at differences between pairs of treatment groups. Spearman's Rank Correlation tests were run between ecological variables and antimicrobial content.

RESULTS

Ecological survey

Ecological data was separated into epiphytic species, *B. longiflorum, D. biflorum,* and *T. fasciola* and terrestrial species, *M. resupinata* and *S. plicata*. This was due to a different number of ecological variables measured between the two groups. *V.*

tahitensis was not included in data analysis because only two individuals were located.

Discriminant analysis for epiphytic species showed significant separation between species (Wilks' Lambda, F=306.0991, NumDF=10, DenDF=530, p<0.0001) (Figure 3). *T. fasciola* and *D. biflorum* are most separated by distance from stream and to a lesser extent, elevation. *B. longiflorum* is separated from the other two species by circumference of tree host, and to a lesser extent, canopy cover. Discriminant analysis for terrestrial species showed less clumping of data, but results were significant (Wilks' Lambda, F=3.5311, NumDF=3, DenDF=86, p=0.0182) (Figure 4).

Plant size for each individual was measured. Spearman's rank correlation was run on plant size and each variable for all species, with no Pearson's r values found to be statistically significant.

Antimicrobial assay

Mean inhibition levels were calculated for each Petri dish using data from each of the ten filter paper vessels. A one-way ANOVA of the positive control (anti-fungal cream), the negative control (ethanol) and the six species showed all eight treatments to be significantly different (F ratio=7.9461, df=7, p<0.0001). Results indicated that the anti-fungal cream had the highest mean inhibition (mean=2.62, SD=0.179) and ethanol had the lowest mean inhibition (mean=0.1, SD=0.1) (Figure 5). A Tukey-Kramer test showed significant differences between the positive control and the negative control. However, only *M. resupinata* was significantly different from both ethanol and the anti-fungal cream, with a mean of 1.26 inhibition level.

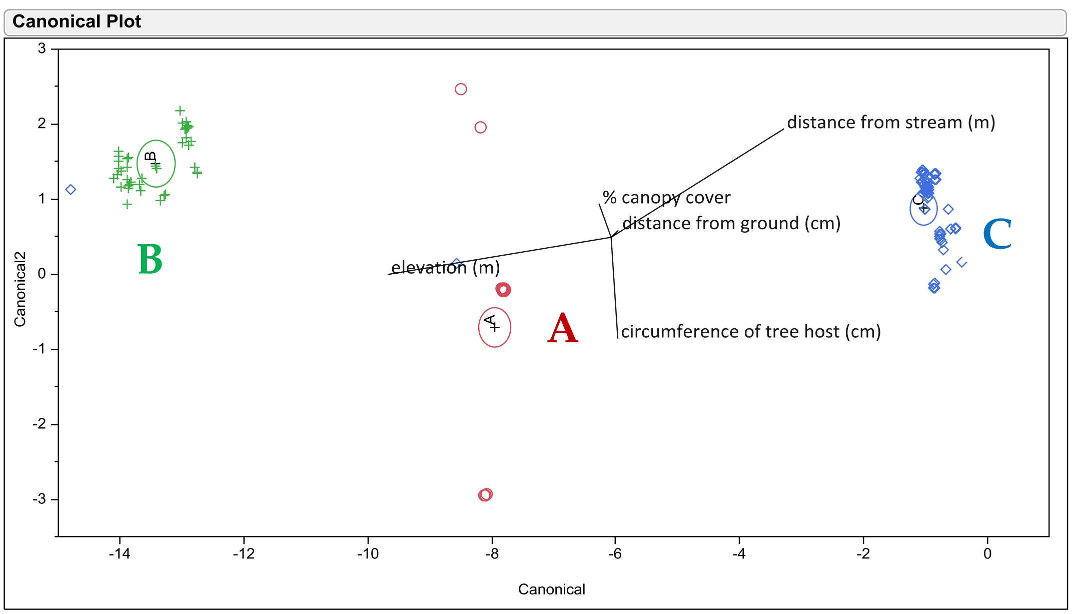


Figure 3. Discriminant analysis for epiphytic species based on the following ecological variables: distance from stream (m), % canopy cover, elevation (m), and circumference of tree host (cm) "A" is *B. longiflorum*, "B" is *D. biflorum*, and "C" is *T. fasciola* (Wilks' Lambda, F=306.0991, NumDF=10, DenDF=530, p<0.0001).

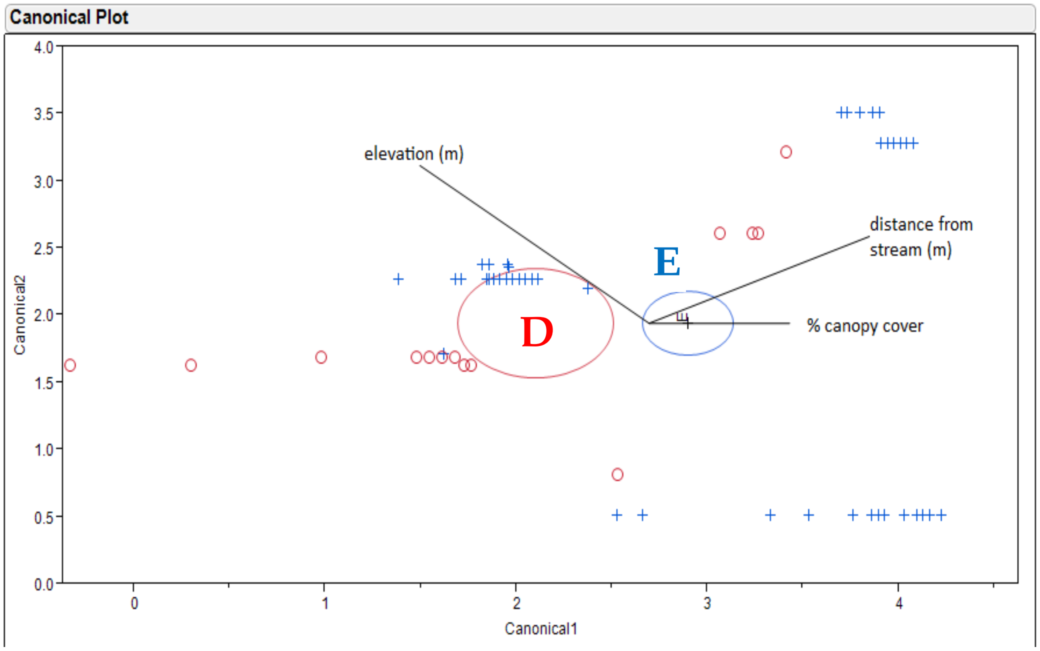


Figure 4. Discriminant analysis plot for terrestrial species. Although Wilks' Lambda results were statistically significant (Wilks' Lambda, F=3.5311, NumDF=3, DenDF=86, p=0.0182), there is little clustering of data present on the canonical plot. "D" is *M. resupinata* and "E" is *S. plicata.*

Spearman's Rank Correlation tests were run on mean antimicrobial activity of each species and means of each of the ecological variables of each species (canopy cover, distance to ground, circumference of tree host, distance from stream, elevation, and plant size). Canopy cover showed a positive correlation with antimicrobial activity (r=0.6, p=0.02848). Distance from stream also showed a positive correlation with antimicrobial activity (r=0.97, p<0.001).

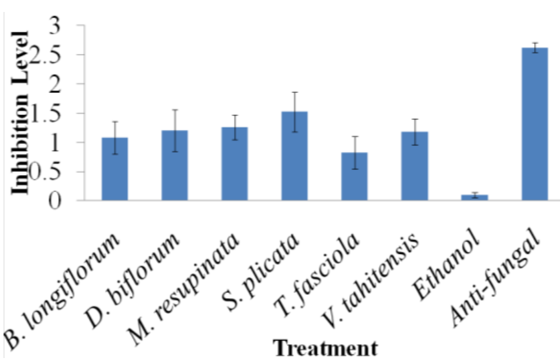


Figure 5. Mean yeast inhibition levels for eight treatment groups with standard error bars. The negative control, ethanol, showed the lowest inhibition with a mean of 0.1 and the positive control, anti-fungal cream, showed the highest inhibition with a mean of 2.62.

Phylogenetic analysis

A supertree of six orchid species of interest was created with prior data from Cameron et al. 1999. All ecological variables and antimicrobial activity were mapped onto supertree using Mesquite (Maddison and Maddison 2010). Possible trends were noted for each of the variables (Figure 6a-b). A Felsenstein's Contrasts Correlation of inhibition level with each ecological variable was analyzed. Inhibition level showed a significant correlation with % canopy cover (r=0.858, df=3, p=0.0314), distance from stream (r=0.845, df=3, p=0.0358), and plant size (r=0.834, df=3, p =0.0395).

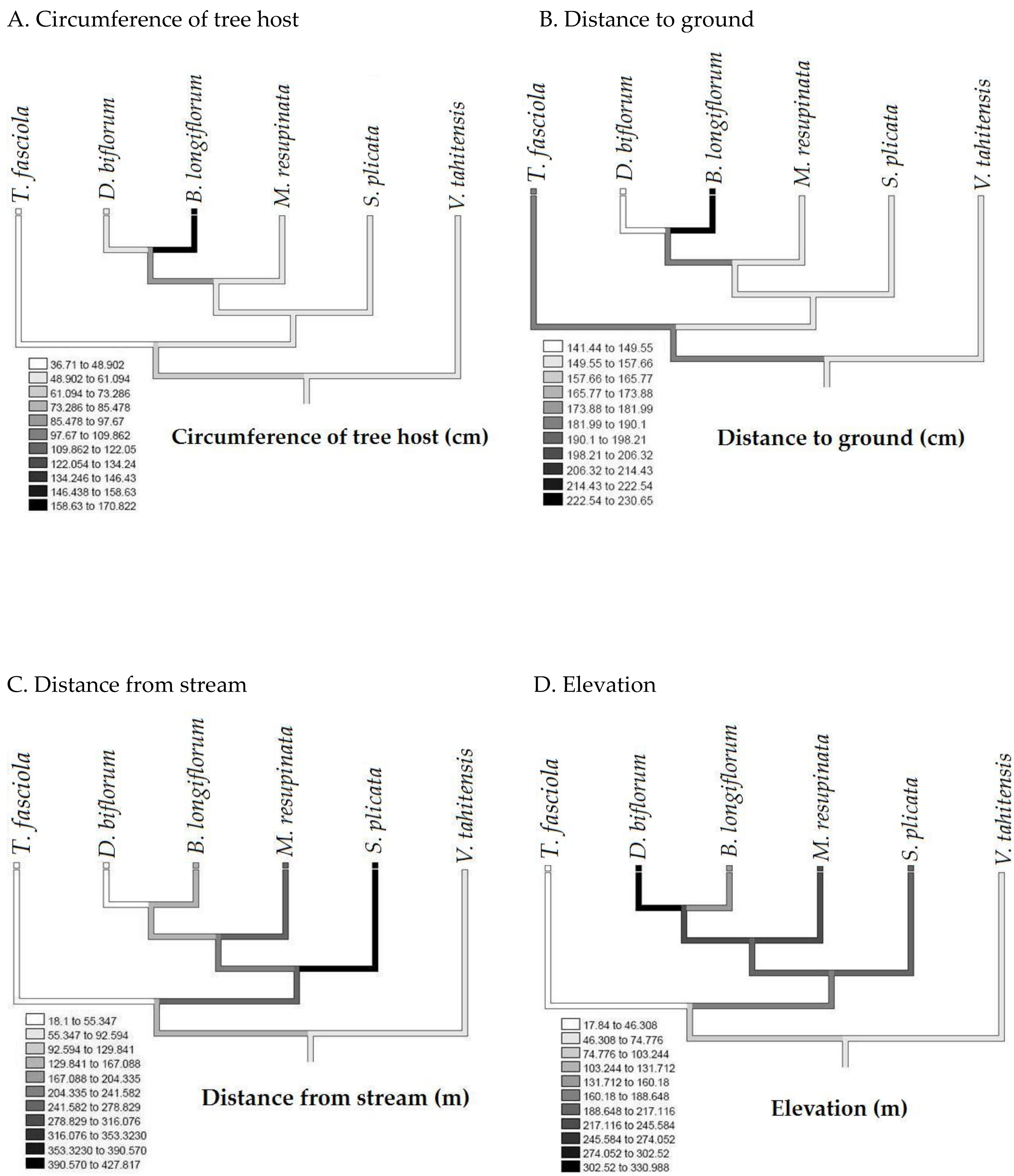
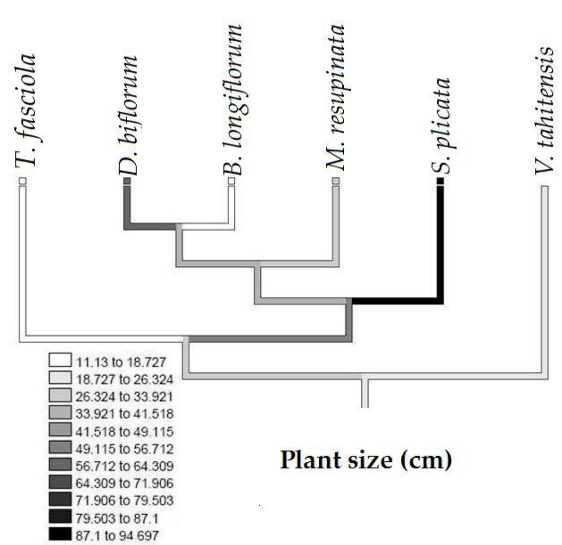
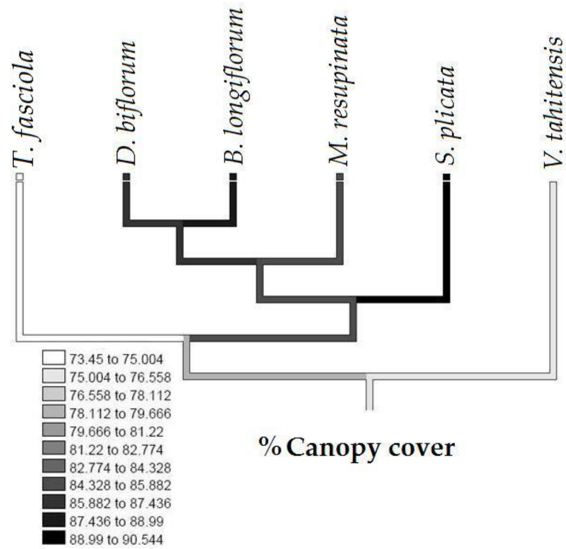


Figure 6a. Supertree of orchids with mapped characteristics. Phylogenies A-B have *T. fasciola, D. biflorum*, and *B. longiflorum* mapped. Phylogenies C-D have all species mapped except *V. tahitensis*.

E. Plant size



F. Canopy cover



G. Yeast inhibition

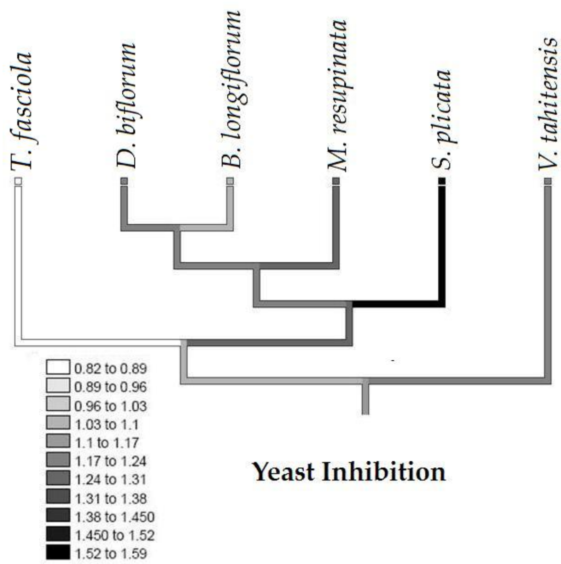


Figure 6b. Continued supertree with mapped phylogenies. Phylogenies E-F have all species but *V. tahitensis* mapped out. Phylogeny G has all species.

Discussion

Ecological survey

The results of this study suggest that ecological variables clearly distinguish the epiphytic species from one another, but do not distinguish as clearly between the terrestrial species. The three epiphytic species each had a distinguishing trait. *B. longiflorum* has a pseudobulb, a large organ found at the base of the leaf which is used for water storage. *T. fasciola* has reduced leaves and instead uses its roots for photosynthesis. Last, *D. biflorum* has leaves and does not have a pseudobulb.

The two terrestrial species, however, showed much less separation based on ecological variables. These two species are planted along the trails for aesthetic purposes (personal interview, Dr. Brent Mishler). As such, studies on these species are not based on natural propagation, but rather human influence. Since epiphytic species were not planted, this difference may suggest possible incongruity of data.

B. longiflorum has a pseudobulb, which swells when filled with water. In addition to water storage, pseudobulbs store mineral nutrients and carbohydrates for growth and reproduction following dormancy (Zimmerman 1990). The discriminant analysis graph showed a clear separation of *B. longiflorum* and *T. fasciola* based on distance from stream, with *B. longiflorum* inhabiting areas farther in distance from a stream than the latter species. Species of the genus *Taeniophyllum* have evolved the trait of leaflessness, and instead use roots for photosynthesis and nutrient storage (Carlsward et al. 2006). Studies on other species of leafless orchids, particularly *Dimerandra emarginata,* show that older stems increase the capacity to store water (Zotz 1999). My field work was conducted during the dry season, specifically during a drought. As such, data collected on orchids should indicate robust and older individuals. Nonetheless, limited access to water and lack of a distinct storage organ such as in *B. longiflorum* demands *T. fasciola* to be close to streams.

D. biflorum is the typical flowering plant, without a pseudobulb and with leaves. As shown on the discriminant analysis graph, it was most distinguished from the other two epiphytes by circumference of tree host, and to a lesser extent, canopy cover. From personal observation, *D. biflorum* preferred *Metrosideros collina* as a host tree, which also contains many other epiphytes. The other two species preferred *Hibiscus tiliaceus* and *Inocarpus fagiferus*, which had much fewer species of epiphytes. Competition of *B. longiflorum* and *T. fasciola* with other epiphytic species may be of lesser concern, due to fewer present in their respective habitats. Furthermore, competition may be limited by the ability to store nutrients by the pseudobulb of the former and to inhabit close distances from streams, for the latter.

The discriminant analysis graph for terrestrial species shows much less clumping of data with more variation between species. Nonetheless, the results are still statistically significant, and the two species are most distinguished by canopy cover. Since terrestrial species are planted along forest floors for decorative purposes (personal interview, Dr. Brent Mishler), there is little explanation for differences in canopy cover. The ecological conditions noted for these species are not due to natural propagation and therefore canopy cover differences may be due to chance.

Antimicrobial assay

While a one-way ANOVA showed statistical significance between my six study species and the negative and positive control, results are inconclusive with a Tukey-Kramer pair-wise comparison. My data suggests that all species have a relatively low inhibition level. This data is inconsistent with previous research on one species, *Vanilla,* and the compound vanillin, suggesting its role in inhibition of yeast, fungi, and bacteria growth in mangoes (Ngarmsak et al. 2006). However, *Vanilla tahitensis,* or Tahitian vanilla, is absent in the wild (Lubinsky et al. 2008). Instead, the distribution of it is restricted to French Polynesia and Papua New Guinea (Lubinsky et al. 2008). There have been no studies documenting possible antifungal uses of this particular species. Future studies may look at possible mutations of the vanillin compound found to be inhibiting yeast growth.

M. resupinata showed significant difference with both the positive and negative controls. With a mean of 1.26, this suggests that *M. resupinata* shows a low inhibition of yeast. Individuals of the genus *Malaxis* have been studied extensively for use in ethnobotany. In India, as discussed in "Charaka Samhita," *Malaxis rheedii* has been used for medicinal reasons (Kala and Senthikumar 2010). Furthermore, *Malaxis muscifera* and *Malaxis acuminta* have been used to treat fever and burning (Singh and Duggal 2009). However, there have been no studies on possible antimicrobial effects of *M. resupinata*. As such, it is difficult to reject or accept the validity of my antimicrobial test. Nonetheless, this study suggests a low level of yeast inhibition by *M. resupinata*, nowhere near the inhibition level of antifungal cream.

Studies on many species of orchids in China show that all orchids are myco-heterotrophic at some point in their life cycle (Liu et al., 2010). This means that they have a symbiotic relationship with fungi, in which they obtain nutrients from the fungus. Commonly, orchids rely on fungus for help with germination, since seeds are limited by energy demands (Yam et al., 2010). Since all orchids rely on fungi for growth, it is surprising that past studies on *Vanilla spp.* have shown antimicrobial effects. However, this may possibly explain the uniform low inhibition levels exhibited by species in my study, with the exception of two species. *T. fasciola* exhibited much higher antimicrobial activity than the other species in question, whereas *S. plicata* showed extremely low inhibition of yeast.

While there are no studies on these two species, what can be concluded by my results may be supported by individual field observations. *T. fasciola* was one of the smallest species I studied, whereas *S. plicata* was the largest. I noted that *S. plicata* exhibited a lot of leaf herbivory. By contrast, *T. fasciola* did not have any leaves, showed no herbivory, and instead used its roots as a photosynthetic organ. While leaf herbivory by insects may not give us a substantial measure of antifungal activity, it can serve as a proxy to possible reasons for antifungal activity. A past study on antimicrobial activity in relation to leaf herbivory showed that medicinal plants that were able to fend off mold (fungus) were not preyed upon by herbivores (Chan 2009). As such, leaf herbivory may serve as a proxy to predicting anti-fungal activity.

Correlations between inhibition level and each of the ecological variables suggests significant relationships between inhibition level with canopy cover and distance from stream. An Independent Contrasts correlation test showed correlations of inhibition level with canopy cover, distance from stream, and plant size. See phylogenetic analysis discussion for interpretation of results.

The maintenance of low yeast inhibition, coupled with only *M. resupinata* showing significant difference between the positive and negative control may be explained by poor experimental design. I based my study on past antimicrobial assays conducted on Mo'orea (Cox 2008 and Chan 2009). While one study produced significant results (Cox 2008), the other showed a similar result to mine, in which few species differed from the positive and negative controls (Chan 2009). Future studies should focus on fine tuning experimental design. Studies should allow yeast to grow for a longer period of time after application of extracts. Use of a more quantitative measuring system would be more appropriate and less biased. Instead of using the level 0-3 inhibition system, which is due chiefly to personal observation, using a microscope to count cells or colonies may be more pragmatic. In addition, use of commercial baking yeast, although readily available, may not have been a good choice. If given the opportunity, *Escherichia coli* should be run alongside commercial baking yeast to compare results of antibacterial and antifungal inhibition.

Phylogenetic analysis

Supertree of orchid species of interest suggested possible trends in some mapped ecological variables. However, only six species were studied, so trends are only suggestive and need to be further examined for evidence of relationships. In addition, only the phylogeny for yeast inhibition contained characteristics for all species.

Distance to ground, elevation, plant size, tree host, and canopy covering showed no distinct trends. Again, since only five species were mapped onto the supertree for these characteristics, it was difficult to see possible trends. For distance from stream, there was a general trend within the node containing *D. biflorum, B. longiflorum, M. resupinata,* and *S. plicata*. *T. fasciola* was an exception in this trend, an increase in distance from stream through evolutionary time.

The phylogeny for yeast inhibition showed a general trend of mid inhibition levels between *D. biflorum, B. longiflorum, M. resupinata,* and *V. tahitensis.* This could partially be explained by poor experimental design. However, there was a significant difference between these groups and *T. fasciola*, which had a considerably low yeast inhibition level. Similarly, *S. plicata* had an unusually high level of yeast inhibition. Possible reasons for low yeast inhibition for *T. fasciola* and high yeast inhibition for *S. plicata* were indicated in the antimicrobial discussion section.

The results for the Independent Contrasts correlations showed significant positive correlations of antimicrobial activity with canopy cover, distance from stream, and plant size. The advent of antimicrobial activity requires the production of secondary compounds. Secondary compounds are costly in metabolic energy expenditure, but Darwinian natural selection maintains that they must provide some benefit to the individual, or the trait would not survive evolutionary time (Williams et. al, 1989). Plant health may serve as a proxy to evaluating possible antimicrobial activity, since energy is needed. If a plant is not healthy, it may not generate enough energy to produce secondary compounds.

The percent of canopy covering over a species increased as there was more yeast inhibition. Plants that had a higher level of yeast inhibition were farther from streams. Last, larger plants were able to have a higher level of yeast inhibition. Since generating secondary compounds requires energy, it is expected that those plants that grow best are most adapted to generating secondary compounds. However, my results are somewhat confounding. It is expected that plants that have more canopy covering would grow best, thereby growing to the largest size. However, it is also expected that those individuals living closest to streams would also grow best; my results suggest otherwise.

Plants living in the tropical montane cloud forest (TMCF) can be used to suggest favorable environmental conditions, since 70% of endemic vascular plant species are found in these "islands within islands" (Meyer 1996). These cloud forests have high canopy covering from the clouds and have two water sources: fog interception and rainfall (Cavelier and Goldstein, 1989). As such, both water and canopy cover are greater in these two environments than compared with environments in lower elevations.

TMCFs can be used as proxies to good environmental standard for plants, suggesting high canopy cover to be positively correlated with inhibition level. However, this rejects my result that greater distances from streams show more yeast inhibition. It would be expected that closer distances from stream, and therefore wetter conditions, would be more favorable.

Plant size showed a significant correlation with inhibition level. I originally thought that plant size would serve as a standard for plant health. However, this would only be relevant within a species. Studies on plant size show that environmental regulation and competition play a large role in plant size (Pashigian 1984). As such, plant size deals with multiple parameters. There is no clear cut explanation for why there would be a correlation between plant size and inhibition level, since individuals within a species also differ between the two variables. This was not accounted for in my study.

A limited number of species were included in my phylogenetic analysis. Significant correlations through the Independent Contrasts test may be skewed due to *T. fasciola* and its extreme habitat preferences when compared with other species. As such, future studies should include a greater number of species and individuals to see distinct trends in phylogeny.

CONCLUSION

This study showed significant differentiation of epiphytic species from one another, and less differentiation of terrestrial species from one another. Antimicrobial testing showed no significant antifungal activity by any species, and phylogenetic analysis showed possible trends with distance from stream and antifungal activity.

Studying ecology is crucial to maintaining biodiversity, especially on islands. Extinction events are common on islands that are subject to common threats, such as invasive species. Understanding habitat preferences and key ecological variables of importance to species can help with habitat restoration and conservation when species are threatened. It is crucial to take these steps now instead of waiting until possible threats can take on full swing. Equally important to preserving biodiversity is to understand different properties of species, such as antifungal properties.

Phylogeny can give us clear hints as possible favorable environments and traits that may run throughout evolutionary time. As such, understanding phylogeny through character mapping may help with predicting conservation techniques for species we have limited data for.

Future studies should focus on increased sample sizes and a greater number of species. Ecological studies on naturally propagating vs. human planted terrestrial orchids may give us a better understanding of favorable environmental conditions for these species. In addition, more genetic work on various *Vanilla* species and vanillin in *Vanilla tahitensis* may be interesting to note.

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