Title

Morphological and Chemical Differences Among Populations of Hibiscus Tiliaceus Along an Elevational Gradient in Moorea, French Polynesia

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MORPHOLOGICAL AND CHEMICAL DIFFERENCES AMONG POPULATIONS OF HIBISCUS TILIACEUS ALONG AN ELEVATIONAL GRADIENT IN MOOREA, FRENCH POLYNESIA

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Abstract. Environmental variables change over elevational gradients and can isolate plant populations. Three varieties of *Hibiscus tiliaceus L.* exist on an elevational gradient in Moorea, French Polynesia. These variety's morphological and chemical characteristics are associated with the differences between their environments. Leaf and flower morphological data were collected and analyzed and found significant differences in petal width and length, anther count, burgundy center color, and leaf width and length between the varieties, particularly between the coastal and mountain types. The increased rainfall and lower temperatures of the high mountains lowered net primary production for the mountain variety as compared to the coastal and mid-mountain varieties. The mid-mountain variety was found to have greater competition for light than the other varieties. These can be linked to the significant size differences in leaves and inflorescence. Chemical differences were analyzed using anti-microbial and anti-cancer bioscreens. Significant differences were found in the anti-microbial bioscreen between the mid-mountain variety, which showed little activity, and both the coastal and mountain varieties. The anti-cancer screen showed increased activity from the coastal and mountain leaves. Chemical differences are influenced by increased insolation and chemical protection from microbes in wet conditions. Anthropological uses of the varieties are linked to the greater size of the coastal type. The plant populations studied are associated with the differences in their environments.

Key words: elevation; environmental pressures; bioscreen; Moorea, French Polynesia; flower; leaf

INTRODUCTION

Ecological variables that inhibit the transfer of genetic information can lead to differentiation between members of the same species (Wright 1943). The biological species concept explains that species are interbreeding groups that are reproductively isolated from other groups. They may be separated by prezygotic isolating factors, which can be factors such as physical environmental barriers and pollination by different insects (Coyne 1992). Different environmental pressures that occur over a variety of habitats can impede seed and pollen dispersal, which may isolate plant populations and drive them apart genetically. These environmental pressures can include differences in wind intensity, insect presence, and proximity to bodies of water (Kudoh and Whigham 1997). Environmental pressures change over elevational gradients and can affect the rate of pollen dispersal (Alonso 2005). The differences in pollen dispersal rates over elevational gradients isolate plant populations at different elevations (Heywood 1991). For example, studies have shown differences in taxa of pollinating insects over elevational gradients (Kearns 1992). Use of different insects for pollination would inhibit transfer of genetic information between different elevational populations. In this paper I discuss the differences in flower morphology, leaf morphology, medicinal properties, and anthropological uses in *Hibiscus tiliaceus* L.

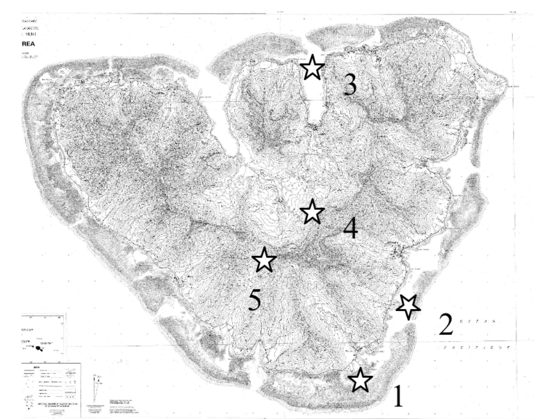

Fig. 1. Sites sampled in this study.

and how these differences are related to the differentiated varieties that have arisen due to elevational isolation. I chose to study *H. tiliaceus* for several reasons.

Hibiscus tiliaceus is a tree that grows in a variety of habitats, from the coastline and along streams up to 800-meter high mountain peaks in the South Pacific. It is a medium size tree up to 15 meters high and is common secondary forest tree. The flowers are 5-merous, with a yellow corolla, a burgundy center at the base of each petal, and staminal column joined to the base of the ovary (Petard 1986, WHO 1998). *Hibiscus tiliaceus* is reported to have a variety of medicinal properties that are used by many peoples in the South Pacific. Medicinal chemicals in *H. tiliaceus* have positive effects on fractured bones, sprained muscles, gonorrhea, skin diseases, eye infections, and stomachaches. The peoples of over 10 South Pacific island groups use the tree medicinally (WHO 1998).

Hibiscus tiliaceus occurs in three wild varieties on the island of Moorea, French Polynesia. These varieties occur over an elevational gradient with *H. tiliaceus* var. *henryanus* occurring along the coastline and low-mountain streams, *H. tiliaceus* var. *sterilis – Paritium trilobatum* occurring in the mid-mountain ranges, and *H. tiliaceus* var. *sterilis* occurring on the higher peaks of the island (Franc 2001). The differences in environmental pressures along an elevational gradient have affected the morphology of these varieties (Petard 1986). Differences in environments along this elevation gradient are associated with these three varieties. The following questions are addressed in this paper: Are populations of *H. tiliaceus* along an elevation gradient differentiated in: (1) flower morphology, (2) medicinal properties, (3) leaf morphology, and (4) anthropological uses between the different varieties.

METHODS

Five study sites were used for this study. The first three are sample sites for *H. tiliaceus* var. *henryanus*, the coastal and stream variety (Fig. 1). The first is adjacent to the Gump Station on the coast. The second is on the Viaroro Stream 300 meters from the coast. The third is 1000 meters north of the Viaroro Stream at the intersection of a small stream and the coast. The fourth is the sample site for *H. tiliaceus* var. *sterilis – Paritium trilobatum*, the mid-mountain range variety, adjacent to Marae Tetiiroa near the Belvedere lookout at an elevation of 100 – 180 meters. The fifth is the sample site for *H. tiliaceus* var. *sterilis*, the high-mountain variety, at the top of Three Coconut trail at an elevation of 394 meters.

Flower morphology

Flowers were collected from each of the five study sites between the hours of 11AM and 4PM over the course of multiple days. Approximately 15 trees were sampled of each variety except for *H. tiliaceus* var. *sterilis – Paritium trilobatum*, in which 10 trees were sampled due to the difficulty of finding this variety. 25 flowers were collected from each species by hand picking, using a noose attached to PVC piping, or collecting after dropping from treetops. The petals were removed from each flower and maximum petal length and petal width was recorded. The maximum length and maximum width were recorded for the burgundy center area on each petal. The direction of petal curve was recorded for each petal. The anthers were removed from the staminal column and counted for each flower. The separation or closure of the five stigma parts was also recorded for each flower.

Data for each aspect of the flower were analyzed with JMP software and a one-way ANOVA and student's t-test was used to determine means, standard deviation, standard error, and p values.

Leaf morphology

Leaves were collected from each of the five study sites between the hours 9AM and 2PM over the time period of a week. 50 leaves were collected from each variety using at least 10 trees from each variety. No more than 5 leaves came from any one single tree. Leaves were all exposed to full sunlight and were at similar stages of mature development. Maximum length and width measurements were taken from each leaf.

Data for each aspect of the leaf were analyzed with JMP software and a one-way ANOVA and student's t-test was used to determine means, standard deviation, standard error, and p values.

Medicinal properties

Extract preparation. Leaf and flower samples were collected from each of the three varieties of *H. tiliaceus*. All plant material was air-dried and then ground into a fine powder.

The leaf and flower powder was each weighed and extracted with 70% ethanol to attain a .1g/mL concentration of plant extract (Vlietinck 1995). The leaf and flower suspensions from each variety were each stored at $4^{O}C$ for the period of 3 days.

Anti-cancer bioscreen. Brine shrimp were hatched and aged 48 hours. 10mL of salt-water and 20 brine shrimp were added to 9, 100mm Petri dishes. 0.2 mL of the leaf and flower extracts of each variety were added to different Petri dishes. 0.2 mL of 70% ethanol was run as a negative control and 0.2mL of *Catharanthus roseus* extract, a proven anti-cancer plant prepared in the same fashion as the leaf and flower extracts, was run as a positive control. After a period of 24 hours the number of brine shrimp killed in each Petri dish was recorded. This test was replicated 6 times.

Anti-microbial bioscreen. 20 mL of sterilized liquid peptone agar was added to each of 24, 100 mm Petri dishes and stored at $4^{O}C$. The Petri dishes were inoculated with a sterile suspension of yeast. 5mm filter paper disks were inoculated with 0.2 ml of each of the 6 plant extracts or control substances. The plant extract disks from each variety were placed on different dishes inoculated with yeast (Dimayuga and Garcia 1991). A disk of evaporated ethanol was used as a negative control and a disk of Ciprofloxacin was used as a positive control. All plates were incubated at $37^{O}C$ for 24 hours. The zones of inhibition around the disk were measured at the end of the period. This test was replicated 9 times.

Data for each aspect of the medicinal bioscreens were analyzed with JMP software and a one-way ANOVA and student's t-test was used to determine means, standard deviation, standard error, and p values.

Environmental data

Environmental data were collected over a 3-week period from late October to mid-November. Sites 1, 4, and 5 were sampled for the three varieties in the form of light (lux), wind speed (m/s), soil temperature (degrees Celsius), and air temperature (degrees Celsius). Data were collected over the period of five different days between the hours of 10AM and 3PM. Three data collections were competed each day with at least 2 minutes separating the samples. Maximums and minimums were recorded for each light and wind sample. JMP software was used to determine means and standard deviations.

Anthropological uses

Local healers were consulted to determine whether the Polynesians used each of the three varieties of *H. tiliaceus* in different ways or for different medicinal purposes.

RESULTS

Flower morphology

The average petal width ranged between 5.4 cm and 7.5 cm (Fig. 2). Petal width is significantly different at $p < .01$ between the coastal, mid-mountain, and mountain varieties. The average petal length ranged between 6.5 cm and 8.0 cm (Fig. 2). The mountain petal length is significantly different at $p < .01$ from the coastal and mid-mountain varieties, which are not significantly different from each other.

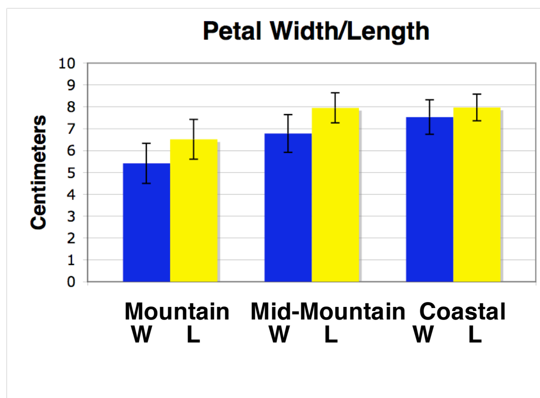

Fig. 2. Average petal width decreases as population site elevation increases, all varieties are significantly different. Mountain petal length is significantly smaller than the mid-mountain and mountain varieties, which are not significantly different.

The average burgundy center width ranged from 14 mm to 18 mm (Fig. 3). The mountain burgundy center width is significantly different at $p < .01$ from the coastal and mid-mountain varieties, which are not significantly different from each other. The average burgundy center length ranged from 17 mm to 23 mm (Fig. 3). Burgundy center length is significantly different at $p < .01$ between the coastal, mid-mountain, and mountain varieties.

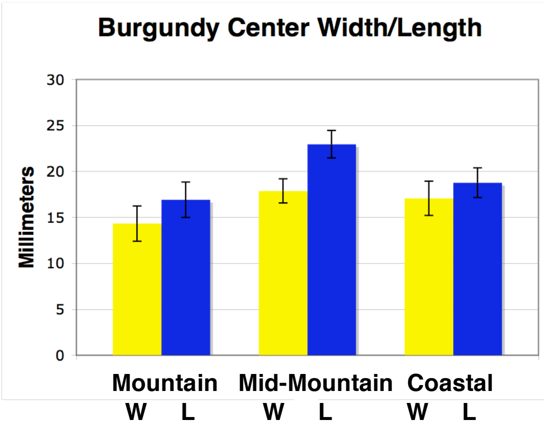

Fig. 3. Burgundy center width and length are largest in the mid-mountain variety. The width in the mountain variety is significantly smaller than the mid-mountain and coastal varieties. The lengths of all varieties are significantly different.

The average anther count ranges from 82 anthers to 92 anthers (Fig. 4). The coastal and mountain varieties are significantly different at $p < .01$, while the coastal and mid-mountain varieties are at $p < .05$. The mid-mountain and mountain varieties are not significantly different.

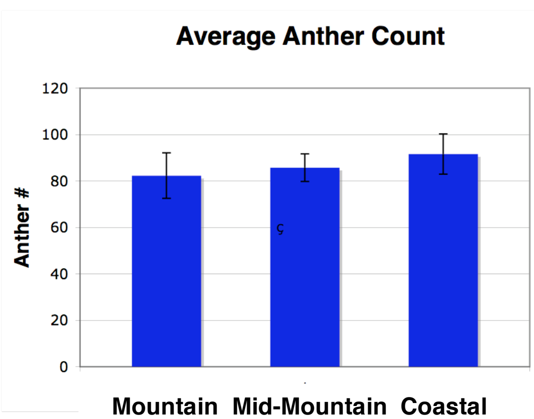

Fig. 4. Average anther count decreases as population elevation increases. The coastal variety average anther count is significantly different from the mountain and mid-mountain varieties, which are not significantly different from each other.

The open stigma parts average ranges from 4% to 96% (Fig. 5). The coastal variety is significantly different at $p < .01$ from both the mountain and mid-mountain varieties, which are not significantly different from each other.

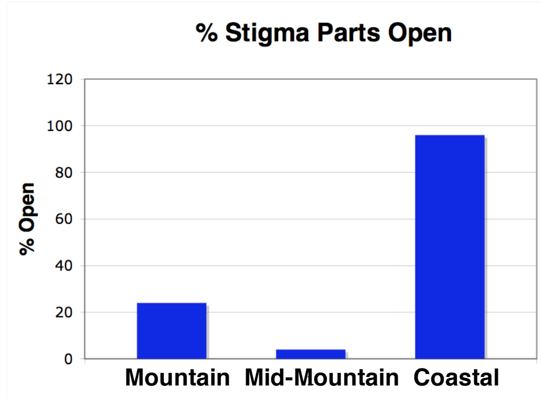

Fig. 5. The coastal percentile of stigma parts open is significantly different from both the mountain and mid-mountain varieties, which are not significantly different from each other.

The rightward petal direction percentage ranges from 40% to 64% (Fig. 6). The varieties are not significantly different.

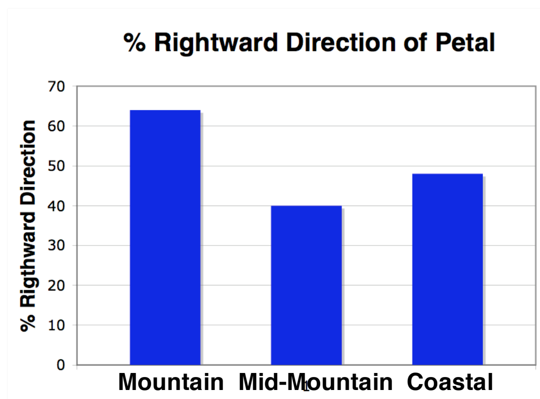

Fig. 6. The percentile of rightward direction of petal curve is not significantly different between the varieties.

Leaf morphology

The average leaf width ranges from 16.2 cm to 22.0 cm (Fig. 7). The mountain variety is significantly different at $p < .01$ from both the coastal and mid-mountain varieties, which are not significantly different from each other. The average leaf length ranges from 16.5 cm to 21.9 cm (Fig. 7). The mountain variety is significantly different at $p < .01$ from both the coastal and mid-mountain varieties, which are not significantly different from each other.

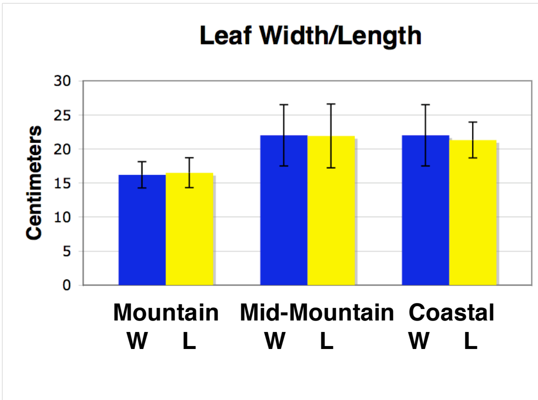

Fig. 7. The width and length of the mountain variety are significantly smaller than both the mid-mountain and coastal varieties, whose widths and lengths are not significantly different from each other.

Medicinal properties

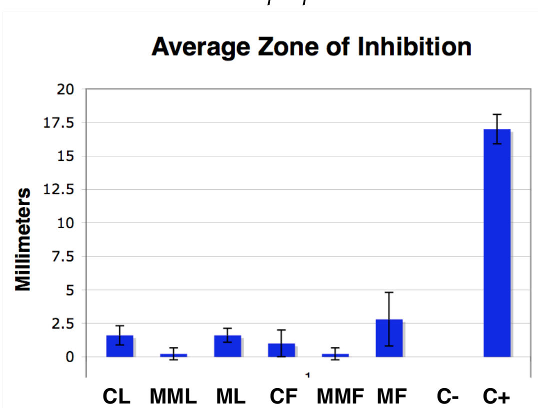

Fig. 8. Average zone of inhibition for the mid-mountain leaf was significantly less than the coastal and mountain varieties, which were not significantly different. Average zone of inhibition for the mountain flower was significantly greater than both the mid-mountain and coastal varieties, which were not significantly different from each other.

Anti-microbial bioscreen. The average radius of the zone of inhibition for the flower varieties ranges from .22 mm to 2.8 mm (Fig. 8). The mountain variety flower effectiveness is significantly different from the mid-mountain variety at $p < .01$ and coastal at $p < .05$. The coastal and mid-mountain varieties are not significantly different. The average radius of the zone of inhibition for the leaf varieties ranges from .22 mm to 1.6 mm (Fig. 8). The coastal and mountain varieties leaf effectiveness is significantly different from the mid-mountain variety at $p < .01$. The mountain and coastal varieties are not significantly different.

Anti-cancer bioscreen. The average percent brine shrimp killed for the flower varieties ranged from 53% to 67% (Fig. 9). The varieties are not significantly different. The average percent brine shrimp killed for the leaf varieties ranged from 60% to 87% (Fig. 9). The mountain variety is significantly different from the mid-mountain variety at $p < .01$, and the coastal variety at $p < .05$. The mid-mountain and coastal varieties are not significantly different.

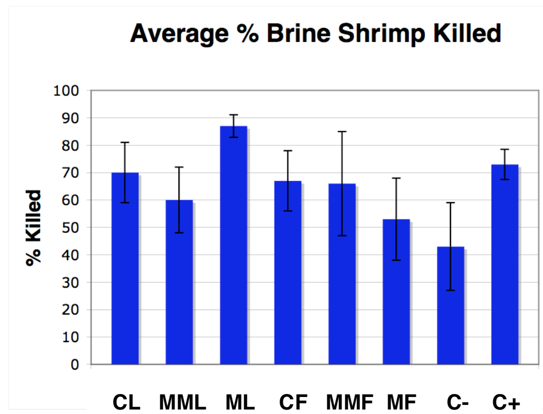

Fig. 9. Average percent brine shrimp killed for the flower varieties was not significantly different for any variety. Average percent brine shrimp killed for the mountain leaf variety was significantly different than the mid-mountain and coastal varieties, which were not significantly different from each other.

Environmental data

The average and standard deviation for light (lux) measurements for the coastal site is 542 lux (318), mid-mountain 290 lux (241), mountain 559 lux (360). The average wind speed (m/s) and standard deviation for the coastal site is 1.2 m/s (1.0), mid-mountain .59 m/s (.70), mountain 1.1 m/s (1.8). The average soil temperature (^{O}C) and standard deviation for the coastal site is 27.6 ^{O}C (2.0), mid-mountain 23.8 ^{O}C (1.4), mountain 23.2 ^{O}C (1.7). The average temperature (^{O}C) and standard deviation for the coastal site is 31.1 ^{O}C (5.0), mid-mountain 29.0 ^{O}C (2.9), mountain 29.2 ^{O}C (2.5).

Anthropological studies

Two interviews were conducted in Moorea on the different medicinal and anthropological uses of *H. tiliaceus* varieties. The coastal variety is most used by the native peoples due to its large size. The large amounts of wood are best for boat building and the large leaves can serve purposes for cooking, eating, and use as bandages. The smaller mountain flowers were not used for these purposes due to their small size and remote comparative location. The coastal flowers can be used to make a boiling water infusion, which is cooled until warm and drank for a period of three days. This can be used as a remedy for menstrual abnormalities. The mid-mountain variety bark can be used to make rope (Papa Mita, personal communication, Nov. 4, 2006). The coastal variety roots can be pulverized and used as a salve for eye infections. Coastal flowers can also be boiled and the resulting gel can be applied to the skin to treat itching, infections, and jellyfish stings. The varieties are recognized by the native peoples as being different types of hibiscus, however the cuttings are used rather than seeds due to the frequency of the tree not inheriting the morphology of the parent tree (Hinano Murphy, personal communication, Nov. 10, 2006).

DISCUSSION

The differentiated varieties of *H. tiliaceus* on Moorea show many statistically significant differences in morphology and chemical effectiveness. The environmental conditions that change from the coastline to the mountaintops has had a large influence on plant characteristics (Korner 1989).

The leaf averages of length and width show coastal and mid-mountain leaves both significantly larger than the mountain variety. Leaf size has been shown to decrease with increasing elevation (Velízquez-Rosas, Meave, and Vízquez-Santana 2002). Net primary productivity decreases as mean annual precipitation increases in tropical islands (Schurr and Matson 2001). The average rainfall of the mountaintops in Moorea is 4.7 times greater than at the coast (800 cm/year mountains, 170 cm/year coast) (Mueller-Dombois and Fosberg 1998). This would explain why the mountain variety has smaller overall leaf size. Mountain conditions are usually more extreme over the course of a year including lower overall temperatures and greater rainfall. The environmental data collected showed that average temperature and soil temperature were lower by 2 degrees and 4 degrees Celsius respectively. Light data was similar but yearlong data shows less light over the course of the year rather than a 3 week period (Mueller-Dombois and Fosberg 1998). This means that overall the mountain peaks present a cooler environment that stresses the plant with heavy rain and low light levels. This would explain why the mountain variety has significantly smaller leaves than the other varieties.

The mid-mountain leaves are similar in width and length to the coastal leaves but occur at a higher elevation. The coastal variety is one of the tallest trees on the coastal habitat while the mid-mountain variety faces greater competition for sunlight from taller trees such as the Tahitian Chestnut. It has been shown that with decreasing insolation leaf size increases, and the mid-mountain variety has large leaves in order to be exposed to as much sunlight as possible (Ackerly, Knight, Weiss, Buron, and Starmer 2002).

Overall the average lengths and widths of the petals can determine flower size. The petal width was shown to decrease as elevation increases and all three varieties were significantly different. Petal length showed similar measurements for the coastal and mid-mountain varieties with significantly smaller measurements for the mountain flowers. Again, since the mountain conditions are stressful and primary productivity is less, smaller flower size can be anticipated. Mid-mountain flowers are overall smaller than the coastal flowers because the greater competition experienced at the mid-mountain ranges leaves less energy for floral size. The coastal variety has more energy to devote to reproductive organs. Anther count closely mirrors trends seen in petal width and overall flower size. The petals protect and house the staminal column. The size of the staminal column is therefore a result of flower size and the number of anthers that can fit on a smaller staminal column is less than the amount that can fit on a larger one. Anther counts on a smaller flower will be less than that of a larger flower. The rightward or leftward curve of the petals in a flower is the same for all the petals in a specific flower. The overall percentage of rightward or leftward curve in the petals of a variety are not significantly different from one another. This is a random effect that does not seem to be influenced by environmental factors.

The yeast and brine shrimp bioscreens show increased toxicity for the coastal and mountain variety leaves and flowers. Statistically the coastal and mountain leaves showed the greatest degree of significant separation of the bioscreens when measuring the effectiveness against yeast. Mountain and coastal leaves also showed increased toxicity in the brine shrimp bioscreen. The mountain and coastal varieties have little competition and therefore the leaves receive a large amount of direct UV-B light in periods of low cloud cover (Sullivan, Teramura, and Ziska 1992). Leaves may expel salts and toxins from the interior of the leaf to protect the inner tissue from extreme insolation (Karimi and Ungar 1989). This leads to higher overall toxicity in leaves exposed to increased sunlight.

The extract of the mountain flower showed significantly increased anti-microbial activity. Anti-oxidants such as DPPH have been found in the flowers of *Hibiscus sabdariffa L.* (Tseng, Kao, Chu, Chou, Lin Wu, and Wang 1997). This antioxidant has been shown to have anti-microbial effects (Yildirim, Mavi, and Kara 2003). The ethanol extracts may have attracted non-polar phenolic compounds from the flower material, which could have also played a role in the anti-microbial bioscreen. The mountain flower in particular could have contained more of these anti-oxidants and phenolic compounds in order for increased disease resistance due to the heavy rainfall and stressful conditions of the mountain ridges (Nicholson and Hammerschmitt 1992).

The anthropological uses of the different varieties seem to be limited to the location of the people. The great majority of Moorean people live near the coast, and almost all of the uses of the tree are limited to the coastal variety. The coastal tree is larger and can provide better uses for cooking and boatbuilding than the smaller mountain variety. The coastal variety can grow larger due to the increase in primary productivity (Schurr and Matson 2001). The shoots of the mid-mountain variety are straight and have smooth bark, which is ideal to strip and make rope. These shoots may exist to climb straight up towards the sky in an effort to collect more sunlight due to competition. Medicinally the use of the coastal variety could be because the local knowledge of the mountain variety was lost after the population moved to the coast, or the other varieties do not present greater medicinal value for the afflictions treated.

The use of cuttings instead of seeds may present insights into whether the varieties are genetically different or merely ecophenotypes, in which all the trees have the same genotype but differ phenotypically due to the environment. Further studies should be conducted to investigate.

The variation in morphology and medicinal effects of *H. tiliaceus* across an elevational gradient in Moorea can provide insights into gene flow and perhaps plant plasticity. The different environmental pressures placed on genetically isolated populations eventually lead to adaptations and differences between them. In the case of this species, sea-dispersed seeds have populated the tropical regions of the world with *Hibiscus* species that have differentiated from each other after gene flow between the populations have ceased (Takayama, Kajita, Murata, and Tateishi 2006).

In conclusion, this is a classic case of observing differences between isolated populations due to differing environmental pressures. It is interesting because the three microhabitats, which forced this single species into three distinct varieties, can all be accessed and studied in a single day. Further studies in plasticity, and perhaps self-pollination in the varieties presenting significant stigma parts closure should be investigated.

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