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Habitat Heterogeneity of *Cycas micronesica* Seed Chemistry in Guam's Forest

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Abstract—Understanding spatial structure of secondary metabolites with abiotic and biotic chorographers of chemical heterogeneity in natural populations may allow deeper insights into their function at the plant and community levels. Cycads are an ancient group of plants characterized by opulent secondary chemistry. *Cycas micronesica* K.D. Hill seed chemistry was determined in seven populations throughout Guam to determine if steryl glucosides would segregate among the habitats. Further, we measured covariables to shed light on habitat characteristics that may help explain the heterogeneity. Seed gametophyte content of steryl glucosides stigmasterol β-D-glucoside and β–sitosterol β-D-glucoside and their precursors stigmasterol and β–sitosterol significantly differed among habitats. Cycad plants from the littoral habitat on sandy soils produced higher seed concentrations of these compounds than the six upland habitats. Concentration among the other six habitats also differed, but was less pronounced. None of the measured habitat characteristics correlated with seed chemistry across the continuum of habitats. However, the littoral site was the most resource-scarce site. Our results are valuable for informing ongoing attempts to answer critical conservation, ecology, and neurotoxicity questions. Resource manipulation studies are urgently needed to validate the hypothesis that resource limitations increase production of these neurotoxins in this endangered species.

Introduction

The chemical ecology of a plant species population is characterized by heterogeneity within individual plants, among plants within each habitat, and among the various habitats that support the population. This spatial structure is partly explained by the ability of individual plants to respond to environmental heterogeneity with phenotypic plasticity of secondary chemistry (Brenes-Arguedas & Coley 2005, Callaway et al. 2003). Phenotypic plasticity resulting from plant ontogeny and heterogeneous size (Bowers & Stamp 1993, Wright &

McConnaughay 2002) and season (Cantonwine & Downum 2001, Covelo & Gallardo 2001, Scogings *et al*. 2004) further magnify chemical spatial heterogeneity at any point in time. Spatial structure of plant chemistry is additionally explained by genetic diversity, especially if habitats that collectively define the population are fragmented (Adler et al. 1995).

Cycads are believed to be the oldest of the extant seed bearing plants, and studying this primitive plant group should provide invaluable answers for increased understanding of various facets of contemporary plant biology (see Brenner et al. 2003). One of those areas may be chemical ecology and the influence of metabolites on interactions of plants with co-occurring flora and fauna. Indeed, *Cycas micronesica* and other cycad species contain a plethora of secondary compounds, some of which have been shown to elicit pathological responses in mammalian systems (Norstog & Nicholls 1997). The high incidence of neurological diseases known as amyotrophic lateral sclerosis-parkinsonism dementia complex (ALS-PDC) has been studied for decades on the island of Guam and elsewhere in the Western Pacific (Kurland 1988, Kurland et al. 1994). The strongest epidemiological correlate with empirical validation for the ALS-PDC of Guam is direct consumption of the *C. micronesica* seed gametophyte as flour (Kurland 1993, Marler et al. 2005a, Shaw et al. 2006), although there are other unsubstantiated hypotheses (e.g. Cox & Sacks 2002). We have isolated numerous gametophyte toxins and determined that the most noxious in relation to neurodegeneration are a variety of phytosteryl glucosides (Shaw et al. 2006). Thus, we have been studying this toxin group and some of its sterol precursors in attempts to understand more about the function of these cycad metabolites and their role in onset of ALS-PDC.

Learning more about the chemical ecology of Guam's cycad population and characterizing the habitat characteristics that may influence chemical heterogeneity recently reached a status of exigency. While the species has long been protected by listing within the Convention on International Trade in Endangered Species of Wild Flora and Fauna (CITES), it acquired provisional endangered red-listing status by the International Union for Conservation of Nature and Natural Resources (IUCN) in May 2006 (Marler et al. 2006a). The current alien arthropod assault on the population that forced this designation was incepted a mere three years ago, revealing the acute nature of ongoing population mortality. Many answers to chemical ecology questions about coevolutionary interactions and the role of metabolites on co-occurring flora and fauna are locked within this species population. Any delay in reporting results then pursuing further questions may render these answers lost for perpetuity.

We have studied spatial distribution of *C. micronesica* seed chemistry within habitats, and reported that variation of steryl glucosides is greatest among tissue types within seeds, intermediate among plants within a habitat, and least among locations within plants (Marler et al. 2005b). In this paper we describe the spatial structure of seed chemistry among seven habitats of Guam's population. This is the first study to assess the spatial heterogeneity of secondary chemistry above the habitat level in natural populations of any cycad species. We addressed the following questions: would the cycad seed sterols and steryl glucosides segregate among the various habitats? Would the resource-limited sandy littoral habitat segregate from the resource-abundant upland habitats? Does phenotypic variation of seed chemistry correlate with habitat proximity at different spatial scales? Would seed chemistry of the southern habitat closest to the epicenter of historical ALS-PDC incidence segregate from that of central and northern habitats? This study was not designed to make predictions concerning the relative level of genetic versus environmental control of chemical phenotype among the habitats. However, we measured abiotic and biotic variables in each habitat to explore their relation to habitat differences in cycad seed chemistry. This report provides the foundation to continue the urgently needed ecological research on this rapidly diminishing plant population.

Materials and methods

STUDY LOCATION AND SPECIES

The study of the spatial patterns in seed chemistry of *C. micronesica* was conducted on the island of Guam. The northern portion of the island is an uplifted coralline limestone plateau rising 184 m above sea level (masl) at the northern extreme. The southern region is comprised of volcanic mountains rising to 406 masl. These mountains gently descend to the shoreline and are dissected with ravines cut by eons of erosion.

The climate is typical of a tropical marine lowland forest (NOAA 2005). Average temperature varies little, with daily minimum ranging from 23-25 ºC and daily maximum ranging from 29-31 ºC. Yearly rainfall is ca. 2500 mm, with about 55 percent occurring during the rainy season from mid-July to mid-November. Trade winds are chronic, come from the east or northeast, and average ca. 4 m/s. Slope and aspect of a habitat determine the extent that these trade winds influence plant physiology. All habitats with eastern facing slopes are strongly influenced by trade winds regardless of their location on the island. The habitats directly along the eastern coastline are also influenced by chronic aerosol deposits of salt water spray. The most active tropical cyclone basin worldwide is in the western region of the North Pacific, giving Guam the dubious distinction of having the greatest risk of exposure to tropical cyclone force winds of anywhere in the United States (Marler 2001). These frequent tropical cyclones are called typhoons throughout Micronesia, and largely define the physiognomy of Guam's forests. Stone (1971) proposed the term "typhoon forests" to describe the island's forests because of the "monotonous regularity" of these typhoons.

The *Cycas* genus is considered basal among cycads (Norstog & Nicholls 1997, Brenner et al. 2003). *Cycas micronesica* is the only native gymnosperm in the Mariana Islands. Moreover, it is the only native *Cycas* species on United States soils. The pachycaulis stems of this arborescent cycad are comprised of

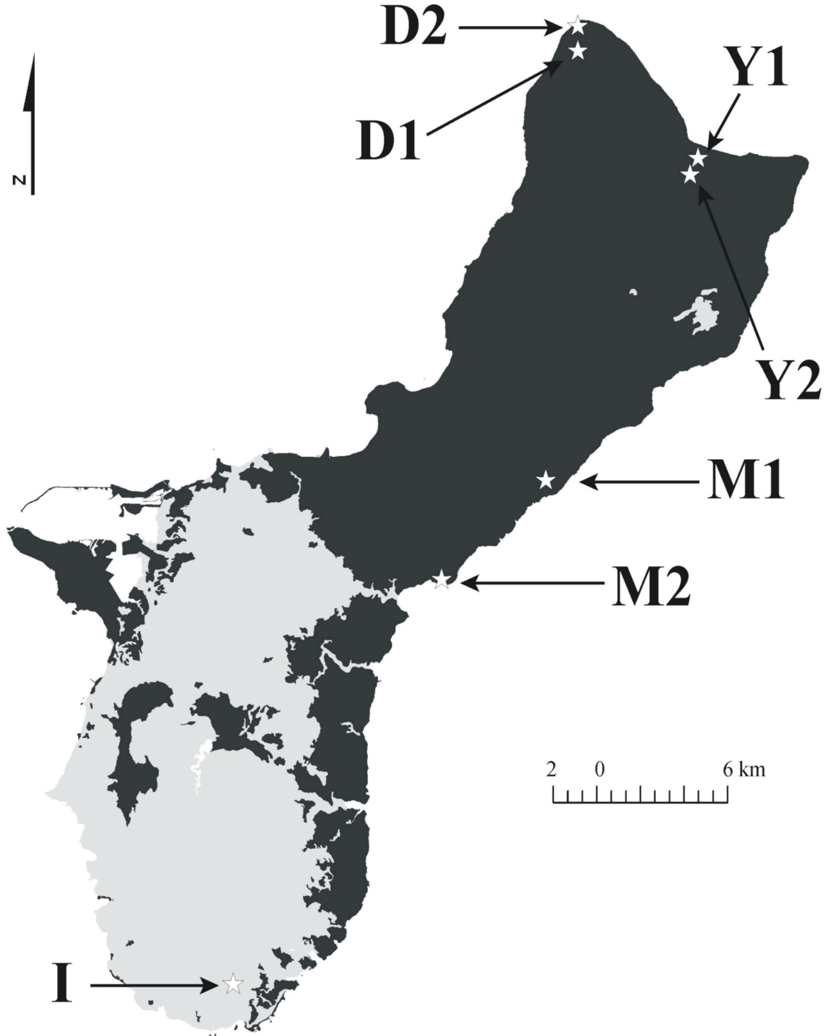

Fig. 1. Map of Guam with habitat locations. Dark areas comprised of various limestone soils. Gray area comprised of various volcanic soils. D = Dededo, I = Inarajan, M = Mangilao, Y = Yigo.

secondary vascular tissue cylinders between persistent parenchymatous pith and cortex tissues (Norstog & Nicholls 1997). Pinnately compound leaves are large and retained for several years in the absence of typhoon or herbivory damage. This and other cycads form symbiotic relationships within a tripartite system with cyanobacteria and mycorrhizae (Marler et al. 2005a).

The threats to this endemic species were restricted to habitat loss until recent activity of introduced herbivores. Feral ungulates impose a major threat to the species in some habitats, and cause structural damage to the plants. Feral deer (*Cervus mariannus* Desmarest) consume primarily leaves (Wiles et al. 1999) and feral pigs (*Sus scrofa* L.) consume fallen seeds and stems following any form of physical damage to accessible stem tissues (Marler et al. 2005*b*). Several arthropods that feed on various tissues of this cycad (Marler & Muniappan 2006) were co-existing with the cycad population prior to 2003 and posed no population-level threat. However, the scale *Aulacaspis yasumatsui* Takagi (Haynes 2006) was first found in 2003 and escaped from the urban landscape in less than one year. More recently the cycad blue butterfly (*Chilades pandava* Horsfield) was introduced. The combined attack of these two alien arthropods is currently threatening the continued existence *C. micronesica* on Guam. The epidemic level of mortality in the Guam population due to these alien pests has prompted the IUCN to designate a provisional conservation endangered status for *C. micronesica* (Marler et al. 2006a). This cycad population may soon qualify for what Janzen (1986) refers to as the "living dead."

HABITAT SELECTION

We selected seven diverse habitats to study spatial structure of *C. micronesica* seed chemistry. The Inarajan habitat is located within the forest of the Ajayan River ravine ca. 2 km from the southern tip of the island (Fig. 1). This was the only study site located on acidic volcanic soils, and was the most isolated site. These soils formed in residuum derived from tuff (very fine, kaolinitic, isohyperthermic Oxic Haplustalfs) and tuffaceous sandstone (Clayey, montmorillonitic, isohyperthermic, shallow Udic Haplustolls) (Young 1988). The forest is highly protected by terrain from exposure to trade winds, and the river elevation at this site is about 25 masl (Table 1). We sampled 17 individuals along the river banks and on the east face of the ravine with aspect of ca. 240°.

The two sites in the Mangilao municipality are located on the east coast of the northern limestone plateau. Mangilao1 is located south of Taguan Point about half way up a series of limestone terraces at about 60 masl (Fig. 1, Table 1). The Ritidian soil series formed in slope alluvium, loess, and residuum overlying coralline limestone (Clayey-skeletal, gibbsitic, nonacid, isohyperthermic Lithic Ustorthents; Young 1988), and aspect was ca. 120°. We sampled 36 individuals at this site. Mangilao2 is located 6.25 km south of Mangilao1 at the edge of the cliff located on the north side of Pago Bay (Fig. 1). The habitat is a homogeneous 40 masl. The soil is Ritidian as with Mangilao1, and the cycad plants were

Table 1. Habitat abiotic characteristics in typhoon forests on the island of Guam. These factors may influence long-term genetic drift or short term phenotypic plasticity of *Cycas micronesica* seed chemistry.

Habitat	Soil series	Soil chemistry	Soil water permeability	Elevation (masl)[A]	Wind exposure[B]	Percent open sky[C]
Inarajan	Akina-Agfayan	acid	low	25-50	very low	35±4
Mangilao1	Ritidian	alkaline	high	55-65	high	26±2
Mangilao2	Ritidian	alkaline	high	40	high	39±5
Yigo1	Ritidian	alkaline	high	150-155	moderate	58±4
Yigo2	Guam	alkaline	high	145	moderate	52±4
Dededo1	Ritidian	alkaline	high	90-100	low	57±3
Dededo2	Shioya sand	alkaline	very high	<10	very low	18±2

[A] m above sea level.
[B] Relative ranking based on direct exposure to eastern tradewinds.
[C] mean ± SE.

restricted to pockets of exposed karst. We sampled 16 individuals at this site. Both of the Mangilao sites are fully exposed to trade winds and aerosol salt spray that impacts the east coast.

The two sites in the Yigo municipality were selected to provide close proximity but contrasting soil series. They are located on the Andersen Air Force Base near the northeast coast of the island, and near the type locality for the species (Hill 1994). Yigo1 is a limestone forest habitat on Ritidian soils at the edge of the cliff at ca. 150 masl (Fig. 1). Since the cliff is about 1 km from the shore and the site is protected due to the orientation of the shoreline, it is not as exposed to the trade winds as are the Mangilao sites (Table 1). Aspect was ca. 210°, and we sampled 35 individuals at this site. Yigo2 is located about 400 m southwest of Yigo1 habitat past the transition between the coastal Ritidian soil series and the Guam soil series. The Guam soils are shallow cobbly clay loams that formed in sediments that overlie coralline limestone (Clayey, gibbsitic, nonacid, isohyperthermic, Lithic Ustorthents; Young 1988). This site is a homogeneous ca. 145 masl, and we sampled 17 individuals in this habitat.

The two sites in Dededo municipality were selected for the same purpose as were the Yigo sites, to provide two soil series in close proximity. They are located on the northwest tip of Guam in the Ritidian Wildlife Refuge (Fig. 1). Dededo1 is located on limestone terraces at about 95 masl. The Ritidian soils are similar to soils in Mangilao1 and Yigo1 sites. Aspect was ca. 300°, and we sampled 34 individuals. Dededo2 was a littoral forest habitat about 200 m from the shoreline in Shioya sand substrate (Carbonatic, isohyperthermic Typic Ustipsamments; Young 1988). This habitat is located about 700 m north of Dededo1 at the extreme edge of the littoral forest. All sampled plants were within 20 m of the first cliff face initiating the limestone terraces that culminate at 180 masl about 1 km from the shore. We sampled 15 individuals in this habitat. Both of these Dededo sites are fully protected from Guam's trade winds because they are on the leeward side of the plateau.

These seven sites are characteristic of typical cycad habitats on the island in terms of soils, exposure, and biodiversity. They represent all major cycad habitats on Guam with the exception of the limestone forests growing on the Ritidian-Rock outcrop soils that cap the tallest of the southern mountains and the littoral forests growing in clay soils along Guam's southwest coastline. All of these habitats have been heavily influenced by past human activity. In terms of direct land disturbance, the Inarajan site is the least and Yigo2 site is the most disturbed. In terms of prevalence of alien plant species, Dededo2 is the least and Mangilao2 is the most disturbed.

PLANT SAMPLING

We initiated this research in 2001-2002 by scouting for and identifying the habitats. We began conducting frequent site visits in order to understand phenology of the populations and create a list of female individuals that initiated a reproductive event in synchrony. All of the individuals we eventually harvested for this project initiated megasporophyll growth in April 2003. We harvested the mature seeds from this coning event in February 2005. Thus, all seeds were a homogeneous 22 months old, thereby ensuring no ambiguities associated with heterogeneous seed age (Marler et al. 2006b).

Eight seeds were collected from each individual, with the exception of Dededo2 where seed set was minimal. No individual in this habitat carried 8 seeds. A composite of gametophyte tissue from the seeds of each plant was frozen as described by Marler et al. (2005b). Among-plant heterogeneity of our target molecules exceeds that of within-plant heterogeneity (Marler et al. 2005b) in Guam's forests, so sampling a large number of plants to characterize a habitat provides more accuracy than ensuring a large number of seeds from each plant. The number of individuals we sampled in each habitat exceeded the minimum of 10 that is sufficient for accuracy using our methods (Marler et al. 2005b).

Tissue was lyophilized then stigmasterol β-D-glucoside (SG), β−sitosterol β-D-glucoside (BSSG), stigmasterol (SS), and β−sitosterol (BSS) were analyzed by Reversed Phase HPLC analysis as described by Marler et al. (2005b). We used ANOVA to determine significance of spatial heterogeneity in phytochemistry among the seven habitats, and present data as mean $\pm$ SE to separate means.

MEASURED HABITAT CHARACTERISTICS

Our objectives did not include determining the causal relationships of environmental variables on secondary chemistry in each habitat. However, we did quantify location characteristics for descriptive purposes. Now that the recent invasion of the two alien arthropod cycad consumers is threatening *C. micronesica* extinction on Guam, these pre-invasion location descriptors have become invaluable for defining the typical cycad habitat before the ongoing downward cascade of ecological responses to the alien disturbance.

Elevation was determined with GPS data and topographic maps. Relative exposure to trade winds was estimated from slope, aspect, and proximity to the east coast. We used a central transect in each habitat to measure average canopy openness. On 10 m intervals we photographed the canopy with hemispherical images in order to quantify percent open sky (see Marler et al. 2005b). Stem height and basal diameter, seed number, and leaf number were measured on each of the cycad plants from which seeds were harvested. We present these biotic and abiotic characteristics of each habitat that may influence phenotypic plasticity as mean ± SE.

Results

Secondary Compounds

Location influenced the concentration of SG in *C. micronesica* gametophyte tissue ($P < 0.0001$), ranging from 668 μg/g in the Dededo2 site to 250 μg/g in the Mangilao1 site (Fig. 2). Yigo1 exhibited higher SG concentration than Yigo2, and Dededo2 exhibited higher SG concentration than Dededo1, despite the close proximity of each pair of locations in these two provinces. Furthermore, Dededo2 SG concentration greatly exceeded that of all other habitats.

Location also influenced the concentration of SS ($P < 0.0073$) and BSS ($P < 0.0052$) (Fig. 2). The general spatial pattern was similar to that of SG, although the variability was not as pronounced (Fig. 2). As for SG, Dededo2 exhibited the greatest concentration of SS and BSS. For the two pairs of locations in close proximity, SS followed the pattern of SG in that Yigo1 site had greater SS than Yigo2, and Dededo2 site had greater SS than Dededo1 site. Dededo2 site was also greater in BSS concentration than Dededo1 site, but the concentration of this sterol in gametophyte tissue did not differ between the two Yigo locations in close proximity. The concentration of BSSG also segregated among the locations ($P < 0.0019$). However, the general pattern was dissimilar to the other three compounds (Fig. 2). Yigo1 exhibited the greatest BSSG concentration, with Mangilao1 and Yigo2 exhibiting the lowest.

We used a variability index (VI, maximum mean – minimum mean / maximum mean) to determine the magnitude of differences among the habitats. Of the four metabolites, SG was the most variable with a VI = 0.63 and BSS was the least variable with a VI = 0.39.

Habitat And Plant Characteristics

Mean plant height and stem diameter were similar among the habitats, with a VI of only 0.35 for height and 0.31 for diameter (Table 2). Mean leaf number per plant ranged from 45 in Inarajan to 82 in Yigo1. The plant variable with the greatest diversity (VI = 0.87) was mean seed number per plant, which ranged from four in Dededo2 to 33 in Yigo2.

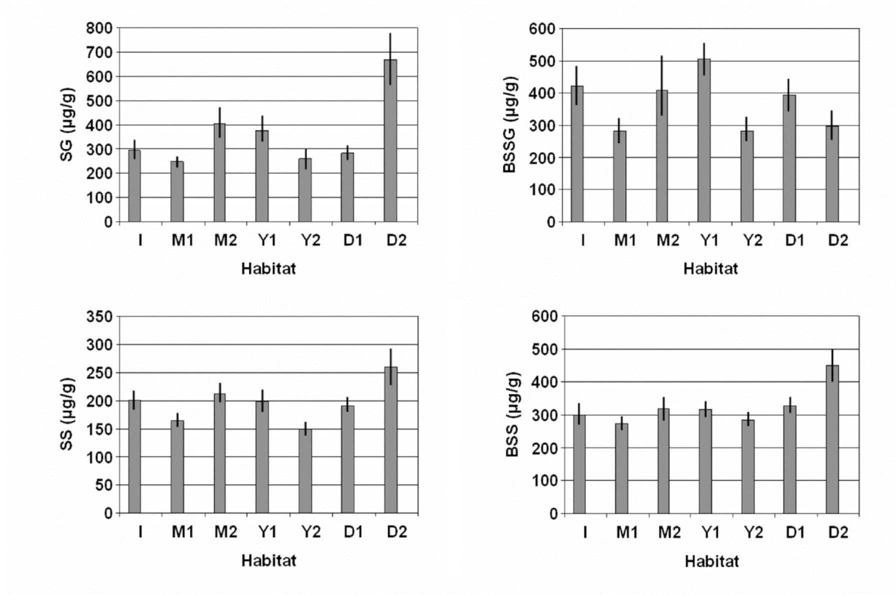

Figure 2. The influence of typhoon forest habitat in Guam on concentration of four sterol or steryl glucosides in *Cycas micronesica* seed gametophyte tissue. SG = stigmasterol β-D-glucoside, BSSG = β−sitosterol β-D-glucoside, SS = stigmasterol, BSS = β−sitosterol. Mean ± SE. Habitat designations are explained in Figure 1.

The habitat variable with the greatest diversity (VI = 0.69) was percent open sky (Table 1). Inarajan, Mangilao1, and Dededo2 sites were adjacent to northern limestone cliffs or steep slopes of southern ravine forests, and these geological features reduced the percent open sky as recorded with the hemispherical photographs. The lowest mean value of 18 percent was for Dededo2, where the plant population was close to the edge of the first limestone cliff terrace. The site with greatest canopy exposure was Yigo1 with 58 percent open sky.

Discussion

Guam's various forest habitats exhibited clear differences in concentration of cycad seed sterols and steryl glucosides. Despite the importance of studying cycads for answering questions about plant ecology and evolution (see Brenner *et al*. 2003, Norstog & Nicholls 1997), this is the first study that we are aware of that reports spatial patterns of secondary chemistry above the habitat level for any cycad species.

The range in sterol and steryl glucoside concentration we describe agrees with earlier reports for seeds about the same age (Marler et al. 2005b). Some of the plant and community characteristics that we measured segregated among the habitats (Tables 1-2). Our objectives in the current study did not address the dir-

Table 2. Habitat biotic characteristics in typhoon forests on the island of Guam. These factors may influence phenotypic plasticity of *Cycas micronesica* seed chemistry. Mean ± SE.

Habitat	Plant ht (cm)	Stem diam. (cm)	Seeds per plant	Leaves per plant
Inarajan	225±13	26±1	14±1	45±3
Mangilao1	290±9	29±1	15±2	53±2
Mangilao2	347±25	31±1	14±3	68±5
Yigo1	343±14	38±2	32±4	82±6
Yigo2	297±12	37±2	33±5	74±3
Dededo1	278±11	32±1	31±3	73±4
Dededo2	233±9	33±3	4±1	68±5

ect influence of each of these variables on seed chemistry, as our methods did not allow us to separate the effect of each from the other covariables that also segregated among the habitats. However, the various comparisons discussed hereinafter provide a compelling foundation for continued research designed to determine those factors most controlling of phenotypic plasticity of plant secondary chemistry in Guam's habitats.

DISTANCE-DEPENDENT CHARACTERISTICS

We calculated distance between each successive pair of habitats to provide a ranking by proximity for six habitat pairs. Approximate distance between adjacent habitat pairs in increasing order, and the corresponding VI for SG (the compound with greatest habitat heterogeneity) are as follows. Yigo1/Yigo2: 400 m, VI = 0.31; Dededo1/Dededo2: 700 m, VI = 0.58; Mangilao1/Mangilao2: 6.25 km, VI = 0.38; Dededo1/Yigo1: 7.25 km, VI = 0.25; Mangilao1/Yigo2: 16.0 km, VI = 0.03; Inarajan/Mangilao2: 21.0 km, VI 0.28. If proximity exerted any control over spatial heterogeneity of SG, we expected the ranking of distance to scale in the direction of VI for each pair. This was not the case, as the two habitat pairs less than 1 km apart exhibited some of the highest VI values. Moreover, Inarajan/Mangilao2 exhibited a VI in the mid-range, yet it was the pair with the greatest distance separating the two habitats.

PLANT CHARACTERISTICS

Our three measures of plant size were stem height and diameter, and leaf number (Table 2). The smallest height and diameter means were from the Inarajan population, yet the Inarajan means for the four compounds were mid-range (Fig. 2). Similarly, the Mangilao2 population was the tallest on average, and the Yigo1 population had the greatest stem diameter, yet the means for the four compounds in these two habitats were also mid-range. Comparing variation in mean leaf number among the habitats with that of seed chemistry reveals similar observations. These results indicate that plant size does not exert enough control over secondary chemistry of cycad seeds in Guam's habitats to swamp other causes for segregation of the four studied compounds among the habitats. However, features of reproductive growth may be related to plant size for many taxa (Niklas 1993, Peters et al. 1988), and ontogeny or plant size influenced secondary chemistry of other species (Bowers and Stamp 1993, Brenes-Arguedas & Coley 2005). Ensuring size related factors are considered in concert with phenotypic plasticity of any trait may be particularly important when comparing fragmented habitat populations. For example, Farrera & Vovides (2004) demonstrate the difference in size categories while quantifying population structure of two fragmented populations of the cycad *Ceratozamia matudai* Lundell in Mexico. If fragmented populations of other cycad species are also dissimilar in size structure, any influence of ontogeny or plant size on seed chemistry would lead to a contrast in seed chemistry among the fragmented habitats. Clearly, further work is needed to clarify the influence of cycad plant ontogeny and size on seed chemistry.

Various resource models such as the growth-differentiation balance or optimal partitioning hypothesis operate in some capacity on the basis that a tradeoff in resource or ecological costs occurs between growth and differentiation (Bloom et al. 1985, Herms & Mattson 1992). Thus, the plant resources mobilized to address the demands for growth of a reproductive event with copious seed set may influence the plant's relative investment into secondary chemistry of the maturing seeds. More resources may be available for secondary chemistry during maturation of a reproductive event characterized by poor seed set. Indeed, Dededo2 was the only site with poor seed set, and it was also the site exhibiting the greatest concentrations of SG, SS, and BSS. However, the ranking of seed chemistry among the remaining six habitats did not correlate well with their ranking of seed set (Fig. 2, Table 2). Studies designed to manipulate *C. micronesica* seed load are needed to resolve the relationship between fecundity and seed chemistry.

ABIOTIC HABITAT CHARACTERISTICS

Shade decreases the ratio of carbon to soil-derived elements by limiting photosynthesis more than element uptake (Herms & Mattson 1992). The carbon-nutrient balance hypothesis (Bryant et al. 1983) suggests this shift in resource ratio may affect secondary chemistry. Although the soundness of the hypothesis has been debated (see Hamilton et al. 2001, Lerdau 2002), numerous studies conform to predictions of the hypothesis (reviewed by Herms & Mattson 1992). Dededo2 site segregated from the other habitats in concentration of SG, SS, and BSS, and it also segregated from the other habitats for canopy openness with only 18 percent open sky (Fig. 2, Table 1). However, the range in open sky for the other six habitats was 26 to 58 percent, and this substantial range did not correlate with respective concentrations of any of the four compounds. Perhaps the influence of light on synthesis of these metabolites is non-linear, and the light requirement was saturated below 26 percent open sky. Other studies reporting the influence of light availability on secondary compounds (Agrell et al. 2000, Brenes-Arguedas & Coley 2005, Cantonwine & Downum 2001, Chacón & Armesto 2006, Cronin & Lodge 2003) indicate this factor and its relationship with soil-derived resources may partly explain some of the habitat differences in cycad seed chemistry.

Our habitat choices allowed a broad comparison of seed chemistry in relation to soil chemistry. The Inarajan site was located in volcanic acid soils that are characterized by an overabundance of aluminum, iron, and other metals (Marler et al. 2005a, Young 1988). The ravine forests in these soils contain some plant species that do not grow in Guam's alkaline soils. The other six habitats were positioned on several of Guam's alkaline substrates. All of these substrates are calcareous, and are characterized by deficiency of iron and other essential plant micro-elements. These limestone forests also contain some plant species that are not found in the ravine forests in southern volcanic substrates. No consistent pattern emerged when the sterols and steryl glucosides in cycad seeds from the Inarajan habitat were compared with that of seeds from the other habitats (Fig. 2). For each of the compounds, at least two of the other habitats exhibited values that did not differ from the Inarajan values. Thus, the highly contrasting soil chemistry that separates the Inarajan site from the remaining sites does not appear to be causal of the reported contrast in sterols and steryl glucosides.

The hydraulic properties of these soils also provide an edaphic gradient among the habitats. Water retention increases along the soil continuum Shioya sand : Ritidian : Guam : Akina-Agfayan (Young 1988). Guam's monsoon climate is controlling of many aspects of plant phenology, and the seasonal differences in plant water availability are directly linked to these localized differences in soil hydraulic properties. However, when the four habitats on Ritidian soils are combined and the mean compared with that of the other three habitats, the resulting pattern of sterol and steryl glucoside concentration does not correlate with the ranking based on water retention. For example, SS is 260 μg/g for Shioya, 192 μg/g for Ritidian, 150 μg/g for Guam, and 201 μg/g for Akina-Agfayan soil series.

We used the four habitats on Ritidian soils to look more closely at other habitat characteristics. An elevation gradient of 40 to 155 masl did not correlate with seed chemistry, nor did the diversity of exposure to tradewinds (Fig. 2, Table 1). Furthermore, cycad plants in the two Mangilao sites must contend with the added environmental pressure of copious aerosol salt spray deposits, and the mean of the values for our four compounds in Mangilao1 and Mangilao2 failed to differentiate from the mean of the values from Yigo1 and Dededo1.

COLLECTIVE RESOURCE LIMITATION

Cycad plants in Dededo2 exhibited unique seed gametophyte chemistry. What is different about the plant population in Dededo2? We have already discussed the potential role of shade, soil chemistry, and soil hydraulics separately. However, disjointed focus on these single factors may collude to obscure the general response of this slow-growing species to the scarce availability of collective resources at the Dededo2 location. Undoubtedly, this littoral location is most limited in light resources, is most prone to severe seasonal limitations in water resources, and is least fertile due to leaching vulnerability and deficient cation exchange capacity (Table 1; Young 1988).

Resource manipulation studies are urgently needed to validate the hypothesis that resource scarcity increases *C. micronesica* secondary chemistry and to disentangle which resources are most influential. Besides our field evidence, the literature lends further support for this contention. First, resource-limited environments favor plant species with conservative strategies that define *C. micronesica* behaviors (e.g. long-lived leaves and relatively high levels of secondary metabolism) and resource limitation often exerts predictable consequences on secondary metabolites (Herms & Mattson 1992). We have already argued (Marler et al. 2005a) that questions concerning cycad biochemistry may best be answered within the context of the various resource models that rely on tradeoffs in resource/ecological costs and growth/differentiation processes, and not within the framework of various chemical defense models. Second, Fine et al. (2004) report a corroborating Amazonian study where the chemistry of various plant species (none of which were cycads) and herbivory were compared between communities on clay versus white sand soils. Their plants from white sand habitats produced more of the measured compounds than plants from clay soil habitats, and they attributed these results to the nutrient limitations of the white sand soils. Third, Sakaki et al. (2001) studied steryl glucoside biosynthesis in yeast, and reported that nutrition stress was among the treatments that induced an increase in production under controlled conditions.

SPATIAL STRUCTURE OF SEED CHEMISTRY VERSUS ALS-PDC EPIDEMIOLOGY

One puzzling facet of ALS-PDC history on Guam is the elevated morbidity and mortality rates in southern Guam, especially within Umatac municipality in southwest Guam (e.g. Garruto et al. 1985, Reed & Brody 1975, Zhang et al. 1990). Decades of attempts to explain the affinity of the disease for southern Guam have failed. One possibility that has not been fully explored is the hypothesis that cycad plants in southern Guam differ from the plants in the rest of the island in quality or quantity of seed neurotoxins. However, seeds from the riparian habitat in southern Guam (Inarajan) exhibited moderate concentrations of our four compounds. Moreover, seeds from the habitat most distant from

Umatac (Dededo2) exhibited the greatest concentrations of the compounds. Taken without consideration of any other interacting factors, these results do not indicate ingestion of cycad seed tissue collected from riparian forests in southern Guam would lead to greater exposure to these neurotoxins or their precursors.

Two areas of further study may clarify the link between consumption of cycad seeds from southwest Guam and a higher incidence of ALS-PDC. First, our recent work indicates a synergistic relationship between the steryl glucosides and aluminum (see Marler et al. 2005a for further discussion). Aluminum is highly available in southern Guam soils to the point of being toxic to many plant species. Thus, ingesting cycad seed gametophyte tissue harvested from southern Guam soils may elicit a concomitant exposure to these neurotoxins and aluminum, which may not occur when ingesting cycad seed tissue from northern Guam. This interaction would not reveal itself in the data we present herein. Second, littoral habitats containing cycad plants abound between Umatac and Agat on Guam's southwest coastline. These forests are conveniently accessed from Umatac by boat, and our Dededo2 littoral site data suggest an elevated exposure to steryl glucosides was likely if Umatac residents historically exploited these forests for cycad flour. However, these southern littoral habitats occur on Pulantat clay soils (Clayey, montmorillonitic, isohyperthermic, shallow Udic Haplustalfs) and do not occur on Shioya sand. Thus, no clear conclusion can be drawn in the absence of further data collected from littoral habitats in southwest Guam.

OTHER POTENTIAL CAUSAL FACTORS

Genetic structure of the island's cycad populations is not known, and may partly explain the reported chemical heterogeneity among the habitats. This species is a member of the *C. rumphii* complex (Hill 1994). The members of this group produce a buoyant tissue layer within the sclerotesta that allows for oceanic dispersal, and successive seed arrivals from parent populations or populations from other islands have probably occurred throughout the evolution of the island's population. Separate founder events may partly explain why our only site that was littoral, only 200 m from the shoreline and less than 10 masl in elevation, segregated from the remainder of the upland habitats so drastically.

Differences in plant-plant interactions among the habitats may also play a role in spatial structure of cycad chemistry. We did not include a floristic survey from each habitat, but we did note during seed harvests that the littoral habitat was the only habitat devoid of an invasive alien forb species. *Chromolaena odorata* (L.) R.M. King & H. Rob., *Mikania scandens* (L.) Willd., and/or *Passiflora suberosa* L. were dominant co-occurring alien species in all of the upland habitats. Plant-plant interactions may influence many community characteristics such as resource availability and habitat structure (Brooker 2006). If coevolution (Janzen 1980) of co-occurring native plant species partly explains genetic drift among Guam's disjunct cycad populations, the recent displacement of native neighbors in the upland habitats by these aggressive alien forb species may be altering cycad seed chemistry phenotype in ways not currently understood.

SUMMARY

This paper is the first to report on the spatial patterns of secondary chemistry among natural populations of any cycad species. The cycad seed gametophyte sterols and steryl glucosides we studied segregated among seven habitats located from the southern to the northern extremes of the island. Habitat proximity did not correlate with phenotypic variability of the island-wide population. The habitat closest to the major loci of historical ALS-PDC peak incidence did not exhibit increased concentration of these neurotoxins. The littoral habitat in close proximity to the coastline contained markedly higher concentrations of cycad seed sterols and steryl glucosides. Heterogeneous biotic and/or abiotic characteristics may partially, indirectly, or interdependently explain habitat-dependent phenotypic plasticity, although no single descriptor correlated with the reported seed chemistry phenotype across the continuum of all seven habitats. The littoral site with elevated seed chemistry was the most resource-limited site. These results support the hypothesis that limited resource availability is associated with increased secondary chemistry of *C. micronesica* seeds. The recent IUCN endangered red-listing and the ongoing epidemic level of *in situ* plant mortality mandate urgency in pursuing further questions about the link between human disease and environmental toxins that are locked within this tropical cycad population.

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CORRIGENDUM

Marler, T. & R. Muniappan. 2006. Pests of *Cycas micronesica* leaf, stem, and male reproductive tissues with notations on current threat status. Micronesica 39(1): 1-9

The caption for Figure 2 has B and C switched. The caption should read:

Figure 2. Phenotype of cycad aulacaspis scale infestation on *Cycas micronesica*. (**A**) Abaxial surface of heavily infested rachis and leaflets. (**B**) Male cone and petiole infestation. (**C**) Petioles collapse following scale infestation.