THE HAWAIIAN C_4 *EUPHORBIA* ADAPTIVE RADIATION:

AN ECOPHYSIOLOGICAL APPROACH TO UNDERSTANDING

LEAF TRAIT DIVERSIFICATION

A DISSERTATION SUBMITTED TO THE GRADUATE DIVISION OF THE UNIVERSITY OF HAWAI‘I AT MĀNOA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN

BOTANY
(ECOLOGY, EVOLUTION, AND CONSERVATION BIOLOGY)

MAY 2011

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DEDICATION

For my Mom:

Beverley Margaret Sporck,

and my Dad:

Karl Ludvig Sporck

For always loving, supporting, and inspiring me.

I cannot put into words what amazing people you both are.

ACKNOWLEDGEMENTS

I am deeply grateful to the many people who made this dissertation possible. Without the support of this remarkable group of people and their belief in me, this research would have never been done, and I would not have grown as a scientist and human being as I have over the past six years while attending the University of Hawaii. First, I would like to thank my advisor, Lawren Sack, who invested immeasurable hours and also generously funded a large portion of this research from his grants. Lawren guided me from the very beginning of this project and saw me and the project through the many steps involved to complete it. Lawren is a prolific young scientist who has incredible energy and enthusiasm for all things he is passionate about. It is impossible not to be inspired while working with him. I have learned a great deal about many things while under Lawren's mentorship.

I would also like to thank my committee members: Chris Lepczyk, David Lorence, Cliff Morden, and Tom Ranker, each of whom has been a helpful contributor to my Ph.D. progress and has encouraged me and offered me different perspectives when confronted with challenges along the way. Chris Lepczyk was my EECB faculty sponsor as well as my university representative. Chris contributed many useful comments to my dissertation manuscript drafts. Since he is a fellow "Michigander," he made me feel especially at home in our conversations, even when the topic strayed from my dissertation into the area of the current state of the Great Lakes Region. I'd also like to thank Chris for asking me to read a book in preparation for my oral comprehensive exam, Aldo Leopold's *A Sand County Almanac*. This is a book that anyone interested in natural resources and conservation should read. Thanks to David Lorence for assisting me in the

field at the National Tropical Botanical Garden on Kauai, as well as for asking me questions about the taxonomic history of the subgenus *Chamaesyce* and the genus *Euphorbia,* and for nudging me to think of the larger picture including taxonomic relationships in general and the future of endangered plants in Hawaii. I thank Cliff Morden for guiding me on several trips to my field study sites on Oahu and Molokai, and also for taking the time to discuss the genetic implications surrounding the topics of rare and endangered plants, and for providing encouragement for my future career.

A special thank you goes to Tom Ranker, who has been extraordinarily supportive of me during my years as a grad student. Tom has provided helpful comments on manuscript drafts, invited me sit in with his lab group to discuss fern science, and talked with me about life in general and the possible directions my future career could go. I have been very grateful to have known Tom during my time at UH, and have been humbled by his wisdom and genuine care he has for the people around him.

This project was a large undertaking, requiring visits to nearly 30 field sites on several islands and involving species that are federally listed as endangered. There was an equally large laboratory component to this project and concepts that were new to me presented themselves on an almost daily basis. In addition, I presented this research at several professional conferences during my time as a graduate student and received integral feedback while in preparation for those events. I would not have been able to successfully complete such a logistically difficult project without the help of many.

For assistance with discussion, field collection, laboratory help, proof reading, and more, I thank: Larry Abbott, Courtney Angelo, Anthony Amend, Naomi Arcand, Chelsea Arnott, Ane Bakutis, Kasey Barton, Patty Bedoya, Lalasha Bialac-Murphy, Paul

Berry, Pat Bily, Joanne Birch, Jennifer Bufford, David Burney, Matt Burt, Amy Carlile, Tina Carvalho, Molly Cavaleri, Marian Chau, Susan Ching, Margaret Clark, Vince Costello, Christine Creese, Dana Crompton, Don Drake, Stephanie Dunbar-Co, Erika Edwards, Jesse Eiben, Michelle Elmore, Steve Evens, Erin Foley, Tom Giambelluca, Julia Gustine, Will Haines, Chris Havran, John Hayden, Faith Inman-Narahari, Aurora Kagawa, Kapua Kawelo, Sterling Keeley, Matt Keir, Tobias Koehler, Joel Lau, Matt Lurie, Nalani Mailheau, Lisa Mandle, Athena McKown, Klaus Mehltreter, Chris Nakahashi, Jessica Pasquet-Kok, Steve Perlman, Kori-Ann Phillips, Bob Pearcy, Jon Price, Whitney Reyes, Allen Rietow, Stephanie Saephan, Dan Sailor, Isabel Belloni Schmidt, Christine Scoffoni, Randi Schneider, Shane Shaw, Aaron Shiels, Laura Shiels, Wayne Souza, Cheryl Squair, Natalia Tangalin, Tiffany Thair, Amanda Vernon, Mashuri Waite, David Webb, Alex Wegmann, Ya Yang, the Oahu Army Natural Resources Management Program, the Pohakuloa Training Area, The Nature Conservancy (TNC) of Hawaii, and the National Tropical Botanical Garden.

Access and collection permits, including threatened and endangered species research permits for several taxa, were granted by the Department of Land and Natural Resources and sampling was conducted with permit holders in the case of Army and TNC jurisdiction lands. This research was funded by National Science Foundation Grants IOB-0546784 and Hawaii EPSCoR 657463, and the University of Hawaii Ecology, Evolution and Conservation Biology Program, Dai Ho Chun Fellowship, UH Office of Alumni and Community Relations, the UH Graduate Student Organization, the Botanical Society of America, and the Hawaii Botanical Society. And, a big thank you to the

University of Hawaii Botany Department for being the place where this goal became a reality!

I thank Laura Jaquish, and Greg LaCross, both professors at Northwestern Michigan College in Traverse City, Michigan. After taking several biology classes from Greg, I was encouraged to look into the possibility of grad school. After taking field botany and plant physiology from Laura, I discovered that I had an innate curiosity to learn more about botany. Thank you both for inspiring me to take my education to the next level and advising me on how to do so. Along that order, I would also like to thank the many teachers and professors I have had over the past 27 years of uninterrupted schooling. In some way, either large or small, you are a part of me realizing this dream.

Thanks also to my incredible comprehensive exam study group: Jennifer Bufford, Marian Chau, Daniela Dutra, Pei-Luen Lu, Shane Shaw, and Tammy Wong, who helped me get through one of the most intimidating parts of graduate school, all while having a bit of fun. We will never think of the Raven et al. *Biology of Plants* text book in the same way again. Extra acknowledgement goes to Marian, my office mate and good friend throughout my time at UH. Marian sat right next to me in our office on the sixth floor of St. John and we worked through many life- and school-related challenges together by discussing, brainstorming, laughing, crying, and eating more dark chocolate than anyone should admit to.

Lastly, I would like to thank all of my family and friends who provided support, inspiration, and encouragement in many different ways over the years either in Hawaii or from afar. Much gratitude goes to: my mom and dad, Beverley and Karl Sporck; my brother Leif Sporck; my grandmothers, Margaret Bailey and Barbara Sporck-Stegmaier;

my grandfather Claus Sporck: and my uncle Donald Bailey. I thank my aunts and uncles, Elissa and Roland Koch, and Solveig and Tom Reiskamp; and all of my cousins and their families. I thank the Koehler family: Dennis, Philip, Sandra, and Thilo Koehler, and their extended families. I thank my support network of friends who have not yet been mentioned: Heather Bandeen, Cecily Barnes, Lauren and Peter Connor, Nicole Davenport, Heather Eijzenga, Jaap Eijzenga, Micah Eijzenga, Mila Eijzenga, Katie Franklin, Danielle Frohlich, Matt Peters, Esther Posner, Madeline Reed, Jessica Souke, Carolyn Telgard, Rae Welch-Johnson, and my canine companions Koa and Magic.

Last, but certainly not least, a heartfelt thank you to Tobias Koehler, who stood by me and loved me while living through my many successes and struggles during these dissertation years. Tobias, thank you for making my life feel like a great adventure and for giving me many reasons to look forward to each new day.

ABSTRACT

Foliar traits, such as properties of venation, stomata, papillae, composition, and gross anatomy can provide important information about plant adaptation to the environment as these traits greatly influence plant physiological processes. Examining leaf traits in relationship to the natural physical environments in which they occur can provide a detailed understanding of plant function and adaptation to a set of given environmental conditions. My research focuses on the native *Euphorbia* subgenus *Chamaesyce* of Hawaii, a group of C_4 eudicots that have diversified across dramatic habitat gradients from one putative herbaceous colonizing species into 29 endemic woody taxa, within the last five million years. This lineage includes a variety of life forms, ranging from sub-shrubs a few centimeters in height, to trees over six meters tall. Members of the radiation are adapted to diverse habitats, including wet, mesic, and dry forests, bogs, and coastal zones. In this dissertation work, leaf anatomy and physiology were explored in an ecophysiological context. I measured a total of 104 leaf traits from 27 Hawaiian *Euphorbia* taxa across five Hawaiian Islands to test the hypotheses that leaf traits are aligned with environmental factors including rainfall, precipitation, humidity, vapor pressure deficit, elevation, and with habitat irradiance, and that leaf traits are coordinated in plant function. In most cases, I found that leaf traits correlated with environmental factors similarly to what has been reported in previous studies of distantly related species sampled within or across communities. I confirmed that the C_4 Hawaiian *Euphorbia* lineage has diversified across habitat types in their overall growth form and that there is exceptional variation in foliar characteristics for these taxa indicating strong adaptation to the diverse environments and habitats. Thus, I found very large variation across taxa in

leaf morphology and nutrient composition; in stomatal distribution, size and densities; the presence of papillae; and venation characteristics. This work captures, in detail, some of the greatest variation for leaf traits across taxa within a genus ever reported and demonstrates the rapid evolutionary diversification of many aspects of leaf structure and function.

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LIST OF ABBREVIATIONS

°C	degrees Celsius
µm	micrometer
%	percent
C_3	C_3 photosynthetic carbon fixation
C_4	C_3 photosynthetic carbon fixation
$\delta^{13}C$	carbon isotope discrimination (‰)
CAM	Crassulacean acid metabolism
C_{mass}	carbon concentration per mass (%)
Chl_{area}	chlorophyll concentration per area (SPAD)
Chl_{mass}	chlorophyll concentration per mass (SPAD g^{-1} m^2)
C:N	carbon: nitrogen ratio
C:P	carbon: phosphorus ratio
cm	centimeter
D	leaf density (g cm^{-3})
1°D	first order vein density (cm cm^{-2})
2°D	second order vein density (cm cm^{-2})
3°D	third order vein density (cm cm^{-2})
E.	*Euphorbia*
ECS_{ab}	abaxial epidermal cell size (µm^2)
ECS_{ad}	adaxial epidermal cell size (µm^2)
FAA	formalin acetic acid
FEV	free ending veinlets
g	gram
GCL_{ab}	abaxial guard cell length (µm)
GCL_{ad}	adaxial guard cell length (µm)
HCl	hydrochloric acid
kPa	kilopascal
L	lamina length (cm)
LA	leaf area (cm^2)
LamL/LamW	lamina length/ lamina width (unitless)
%LM	percentage of missing vein length
LMA	leaf mass per area (g m^{-2})
m	meter
MajVD	major vein density (cm cm^{-2})
MajV SAPA	major vein surface area per leaf area (unitless)
MajV SAVOL	major vein surface area: vein volume (mm^{-1})
Maj VLVOL	major vein length per vein volume (mm^{-2})
MajV VPA	major vein volume per leaf area (mm)
MAP	mean annual precipitation (m)
MARH	mean annual relative humidity (%)
MAT	mean annual temperature (°C)
MinVD	minor vein density (mm mm^{-2})
MinV SAPA	minor vein volume per leaf area (mm mm^{-2})

MinV SAVOL	minor vein surface area: vein volume (mm^{-1})
Min VLVOL	minor vein length per vein volume (mm^{-2})
MinV VPA	minor vein volume per leaf area (mm)
MinW	minor vein width (μm)
MFVPD	mole fraction vapor pressure deficit (unitless)
M ht	mature plant height in the field (m)
mm	millimeter
MV	missing vein average length (μm)
ML LD	missing vein length density ($cm\ cm^{-2}$)
$\delta^{15}N$	nitrogen isotope discrimination (‰)
N_{area}	nitrogen concentration per area ($g\ m^{-2}$)
N_{mass}	nitrogen concentration per mass (%)
N:P	nitrogen: phosphorus ratio
NaOH	sodium hydroxide
%OS	percent open sky (%)
P^2/A	$perimeter^2$/leaf area
P_{area}	phosphorus concentration per area ($g\ m^{-2}$)
PCA	principal components analysis
P_{dia}	papillae diameter (μm)
P_D	papillae density (mm^{-2})
PL_{ab}	abaxial stomatal pore length (μm)
PL_{ad}	adaxial stomatal pore length (μm)
PL_{tot}	total stomatal pore length, both surfaces (μm)
P_{mass}	phosphorus concentration per mass ($g\ m^{-2}$)
%PSAM	percentage of projected surface area missing
SAPA	vein surface area per leaf area (unitless)
1° SAPA	first order vein surface area per leaf area (unitless)
2° SAPA	second order vein surface area per leaf area (unitless)
3° SAPA	third order vein surface are per leaf area (unitless)
SD	stomatal density (mm^{-2})
SD_{ab}	abaxial stomatal density (mm^{-2})
SD_{ad}	adaxial stomatal density (mm^{-2})
SD_t	total stomatal density, both faces (mm^{-2})
$\%SD_{ab}$	percentage of stomatal density on abaxial surface
$\%SD_{ad}$	percentage of stomatal density on adaxial surface
$\%SD_{amphi}$	degree to which percentage of stomatal density is distributed on both surfaces
SPI	stomatal pore index
SPI_{ab}	abaxial stomatal pore area per leaf area index
SPI_{ad}	adaxial stomatal pore area per leaf area index
SPI_{tot}	stomatal pore area per leaf area index for both faces
T	lamina thickness (μm)
TotVD	total vein density (mm^{-2})
TotVPA	total vein volume per surface area of leaf ($mm\ mm^{-2}$)
TotV SAPA	total vein surface area per leaf area (unitless)

TotVLVOL	total vein length per vein volume ratio (mm^{-2})
TotVPSAPA	total vein projected surface area per leaf area (unitless)
TotVSAVOL	total vein surface area: vein volume (mm^{-1})
VI	vein island
%VI	percentage of total vein that is made up of vein islands
VI	vein island average length (mm)
VILA	vein island length per area (mm mm^{-2})
VINA	vein island number per area (mm^{-2})
%VM	percentage of vein volume missing
1°VPA	first order vein volume per leaf area (mm)
2°VPA	second order vein volume per leaf area (mm)
3°VPA	third order vein volume per leaf area (mm)
VPD	vapor pressure deficit (kPa)
VPSAPA	vein projected surface are per leaf area (unitless)
VP_{sat}	Saturated vapor pressure (kPa)
1°W	first order vein width
2°W	second order vein width
3°W	third order vein width
1°	first order
2°	second order
3°	third order
4°	fourth order
5°	fifth order

GENERAL INTRODUCTION

In one of the earliest known encounters by botanists recognizing the noteworthy diversity within Hawaiian *Euphorbia* (called *'akoko* in the Hawaiian language), was Charles Gaudichaud-Beaupré (1826-1830), writing about his travels Hawaii with Louis de Freycinet, specifically commented on the variability of "*l'euphorbia*," describing variation from a herbaceous coastal forms to a tree-shaped form in the mountains (Koutnik, 1987). It seems plausible that this early observation sparked an interest in this lineage for other botanists at the time. Since then there have been various studies of the Hawaiian *Euphorbia* lineage or selected members. After reading some of the earlier papers on this unique group of Hawaiian endemics, I was excited to commence a study and this dissertation work grew out of that excitement and recognition of what a truly exceptional group of plants the Hawaiian *Euphorbia* are. This introduction briefly reviews the taxonomy of the group, previous studies, and the major questions addressed in my dissertation.

A SUMMARY OF THE TAXONOMIC HISTORY

Euphorbia is the largest genus in the plant family Euphorbiaceae, with over 2000 species worldwide. The Hawaiian C_4 *Euphorbia* lineage is part of subgenus *Chamaesyce*. (Notably, *Euphorbia haeleeleana* is an endemic Hawaiian species from a different colonist, of the subgenus *Euphorbia* (Herbst, 1971; Yang, Y, personal communication;). Indeed, there are three independent natural colonization (*Chamaesyce* clade of *Euphorbia* (29 taxa); *Euphorbia* clade of *Euphorbia* (one species, *E. haeleeleana*), and *Claoxylon sandwicense*) of endemic Hawaiian plants from the family Euphorbiaceae). Considered worldwide, the subgenus *Chamaesyce* includes nearly 300 species and, based on

molecular evidence, is nested deeply within *Euphorbia* (Steinmann and Porter, 2002). The recognition of *Chamaesyce* as a subgenus within *Euphorbia* or as a separate genus has changed over time and has been a subject of debate since the early 1800s (Koutnik, 1987). In the first thorough taxonomic treatment of *Euphorbia*, Boissier recognized seven Hawaiian *Chamaesyce* species (Boissier, 1862). In a 1938 revision of the Hawaiian taxa (Sherff, 1938), the lineage was described as including some 60 taxa under the genus name *Euphorbia*, however, Sherff's work also brought attention to the problems that exist in understanding the group taxonomically (Koutnik, 1987). Various authors (Gray, 1821; Hassall, 1977; Koutnik, 1987) have considered *Chamaesyce* as a discrete stand-alone genus because of several features that distinguish it from the rest of *Euphorbia s.l.* The abortion of the main axis at the onset of the first true leaves occurs with few exceptions and has been the trait typically used to justify retaining *Chamaesyce* at the generic level. Perhaps an even more compelling justification for recognizing *Chamaesyce* at the generic level is the presence of C_4 leaf photosynthesis in all but three of species in the clade. native to North and/or Central America (and now thought to be basal species to the *Chamaesyce* clade of *Euphorbia*; Sage et al., in press), while all other species of *Euphorbia*, and all species in the plant family Euphorbiaceae for that matter, exhibit C_3 or Crassulacean Acid Metabolism (CAM) metabolism in their leaves (Koutnik, 1987; Sage et al., 1999). Several studies that came in the years that followed, including treatments by Gray, Boissier, Sherff, Hassall and Koutnik also accepted the genus as stand-alone (e.g., Chaw and Koutnik, 1990; Wagner et al., 1999; Morden and Gregoritza, 2005; Morden and Motley, 2005). Recent molecular work indicates, however, that the *Chamaesyce* clade is nested within *Euphorbia*. Thus, Steinmann and Porter classified *Chamaesyce* as a

subgenus nested deeply within *Euphorbia* (Steinmann and Porter, 2002; Bruyns et al., 2006; Yang and Berry, in review). A 2010 study titled *Hawaiian angiosperm radiations of North American origin*, recognizes the lineage under the modern classification as *Euphorbia*, subgenus *Chamaesyce* (Baldwin and Wagner, 2010).

A HISTORY AND SUMMARY OF PREVIOUS DEVELOPMENTAL, ANATOMICAL AND PHYSIOLOGICAL STUDIES OF THE HAWAIIAN *EUPHORBIA*

The first detailed examination of the anatomy of member of the Hawaiian *Euphorbia* lineage to my knowledge is an unpublished master of science thesis from the University of Hawaii Botany Department (Komkris, 1963). Komkris describes the anatomy and morphology, including ontogeny of the coastal strand species, *Euphorbia degeneri*. Komkris compared leaf, wood, and floral anatomy for populations at three different sites (Makapuu Beach and Diamond Head on Oahu, and Moomomi Beach on Molokai). The main goal of that study was an attempt to aid in taxonomic clarification and to better understand characters used to separate *Chamaesyce* from *Euphorbia.* The author stated:

> *"[that her findings] are not of sufficient magnitude to justify the retention of this species in the genus* Chamaesyce*. It is suggested that* Chamaesyce *be considered as a subgenus of the genus* Euphorbia*."*

Relevant to my dissertation work is Komkris' reporting of the presence of foliar water storage cells, papillae, and stomata being confined to the adaxial leaf surface. Komkris provided some detail on the water storage tissue as a potential specialization for coping

with xeric habitats, but the functional significance of papillae and hyperstomaty were not explored, nor was attention brought to the fact that hyperstomaty is an unusual finding.

Sherwin Carlquist paid particular attention to the Hawaiian *Euphorbia*, first in his well-known 1967 study focusing on plants with long-distance dispersal, in which the Hawaiian *Euphorbia* are discussed as likely being dispersed to Hawaii by way of birds (Carlquist, 1967). In 1970, Carlquist conducted a study of *Euphorbia* wood anatomy including 13 Hawaiian taxa (Carlquist, 1970). The main finding of this study was that vessel element length was highly variable across habitats types, and specifically, longer vessel elements were correlated with wetter habitats in the Hawaiian species. It is important to note that a survey of the seed testa of a subset of the *Chamaesyce* clade of *Euphorbia* has been conducted. This survey confirmed the fact that many taxa in the group possess a mucilaginous coating when moistened which would allow seeds to potentially stick to birds (Jordan and Hayden, 1992). This study included six Hawaiian taxa: of those six, one (*E. celastroides* sp.) retained the sticky seed coat, while five (*E. clusiifolia*, *E. degeneri*, *E. halemanui*, *E. remyi* sp., *E. rockii*) apparently lost this feature. In his 1992 book, *Hawaii, A Natural History*, Carlquist devoted part of a section on arborescence to discussion of the Hawaiian *Euphorbia*. He described the situation in which *Euphorbia* in Hawaii has likely evolved from a prostrate mat-form into a wide range of woody species including full-fledged trees (Carlquist, 1992).

In 1971 Derral Herbst completed his doctoral work in the University of Hawaii Botany Department, examining the foliar ontogeny of disjunct veins in *E. herbstii* (at that time known as *E. forbesii*; Herbst, 1971). Before he had graduated, Herbst published a short note on his doctoral findings, in the journal *Science* (Herbst, 1971), in which he

reported on the presence of unusually high numbers of disjunct veins in certain species of Hawaiian *Euphorbia*. As a part of this doctoral work, Herbst completed a survey of 128 *Euphorbia* taxa, all from herbarium specimens. These included 104 taxa from the *Chamaesyce* clade and the rest from other ancestry within *Euphorbia*. In taxa from the *Chamaesyce* clade, he found disjunct veins to be present 84% of the taxa and 50% of the taxa for other euphorbias, though in the euphorbias that were not part of the *Chamaesyce* clade, the isolated veins were morphologically different, usually consisting of one large terminal trachied only (Herbst, 1971). It is important to note that he reported that though present, disjunct veins occurred in very low numbers (less than ten per leaf for many taxa) for most of those taxa. For six species of higher moisture habitats in Hawaii, he reported that disjunct veins were found in abundance and as a normal part of the leaf anatomy.

A second part of Herbst's dissertation was prepared as a published manuscript (Herbst, 1972), focusing on the foliar ontogeny of one of those species which exhibited exceptionally high numbers of disjunct veins (*E. herbstii*). The ontogenetic examination revealed that *E. herbstii* showed normal leaf vein procambium development and that,

> *"the disjunct veins become isolated early in the histogenesis of the intersecondary veins when certain procambial cells fail to differentiate into vascular tissue. It appears that there cells develop into normal parenchymatous cells of the ground tissue."*

Herbst also notes that growing this species under differing conditions had no significant impact on the presence or abundance of disjunct veins. Thus, it cannot be said that disjunct vein development is dependent on water availability even though across species higher disjunct vein densities are correlated with wetter habitat types. He added that the

trait now appears to be genetically fixed, and thus it is possible that the diversity in disjunct vein abundance across the radiation was selected by environmental factors.

The way plant physiologists look at C_4 photosynthesis was forever altered when wet forest C_4 trees were observed in the Hawaiian *Euphorbia* by Robert Pearcy and John Troughton (Pearcy and Troughton, 1975). This discovery was initially made when Pearcy noticed the enlarged bundle sheath cells in the cleared leaf micrograph figure in Herbst's 1971 *Science* paper, and recognized their appearance as typical of Kranz anatomy, which had recently been linked with the C_4 syndrome (Downton and Tregunna, 1967; Pearcy, personal communication). Pearcy and Troughton conducted tests of isotopic signatures which verified that the Hawaiian *Euphorbia* is indeed C_4. Trees with C_4 leaves had never been reported prior to this finding, and this discovery would not have been predicted because C_4 plants usually occupy hot, high irradiance environments (Sage, 2004). Many of the Hawaiian *Euphorbia* occupy mesic to wet forest habitats and are shrubs and trees in growth form.

After the confirmation of the Hawaiian lineage as C_4, a series of papers on the physiology of a subset of species were published (Robichaux and Pearcy, 1980, 1980; Pearcy et al., 1982; Robichaux and Pearcy, 1984; Pearcy et al., 1985; Pearcy and Franceschi, 1986). First, the photosynthetic responses of the Hawaiian species *E. herbstii* was compared to those of C_3 species *Claoxylon sandwicense* and this work confirmed that even under shade conditions, which are unusual for C_4 plants to occupy, the C_4 species had higher rates of CO_2 intake than the C_3 species (Robichaux and Pearcy, 1980). An additional study was conducted by Robichaux and Pearcy in 1980, comparing four native *Euphorbia* taxa and reporting diversity in photosynthetic characteristics that

corresponded to the habitat types they occupied (Robichaux and Pearcy, 1980). The study of CO_2 exchange included a common garden experiment of 11 Hawaiian taxa of diverse habitats. Across species, photosynthetic capacity was closely correlated with nitrogen concentration, with the lower photosynthetic rates found for wet forest species. The authors suggested that this lower photosynthetic capacity may be a reflection of adaptation to low irradiance, or possibly to reduced nitrogen availability (Pearcy et al., 1982). The common garden design allows us to conclude that many of the diverse traits of these species are indeed genetically fixed rather than driven by plastic changes in differing environments.

A field-based study was conducted on the photosynthetic differences between species of two Hawaiian adaptive radiations that occur in similar habitat types across an elevation gradient, one C_3 and one C_4 (*Scavola* and *Euphorbia*, respectively; Robichaux and Pearcy, 1984). That work confirmed higher photosynthetic rates in C_4 *Euphorbia* than in C_3 *Scaevola* across habitats, and also higher photosynthetic rates in more light-exposed habitats. In 1985, a study was published examining the CO_2 uptake during sunflecks of native forest understory trees *E. herbstii* and *Claoxylon sandwicense* (Pearcy et al., 1985). The results indicated that the C_4 *Euphorbia* species was able to reach maximum photosynthetic rate more quickly than the comparator C_3 species. In a 1986 study of photosynthetic and chloroplast characteristics (Pearcy and Franceschi, 1986), *E. herbstii* and *C. sandwicense* plants were grown in full sun and shade conditions to simulate their native habitats. For both species, the trees grown in shade showed typical light response for shade plants (i.e. low light saturation points and low dark respiration points). The Hawaiian *Euphorbia* species however, was better equipped to respond to

high light and did not exhibit photo-inhibition in full sun unlike *C. sandwicenese*. Chloroplasts of *E. herbstii* also showed greater ability to change chloroplast characteristics when exposed to differing light levels compared the *C. sandwicense*. These studies indicated that the C_4 photosynthesis in Hawaiian *Chamaesyce* persisted even in shade species. Indeed, these species maintain an advantage in photosynthetic rate not only in high irradiance, but also, counter-intuitively, in deep shade, despite the greater anatomical and biochemical cost of the C_4 system and its apparent lack of benefit per se when irradiance is low, temperatures are cool, and soil moisture is high—and thus CO_2 is not limiting. The C_4 *Euphorbia* were apparently able to maintain their advantage even in deep shade by showing parallel sun-shade adaptation and acclimation of leaf physiology as C_3 species, contributing to their ability to maintain faster photosynthetic rates than C_3 species even in deep shade.

OVERVIEW OF DATA CHAPTERS

Chapter 1

The Hawaiian *Euphorbia* (Euphorbiaceae) is a lineage of C_4 eudicots that radiated from one colonizing species into approximately 30 currently recognized taxa (Wagner et al., 1999). This group includes representative taxa on all main Hawaiian Islands embodying varying life forms, from woody sub-shrubs to trees over 6 m in height and with taxa adapted to bog, coastal strand, dry, mesic, and wet forest habitats. The foliage of the Hawaiian *Euphorbia* taxa vary greatly as has been demonstrated to some degree in previous work. This study takes a novel and detailed focus on the diversification of the leaf surfaces. For 26 Hawaiian *Euphorbia* and three non-native weed taxa, I examined

stomatal and epidermal traits using light microscopy, scanning electron microscopy (SEM) and porometry. I quantified stomatal number, dimensions and distribution, epidermal cell sizes and density of papillae trichomes, traits that have functional significance. I tested for correlations of leaf surface features with habitat, climate, and with other key leaf functional traits, including leaf size and thickness, drawing conclusions about the integrated adaptation across the lineage.

Chapter 2

Because of geographic isolation and habitat-type diversity across the islands in Hawaii, there is a unique opportunity to study biological relationships in a detail that not possible elsewhere. This portion of the dissertation focuses on how leaf venation traits have diversified in *Euphorbia* across these climatic gradients in Hawaii. Understanding leaf venation architecture diversity within and across lineages gives powerful evidence of functional adaptation to habitat types over evolutionary timescales. This chapter greatly expands upon Herbst's doctoral work which focused on leaf venation ontogeny of one native *Euphorbia* species *E. herbstii*. Until this current work, no previous study had quantified the venation architecture across the lineage, including quantifying the disjunct veins or "vein islands," as I will refer to them hereafter, or the relationship of veins to environment for this scientifically important radiation. For 27 of 29 native *Euphorbia* taxa, I chemically cleared leaves and quantified 40 traits relating to venation architecture, including densities of all vein orders (i.e., length/area) and of vein islands. I tested for correlation of venation traits with climate and habitat, and with other aspects of leaf

structure and composition, drawing new conclusions about the adaptation of venation architecture that can be applied to other plant species elsewhere.

Chapter 3

Leaf composition and anatomical traits provide essential information of plant adaptation to environment and physiological function. This chapter focuses on the diversification of foliar composition traits for 26 of the 29 native endemic *Euphorbia* taxa. I tested for correlations across taxa of leaf composition with climate and habitat, and with key leaf structural traits. Environmental characteristics apparently play an important role in influencing leaf composition diversification. Leaf composition traits examined in this study included leaf mass per area, carbon and nitrogen isotope ratios and chlorophyll, nitrogen (N) and phosphorus (P) concentrations per area and per mass, and ratios of chlorophyll to N, N to P, and carbon to P, as well as gross morphological traits such as plant height and stem diameter. A variety of environmental factors such as irradiance level, mean annual precipitation, mean annual temperature, mean annual relative humidity, vapor pressure deficit, and elevation were examined to better understand how the environment may have driven leaf composition diversification. This work allowed testing of general hypotheses for the adaptation of leaf composition which have been established across diverse species and communities, but rarely within lineages, and never for a radiation recently evolved across such a striking range of climates and habitats.

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CHAPTER 1

EXCEPTIONAL DIVERSIFICATION OF THE LEAF SURFACES IN THE C_4 HAWAIIAN *EUPHORBIA* LINEAGE ACROSS CLIMATES AND HABITATS

ABSTRACT

The Hawaiian *Euphorbia* (Euphorbiaceae) is a lineage of C_4 eudicots that radiated from one colonizing species into approximately 30 taxa. This group includes representative taxa on all main Hawaiian Islands embodying varying life forms, from creeping woody sub-shrubs to trees over 6 m in height and with taxa adapted to diverse habitats (bog, coastal strand, dry, mesic, and wet forests). The leaves of the Hawaiian *Euphorbia* taxa vary greatly, and the diversification of the surfaces is exceptional for a lineage. Typically, angiosperm species have stomata distributed only on the leaf lower surface (hypostomaty), or on both surfaces (amphistomaty), whereas the distribution of stomata only on the upper surface (hyperstomaty) is rare, previously documented in aquatic plants, grasses with leaves that curl, and high elevation herbs that fold their leaves to prevent water loss. For 26 Hawaiian *Euphorbia* and three non-native weed taxa, I examined stomatal and epidermal traits using light microscopy, scanning electron microscopy (SEM) and porometry. I quantified stomatal number, dimensions and distribution, epidermal cell sizes and density of papillae (waxy, nipple-like projections on the leaf surface). I tested for correlations of leaf surface features with habitat, climate, and with leaf functional traits, including leaf size and thickness. The Hawaiian *Euphorbia* evolved a large diversity in stomatal density, size and distribution, including taxa representing all three of afore mentioned stomatal distributions types (12 amphistomatous, nine hypostomatous, and five hyperstomatous taxa). Hawaii's isolated location and climatic gradients apparently have driven stomatal diversification in *Euphorbia* beyond that of any lineage previously characterized. Stomatal traits showed

significant relationships with climate, including the positive relationship of % stomata on the adaxial surface ($\%SD_{ad}$) with, mean annual temperature (*MAT*) and vapor pressure deficit (*VPD*) and %open sky, and negative relationship with mean annual precipitation (*MAP*).

INTRODUCTION

The Hawaiian *Euphorbia* subgenus *Chamaesyce* (Euphorbiaceae) is a remarkable example of adaptive radiation including 29 currently recognized taxa (Table 1.1), descended from a single species colonist within the last five million years (Price and Clague, 2002), likely from a herbaceous Caribbean or Central Mexico taxon (Yang and Berry, 2007, 2011). Native C_4 *Euphorbia* taxa are found across the Hawaiian Islands and include many single island endemics. (Note: there is one additional native Hawaiian *Euphorbia* species, *E. haeleeleana* which is C_3 photosynthetic and not a part of the *Chamaesyce* subgenus of *Euphorbia*, thus not included in this study). These taxa are all C_4 (being a part of the largest C_4 clade among the eudicots; Sage, 2004) and occupy a diverse range of habitats and vary greatly in vegetative form and in height, and have adapted across a wide range of habitats which suggests they may be a model for trait evolution. Information about their adaptation is essential given the rarity of the taxa, including seven federally listed endangered species (see U.S. Fish and Wildlife Service endangered species listings here: http://www.fws.gov/endangered/). Very little is known about the biology of these intriguing taxa, thus from a wealth of knowledge and scientific research point of view, studies exploring how these taxa function are essential. It is of interest to the goal of this study that, "scientific research" is one of the most emphasized items in Hawaii's revised statutes for the conservation of aquatic life, wildlife and land plants (Hawaii Revised Statutes). Gaining a clear picture of the anatomy, physiology, and the habitats in which these plants are best adapted to live in is the foundation on which

conservation and restoration practices are built. Additionally, in the face of imminent climate change, recommendations for the most vulnerable of these taxa may be needed.

Several earlier studies have focused on the Hawaiian *Euphorbia* (Herbst, 1971, 1972; Pearcy and Troughton, 1975; Robichaux and Pearcy, 1980, 1980, 1984; Pearcy et al., 1985), but none have investigated their leaf surfaces. Such a study is timely, because leaf surface traits are important in determining gas exchange rates and climatic adaptation (Beerling, 2007). Further, determining trait adaptation has become a key approach to understanding plant evolution and ecology. Trait-environment and trait-trait relationships may represent efficient "design principles," and these have been demonstrated for leaves of species within and across communities (e.g. Givnish, 1987; Niinemets, 2001; Wright et al., 2004), but much less so for closely-related taxa within lineages. A few studies, however, highlight evolutionary trait diversification (e.g. Edwards, 2006; Dunbar-Co et al., 2009). The focus of this study was on the characteristics of stomata, epidermal cells, and papillae across the dramatic environmental gradients occupied (i.e. 10 - 1695 m range in elevation) by Hawaiian endemic *Euphorbia* taxa.

Stomatal distribution is of key importance in plant physiology, with a strong influence on gas diffusion into and out of the leaf, temperature relations, and thus photosynthetic and transpiration rates (Salisbury, 1928; Parkhurst, 1978; Mott et al., 1982; Smith et al., 1997). Stomatal distribution categories include *hypostomaty,* i.e., stomata distributed only on the abaxial (lower) leaf surface, *amphistomaty*, i.e., on both surfaces, and *hyperstomaty*, i.e., stomata only on the adaxial (upper) surface. Hypostomaty is the most common distribution type (Meidner and Mansfield, 1968; Willmer and Fricker, 1996; Casson and Gray, 2008), with theoretical models suggesting

hypostomaty to be advantageous in the shade because it prevents excess water loss, stomatal damage, and overheating during exposure to sunflecks (Smith, 1986; Peat and Fitter, 1994; Martin and Glover, 2007). Amphistomaty, on the other hand, is common for grass leaves and for dicot herbs of sunny habitats, including high elevations. For vertical and unifacial leaves amphistomaty would enable faster photosynthetic rates and allow both sides of the leaf to photosynthesize effectively at different times of the day. Amphistomaty is also common in thick leaves as a means to minimize distance for CO_2 diffusion from the surface (Mott et al., 1982). Amphistomaty tends to be the most common stomatal distribution type for plants with C_4 leaves, and thus it occurs in plants with the highest photosynthetic rates (Mott et al., 1982). Finally, amphistomaty might enable more effective cooling by allowing more evaporation per leaf area.

By contrast, the hyperstomaty distribution type describes leaves in which stomata occur only on the adaxial surface. Hyperstomaty is uncommon, typically only found in some aquatic species (e.g., *Nymphaea alba*), and some plants that curl or appress their upper surfaces, such as, grass leaves that curl, conifer scale or needle leaves that appress to the stem (e.g., *Juniperus*), high elevation or xeric herbs with collapsing rosettes such as *Primula glutinosa*, and some aquatic sedge and grass species (e.g., *Carex aquatilis* and *Spartina* spp; Gupta et al., 1968; Standley, 1986; Korner et al., 1989; Roth, 1992; Hardy et al., 1995; Ickert-Bond, 2000; Maricle et al., 2009). This distribution type has previously not been found in species with typical plagiotropic leaves (leaves that tend to grow at an oblique or horizontal angle).

The rarity of hyperstomaty has remained largely unexplained, though some have suggested that this would lead to rapid water loss, photodamage of stomata, or blockage

of the stomatal pore by various biotic or abiotic particles (Parkhurst, 1978; Smith et al., 1997). During the course of this study I discovered five hyperstomatic taxa. considering that stomatal guard cells are less translucent than other epidermal cells because of their higher chloroplast numbers (Willmer and Fricker, 1996), I proposed the additional hypothesis that hyperstomaty might come at the cost of blocking light from the mesophyll if the stomata occupied a substantial proportion of the leaf surface. In addition, I hypothesized that if stomatal distribution was variable among Hawaiian *Euphorbia*, shade taxa would have hypostomatous leaves, amphistomaty would be found in taxa growing in sites of high irradiance and subject to soil or atmospheric drought, and at higher elevations and temperatures, and thicker leaves would be amphistomatous. I did not expect to find hyperstomaty in the Hawaiian *Euphorbia* because prior to this study, it had never been found on broad-leaved terrestrial eudicots. Notably, there have been previous reports of hyperstomaty in plagiotropic leaves of one other *Euphorbia* species*, E mesembryanthemifolia*, native to coastal sites of the Caribbean (Gaucher, 1898; Roth, 1992), but not in any other *Euphorbia* taxa (Raju and Rao, 1977), including those closely related to the putative ancestor of the Hawaiian euphorbias, the herbaceous species from Central Mexico and the Caribbean, *E. stictospora*, *E. velleriflora*, *E. mendezii*, and *E. leucantha* (Yang and Berry, 2007, 2011).

I tested leaf trait correlations across taxa occupying, bog, coastal, dry forest, mesic forest, and wet forest sites. The overall aim was to test hypotheses for the correlation of leaf surface traits with environmental variables and with other key leaf functional traits (Table 1.2). Based on studies of typical variation in stomatal traits of plants within species grown in different conditions, or of species or communities native

to different environmental conditions I hypothesized linkages of stomatal distribution and other epidermal traits with climate, especially with "sunny and dry" versus "shady and moist" environments (Table 1.2). For the present study, I hypothesized that (i) hyperstomaty and amphistomaty would correlate with sunny and dry environments, because species in those conditions tend to have thicker leaves adapted for greater cooling (Givnish, 1987); (ii) stomatal size should be greater in sunny and dry environments because smaller stomata may be able to close more rapidly and/or effectively and thus should be advantageous in hotter or drier climates (Franks et al., 2009); and (iii) stomatal density (SD_t) would be lower in sunny and dry environments since water could be lost from leaky stomata if that hypothesis holds true (Muchow and Sinclair, 1989). I also hypothesized that (iv) total stomatal pore area per leaf area (whether achieved with high stomatal density or large size) should be higher in sunny and dry environments to allow cooling, and/or to facilitate CO_2 assimilation in more productive and competitive environments. On the other hand, C_4 plants, which can achieve high assimilation rates with relatively small apertures due to their biochemical CO_2 concentration mechanism, might be expected to show weaker trends of stomatal pore area with climate.

I hypothesized that stomatal traits would also correlate with other leaf traits. Trait linkages may be structural or functional, or arise for independent traits that are co-selected by the same environment (Givnish, 1987; Sack et al., 2003; Dunbar-Co et al., 2009). I hypothesized that (v) total stomatal density (SD_t), amphistomaty ($\%SD_{amphi}$), percent stomatal density on the adaxial surface ($\%SD_{ad}$) and total stomatal pore index (SPI_t) would be correlated with other traits related to sunny, dry environments (Table

1.2), and thus with smaller and thicker leaves of higher leaf mass per area (*LMA*), higher delta ^{13}C ($\delta^{13}C$) and higher concentrations of nitrogen (N), phosphorus (P), and chlorophyll per mass and per area (N_{mass}, N_{area}, $P_{mass,}$ P_{area}, Chl_{area}, Chl_{mass}) and higher leaf density (*D*), and lower chlorophyll: nitrogen (*Chl:N*)(Dunbar-Co et al., 2009).

I used published data for 11 Hawaiian *Euphorbia* taxa grown as seedlings in a common garden (Pearcy et al., 1982) to compare with traits that were not included in my study. I tested hypothesis (vi) that SPI_t and percent (%) SPI_{ad} would correlate with gas exchange rates. This relationship is expected because SPI_t would increase the maximum stomatal conductance to diffusion of water vapor and CO_2 and $\%SPI_{ad}$ would reduce CO_2 diffusion resistance due to shorter path length to the palisade mesophyll tissue.

Some of the stomatal traits and their relationships with environmental variables may be influenced by papillae, waxy nipple-like projections of the cell wall on the leaf surface, which are present on the adaxial surface in many *Euphorbia* species (Kakkar and Paliwal, 1972; Raju and Rao, 1977, 1987). Papillae may reflect light, preventing leaf overheating and photodamage (Jordan et al., 2005). In light of this potential benefit, I hypothesized (vii) that papillar density (P_D) and size (P_{dia}) would be greater in open-establishing species, and in taxa of high mean annual temperature (*MAT*) and vapor pressure deficit (*VPD*), and of low mean annual precipitation (*MAP*), because smaller cells can be an adaptation to desiccating conditions (Cutler et al., 1977; Table 1.2). Papillae also may protect stomata from wind, rain, particles and from excess irradiance (Wagner et al., 2003; Jordan et al., 2005). Equally, papillae may confer a self-cleaning ability to the leaf surface and protect the stomata from intrusion of liquid water and particles, allowing stomata to remain functional during or immediately after short-

duration rainfall events, via the "lotus effect" (Wagner et al., 2003; Figure 1.1); see demonstration at http://www.youtube.com/watch?v=cS1t3rMdPKw&feature=related). Because of the probable benefit papillae provide to stomata on the upper leaf surface to increase the "lotus effect," keeping the upper surface clean, free of excess water and debris, and potential epiphytic growth I hypothesized (viii) a correlation of papillae density with adaxial stomatal density.

I hypothesized that (ix) cell sizes would be smaller in sunny, dry environments and related to other traits selected in those environments. Due to the physiological constraints of cell size and water stress, smaller cells are better suited to maintain a low osmotic potential relative to larger cells, which allows for superior turgor maintenance under such conditions (Cutler et al., 1977; Table 1.2). Further, I hypothesized that (x) cell size traits would be related, such that abaxial epidermal cell size (ECS_{ab}), adaxial epidermal cell size (ECS_{ad}), abaxial guard cell length (GL_{ab}), adaxial guard cell length (GL_{ad}), adaxial stomatal pore length (PL_{ad}), abaxial stomatal pore length (PL_{ab}), and papillae diameter (P_{dia}) would all be correlated. I also expected (xi) allometric constraints to dictate that larger leaves should have larger cells, and thus larger ECS_{ad}, ECS_{ab}, Gl_{ab}, PL_{ab}, Gl_{ad}, PL_{ab}, and P_{dia}.

I expected (xii) under allometric constraints that larger epidermal cells would correspond to larger stomata, spaced further apart, and thus to lower stomatal density (*SD*) and, thus that larger ECS_{ab} should correlate with lower SD_{ab}, higher PL_{ab} and GCL_{ab}, and larger ECS_{ad} should correlate with lower SD_{ad}, and higher GL_{ad} and PL_{ad}. This development should produce a negative relationship between *SD* and *GCL* (and *PL*) on either leaf face (Salisbury, 1928; Grubb et al., 1975; Sack et al., 2003; Franks et al.,

2009). Additionally, depending on the slope of the relationship between *SD* and stomatal size, ECS_{ad} and ECS_{ab} should correlate positively or negatively with SPI_{ad}, and SPI_{ab}, respectively, and, (xiii) larger ECS_{ad} should have larger, fewer papillae.

I compared the native Hawaiian taxa with three naturalized species of *Euphorbia* also from the *Chamaesyce* clade (*E. hirta*, *E. hypericifolia* and *E. prostrata*). These three species are considered to be similar to the ancestral colonist which are also thought to be herbaceous and weedy (Morden and Motley, 2005; Yang and Berry, 2007, 2011). I hypothesized that (xiv) the native species would show much wider variation than the herbaceous weeds.

This series of hypotheses allowed testing of fundamental principles of plant design and adaptation to enrich our understanding of plant-climate interactions. This unique evolutionary model allows tests within one lineage of closely-related species recently radiated across an exceptional range of climate gradients, and possessing extraordinary diversification in form and physiology.

METHODS

Taxa, sites and collection of material

Leaf samples were collected from populations of 29 *Euphorbia* taxa (26 of the 29 recognized endemic taxa, plus three weed non-native taxa) on the five high Hawaiian Islands of Hawaii, Kauai, Maui, Molokai, and Oahu (Figure 1.2). Habitat types were categorized as bog, coastal, dry forest, mesic forest, wet forest (Wagner et al., 1999; Table 1.1), with the additional "weed" group for three non-native species that occur in landscaped habitat on the university of Hawaii campus. When taxa existed in multiple

populations, I sampled (sampling methods described below) in populations of typical habitat for the taxon, according to descriptions in Wagner et al. (1999). Seven taxa are federally listed endangered species, and many others are rare and/or recommended for candidacy to become threatened or endangered species in the United States (Table 1.1). Many of the taxa only occur in remote locations. Access and collection permits, including threatened and endangered species research permits, were obtained from the State of Hawaii, Department of Land and Natural Resources (Table 1.1) or sampling was conducted with permit holders in the case of United States Army and The Nature Conservancy (TNC) lands. For *E. celastroides* var. *celastroides* and *E. c*. var. *lorifolia*, I sampled plants in cultivation at the National Tropical Botanical Garden (NTBG), and for *E. herbstii* I sampled out-plantings. Additional plants of *E. celastroides* var. *amplectens* were sampled at NTBG for the study of gas exchange.

I collected from healthy reproductively mature plants (except *E. herbstii* out-plantings which may not have been reproducibly mature yet). I recorded plant height, diameter of the main stem 10 centimeters (cm) from the base (except for certain species that were shorter in overall height I measured 2-4 cm from base for *E. degeneri*, *E. deppeana*, *E. skottsbergii* var. *skottsbergii*, *E. s*. var *audens*, and the three weed species), approximate leaf number, soil type, the dominant associated plants growing at the habitat site within approximately a 10 meter radius of the plants, and the percent open sky (%*OS*). The percent open sky (equivalent to 100% minus the "canopy closure" *sensu* (Jennings et al., 1999) was visually assessed to the nearest 5%; such visual canopy cover estimates involve a level of uncertainty, but have been found to correlate with measurements using a densiometer or hemispherical photography (Korhonen et al., 2006;

Paletto and Tosi, 2009). I recorded the elevation and coordinates for wild populations using a Global Positioning System (GPS) device (Garmin 60CSx, Garmin, Kansas City). For each sampled population, I determined *MAT*, *MAP*, and mean annual relative humidity (*MARH*) using a Geographic Information System (GIS) climate model based on climate station data (Hawaii Digital Climate Map System; (Giambelluca and Cuo, unpublished work). I determined mean annual vapor pressure deficit (*VPD*) from MAT and MARH (Waite and Sack, 2010). Vapor pressure deficit is a measure of atmospheric drought, the driving force for evaporation, and it can be quantified as an absolute pressure difference in kPa, or as a mole fraction normalized by atmospheric pressure. Across the study populations, the two *VPD* measures were highly correlated (R^2=0.998; $P < 0.001$). I present correlation results for both but in the text discuss correlations with absolute *VPD*.

I collected five to twenty leaves from five individuals of each taxon (fewer leaves for rare and endangered taxa), and only from three plants in the cases of *E. arnottiana* var. *integrifolia* and *E. remyi* var. *remyi* because only three plants were found. Fully exposed leaves were selected from the most recent mature flush, several nodes below the apex. Leaves were transported in plastic bags to the laboratory.

Leaf traits: dimensions and composition

I measured mean leaf area (*LA*) for at least three leaves per individual (using a LI-3100 leaf area meter; LI-COR Biosciences; Lincoln, Nebraska, USA). Leaves were oven dried for over 72 h at > 70^{o}C. After drying, I measured dry mass for calculation of leaf mass per area (= leaf area / dry mass; *LMA*).

I measured chlorophyll concentration per area on fresh leaves (Chl_{area}; using a SPAD meter; SPAD-502; Minolta Co., Japan), averaging two measurements for each of

three to five leaves per individual. I determined foliar nutrient composition for three to 15 leaves per taxon. Dried leaves were ground into a fine powder in a Wiley mill with mesh size 20. Leaves were analyzed for concentrations of nitrogen (*N*) and phosphorus (*P*) per mass (N_{mass} and $P_{mass,}$ respectively), and for carbon (*C*) isotope ratio ($\delta^{13}C$) using high temperature combustion in an elemental analyzer (Costech ECS 4010; Valencia, CA, USA), with effluent passed into a continuous flow isotope ratio mass spectrometer (ThermoFinnigan Delta V Advantage with a Conflo III interface; ThermoFisher Scientific; Waltham, MA, USA; Fry et al., 1996). Samples were dry ashed in glass vials (Miller, 1998), dissolved in 1 molar (M) hydrochloric acid (HCl) and analyzed for P_{mass} using inductively-coupled plasma-optical emission spectrometry (Varian Vista MPX Instrument, Varian InE., Palo Alto, CA USA; (Porder et al., 2005). Chlorophyll per mass (Chl_{mass}) was calculated as Chl_{area} divided by *LMA*; concentrations of nitrogen and phosphorus per area (N_{area} and P_{area} respectively) were determined as, N_{mass} and P_{mass} multiplied by *LMA*. Chlorophyll: nitrogen ratio (*Chl:N*) was calculated as Chl_{area}/N_{area}.

I measured leaf thickness (*T*) midway along the leaf between midrib and margin (using digital calipers; model 14-648-17, Fisher Scientific; Pittsburgh, PA). Thickness measurements were made on leaves that were preserved in formalin-acetic acid-alcohol (*FAA*; 37% formaldehyde, glacial acidic acid, 95% ethanol, and deionized water in a 10:5:50:35 mixture). Leaf density was determined as *LMA* divided by *T* (Niinemets et al., 2007).

Leaf traits: stomatal and epidermal anatomy

I measured stomatal traits on adaxial and abaxial faces of leaves for three leaves of each taxon. Leaves were fixed and stored in *FAA*, and prepared for scanning electron

microscopy (SEM) by further fixing sections taken centrally in the leaf between midrib and margin. This was done by immersion in 2.5% glutaraldehyde in 0.1M sodium cacodylate buffer at room temperature, washing in 0.1M cacodylate buffer, post-fixing for 1 h in 1% osmium tetroxide in 0.1M cacodylate buffer, dehydrating in a graded ethanol series, and critical point drying. Leaf sections were then mounted on aluminum stubs with conductive carbon tape, sputter coated with gold/palladium, and photographed at 40×, 200×, and 2500× at an accelerating voltage of 15kV (using a Hitachi S-800 Field Emission SEM; Hitachi, Tokyo, Japan). I quantified stomatal and epidermal characters using digital imaging software (Image J software; http://rsb.info.nih.gov/ij/). For stomatal guard cell and pore lengths and densities I measured one stoma per image and counted all stomata in the image for three images per leaf, and averaged across the three leaves per species. Stomatal pore area index (*SPI*), a dimensionless index of pore area per lamina area was determined as pore length^2 × stomatal density (Sack et al., 2003; Sack et al., 2005). I also quantified the percentage of stomatal density and of *SPI* on the adaxial surface (%SD_{ad} and %SPI_{ad} respectively), and the degree of amphistomaty %SD_{amphi}) where 0% represented hyper- or hypostomaty and 100% represented amphistomaty:

$$\%SD_{amphi} = \min(\%SD_{ad}, 100-\%SD_{ad}) / \max(\%SD_{ad}, 100-\%SD_{ad}) \times 100\% \quad \text{(eqn. 1)}$$

Additionally, for species in which cell epidermis was not covered by dense wax, I determined the average epidermal cells size by measuring three cells per leaf surface (upper and lower surfaces), for three leaves per taxa. For the same three leaves per taxa, I determined average epidermal cell density by counting the number of epidermal cells in a known area. Papillae occurred on the adaxial surface for 13 native taxa. For papillose

taxa, I quantified papilla diameter, averaging for three papillae per image for three images per leaf, and papillar density by counting papillae for one image per leaf for three leaves per taxon. For four taxa (*E. degeneri, E. olowaluana*, *E. sparsiflora,* and *E. celastroides* var. *stokesii*), adaxial cell dimensions and numbers could not be quantified due to the very high papillar density. For example, for *E*. *celastroides* var. *stokesii*, I reported a P_D of 5854 papillae per mm^2 on average for the taxon.

Porometry and measurements of isolated epidermis

To confirm with independent methods of the significant differences in stomatal distribution discovered with the SEM measurements, and to determine their correspondence with stomatal conductance, in Jan 2008 I conducted an additional study of gas exchange and characters of isolated epidermis, for three taxa of contrasting stomatal distribution (*E. celastroides* var. *amplectens*, var. *celastroides*, and var. *lorifolia*) at National Tropical Botanical Garden, Kauai. I measured stomatal conductance for five leaves per taxon, under full sun, at ambient temperature and relative humidity (ranging 22.7 -23.2 °C, and 71.4-73.0%, respectively), for both leaf faces (using an AP4 porometer; Delta-T, UK). Additional leaves were transported to the laboratory, and for three leaves per taxon, epidermal peels were removed from both leaf surfaces using forceps and razor blade, and photographed at 40× and 400× magnification under a light microscope (microscope: SM-LUX Leitz; Wetzlar, Germany, and camera: Nikon Coolpix 4500; Nikon; Tokyo, Japan). The $\%SPI_{ad}$ was measured for images of the epidermal peels as described above. The $\%SPI_{ad}$ from those measurements was compared with the % of total stomatal conductance accounted for by the adaxial surface, which was calculated from the porometer measurements.

Statistics

For each trait, I used three nested analyses of variance (ANOVAs) to test trait differences among taxa, and among (1) vegetation type categories (Table 1.1), (2) open- versus shade-establishing species, and (3) stomatal distribution categories (hyper-, hypo-, and amphistomatous). All analyses were performed using Minitab Release 15 software (Minitab, State College, PA). Each ANOVA was repeated with and without including the three naturalized weed species. Data were log-transformed before ANOVAs to increase normality and homoscedasticity (Sokal and Rohlf, 1995; Zar, 1999). I tested trait-environment and trait-trait linkages hypothesized *a priori* (see *Introduction*), and for a conservative assessment I determined correlations as significant only when both Pearson (r_p) and Spearman (r_s) coefficients were significant. I additionally prepared a correlation matrix to reveal the inter-correlative structure of the traits (Givnish et al., 2004; Edwards, 2006). Because I tested only hypothesis a priori I did not do a Bonferroni correction, however I recommend a Bonferroni-type correction before "mining" for additional trait correlations that were not hypothesized, given the danger of an inflated false discovery rate (Garcia, 2003; Moran, 2003). Significant correlations among inter-correlated variables were further resolved using partial correlation analyses (*corpcor* package; R 2.6.1; http://www.r-project.org; Sokal and Rohlf, 1995), indicating the relationship between two variables holding other variables constant.

I tested for correlations between my data and data from Pearcy's 1982 published dataset. Pearcy's study included gas exchange data for 11 native Hawaiian taxa, a trait that I did not look at in my study. I used Pearson and Spearman correlations to test for significance of hypothesized relationships.

RESULTS

Variation in climate and establishment irradiance across native Hawaiian euphorbias

The 26 native *Euphorbia* taxa occupied a wide range of climatic conditions with extreme values for elevation and mean annual temperature (*MAT*) occurring in *E. skottsbergii* var. *skottsbergii* (19.2 m and 23.8˚C) and for *E. olowaluana* (1695 m and 13.3˚C). Taxa varied nine-fold in percent open sky (%*OS*), from *E. arnottiana* at 11%, to eight taxa at 100%. Taxa varied three-fold in vapor pressure deficit (*VPD*), from *E. remyi* var. *remyi* at 0.32 kPa to *E. degeneri* at 0.90 kPa. The variation in mean annual precipitation (*MAP*) across the habitats of the taxa was noteworthy, with a 23-fold range from *E. skottsbergii* var. *audens* at 424 mm on the dry leeward coast of west Molokai, to *E. remyi* var. *kauaiensis* at 9704 mm in the wet forest at "Blue Hole" below the summit of Waialeale (which means "overflowing water"; Pukui et al., 1974) in central Kauai, one of the rainiest places on earth (Ramage and Schroeder, 1999). The field locations for the three weed species (Table 1.1) collected in Manoa Valley at 64-84m elevation ranged narrowly in climate for field locations; for *MAT*, *MAP*, *VPD* and %*OS*, the mean values were 23°C, 1746 mm, 0.83 kPa, and 89%.

Across taxa, elevation correlated positively with mean annual relative humidity (*MARH*) (r_s and r_p = 0.70-0.91; $P < 0.001$), and negatively with *VPD* (r_s = -0.94; r_p = -0.86 to -0.94; $P < 0.001$). The *MAT* correlated positively with *VPD* and mole fraction vapor pressure deficit (*MFVPD*) (r_s and r_p= 0.86 - 0.97; $P < 0.001$). The *MAT* correlated negatively with *MARH* (r_s = -0.77; r_p = -0.70 to -0.75; $P < 0.001$). The *MAP* correlated positively with *MARH* (r_s and r_p = 0.44-0.55; $P < 0.001$), and negatively with *VPD* and *MFVPD* (r_s and r_p =- 0.39 to -0.52; $P < 0.05$). The *MARH* correlated negatively with *VPD*

and *MFVPD*, (r_s and r_p = -0.67 to -0.96; $P < 0.001$). The *VPD* and *MFVPD* correlated positively with %*OS* (r_s and r_p = 0.43-0.50; $P < 0.05$).

The climate differed among the five vegetation types in which *Euphorbia* occurred, and among the taxa within given vegetation types (ANOVAs; $P < 0.001$; Appendix A1-1). Elevation varied on average from 50 m for coastal vegetation to 814 m for wet forest. The *MAP* varied ten-fold across vegetation types from 646 mm for dry forest to 6469 mm for wet forest. The %*OS* varied from 52% for mesic forest to 95%-100% for coastal and bog taxa. The wet forest and coastal vegetation had the extreme values for *MAT* and *VPD* ranging from 17.3°C to 23.5°C, and from 0.36 to 0.87 kPa, respectively.

The climate variables differed between open- and shade-establishing taxa (ANOVAs; $P < 0.001$; Appendix A1-1). Elevation ranged on average from 408 to 784 m from open to shade-establishing taxa. The *MAT* values for shade- and open-establishing taxa were 18.2°C and 21.2°C, respectively, and the %*OS* and *VPD* were two to three-fold higher for taxa of open habitats, ranging from 43 to 92% and 0.018 to 0.055 kPa, respectively. The *MAP* ranged on average from 1236 to 2943 mm from open- to shade-establishing taxa.

Leaf trait variation among taxa

Across the Hawaiian *Euphorbia,* the 19 measured stomatal and leaf epidermal traits varied significantly (ANOVAs; $P < 0.01$; Table 1.1, Appendix A1-1, and Figure 1.3 - Figure 1.10). The stomatal densities on both leaf surfaces varied greatly, as did distribution between the two surfaces; the %SD_{ad} ranged from 0% to 100%. The SD_{ab} ranged from zero for the five hyperstomatous species of mesic forest, coastal strand, and

bog, to 806 stomates per mm^2 for *E. rockii*, a wet forest taxon. The SD_{ad} ranged from zero for the nine hypostomatous taxa of mesic and wet forests to 642 stomates per mm^2 for *E. celastroides* var. *lorifolia*, a mesic forest taxon (Figure 1.4). Fifteen of the 29 taxa were amphistomatous (Figure 1.4); $\%SD_{amphi}$ ranged from 0% (hyper- or hypostomatous) to 66% for *E. celastroides* var. *amplectens*, a mesic forest taxon (Figure 1.4). This variation resulted in a ten-fold range in SD_t (Figure 1.3). Stomatal size varied substantially but with a smaller range than stomatal density, and with greater variation on the abaxial face. Thus, the PL_{ab} and PL_{ad} varied across species by five- and two-fold on average respectively, and the GL_{ab} and GL_{ad} by four- and two-fold, respectively. As a consequence of this variation in stomatal density and size, SPI_t varied 16-fold. Stomatal density showed a similar range on both faces; the SPI_{ad} ranged from zero to 0.06 for *E. celastroides* var. *celastroides*, a mesic forest taxon, and the SPI_{ab} ranged from zero to 0.04 for *E. remyi* var. *remyi*, a wet forest taxon (Figure 1.3 and Figure 1.4).

To further assess the potential significance of variation in stomatal distribution described above from SEM, I analyzed images of detached, fresh epidermis, and also calculated % adaxial stomatal conductance, using porometry, for common garden plants of three taxa that showed significant variation in $\%SD_{ad}$, *E. celastroides* var. *amplectens,* var. *celastroides* and var. *lorifolia*. All three methods showed the same pattern, supporting the finding of variation among taxa in $\%SPI_{ad}$ from 0% to 100%, and its correspondence with differences in stomatal conductance (Figure 1.5).

Adaxial papillae occurred in 13 of the 26 native taxa. Papillar density (P_D) ranged from zero to 7617 papillae mm^{-2} for the mesic forest taxon *E. celastroides* var. *lorifolia* (Figure 1.8), and papillar diameter (P_{dia}) had a two-fold range. Epidermal cell sizes on the

adaxial and abaxial faces (ECS_{ad} and ECS_{ab}) also varied widely across taxa with nine- and four-fold ranges, respectively.

Values for weeds relative to natives

For 15 of 17 leaf surface traits, the mean values for the weeds *E. hirta*, *E. hypericifolia*, and *E. prostrata* fell centrally within the range of values for the Hawaiian species. For PL_{ad} and GL_{ad} the weeds had values slightly lower than the lowest value for the native taxa. The three weed species did not have papillae (Appendix A1-1).

Variation of leaf surfaces across vegetation types

Many leaf features varied significantly on average across vegetation type (ANOVAs; for vegetation type $P < 0.05$, except GL_{ad}; for taxon nested within vegetation type, $P < 0.01$; Appendix A1-1). On average, SD_t was three-fold higher in wet than dry forest. The SD_{ab} varied more across vegetation type than SD_{ad}, from zero and 39 stomata mm^{-2} for bog and coastal vegetation, respectively to 590 stomata mm^{-2} for wet forest taxa, and from zero for wet forest to 182 stomata mm^{-2} for mesic forest taxa. The GL_{ab} and PL_{ab} varied two-fold from wet forest to coastal vegetation while the PL_{ad} varied 1.25-fold from dry forest to coastal vegetation. There was a two-fold range in SPI_t from dry forest to coastal vegetation. The SPI_{ad} ranged from zero for bog and wet forest habitats to 0.019 for mesic habitats and the SPI_{ab} ranged from zero for bog habitat and 0.005 for coastal habitat, to 0.023 for wet forest habitats. The $\%SPI_{ad}$ ranged from zero for *E. sparsiflora* to 100% for wet forest taxa. There was significant variation across vegetation types in papillar size and density. The P_D ranged from zero for wet forest taxa to 4249 mm^{-2} for bog and 1750 mm^{-2} for mesic forest, and P_{dia} varied up to 6.0 μm on average for dry forest taxa and

10 µm for bog taxon *E. sparsiflora.* The ECS_{ad} varied two-fold from dry forest to coastal vegetation, and ECS_{ab} varied three-fold from wet forest to coastal vegetation.

Variation in leaf surfaces relating to open versus shade regeneration

Leaf features varied significantly between species that establish in open habitat versus shaded habitats (ANOVAs; for open versus shade- establishing category, $P < 0.05$ except for PL_{ad} and for ECS_{ad}, for taxon, $P < 0.01$; Appendix A1-1). The $\%SD_{ab}$ was three-fold higher for shade- than open-establishing taxa, whereas the $\%SD_{amphi}$ was three-fold higher for open- than shade-establishing taxa. For SD_t, SD_{ab}, SPI_{ab}, GL_{ad}, and ECS_{ab}, shade-establishing taxa had higher values on average than open-establishing taxa; for P_D, SD_{ad} , $\%SD_{ad}$ SPI_{ad}, $\%SD_{amphi}$, $\%SPI_{ad}$, SPI_t, P_{dia}, PL_{ab}, and GL_{ab}, open-establishing taxa had higher values.

Variation in plant and leaf traits relating to stomatal distribution category

The climate differed among taxa of the three stomatal distribution categories (*SDC*; hypo- amphi-, and hyperstomatous, ANOVAs; $P < 0.001$; Appendix A1-1). Mean elevation varied from 394 m on average for hyper- to 734 m for hypostomatous taxa. The %*OS*, *MAT*, and *VPD* varied from 37%, 18.6°C and 0.40 kPa for hypo- to 83%, 21.1°C, and 0.64 kPa for hyperstomatous taxa. The *MAP* varied from 1006 to 1864 to 3733 mm from amphi- to hyper- to hypostomatous taxa.

On average, taxa of different stomatal distribution categories varied significantly in other plant traits (ANOVAs; for *SDC*, $P < 0.05$ except for ECS_{ab}, P_{dia}, and PL_{ad}, Appendix A1-1). This finding is consistent with the hypo-, hyper- and amphistomatous taxa being typical differing habitat types. Hypostomatous were prevalent in moist

habitats, hyperstomatous in intermediate habitat types, and amphistomatous distribution dominated in dry habitats.

Plant height, stem diameter, P_{mass}, and GL_{ad} varied 1.1 to two-fold from hyper- to amphistomatous taxa. The Chl_{area}, *LMA*, N_{area}, PL_{ab}, GL_{ab}, and SPI_t varied 1.2 to two-fold from hypo- to hyperstomatous taxa, whereas the Chl_{mass}, *Chl:N*, and ECS_{ad} varied two to three-fold from hyper- to hypostomatous taxa. The *LA* and *T* varied seven-fold and 1.3-fold, respectively, from amphi- to hypostomatous taxa. The *D*, N_{mass}, C_{mass}, P_{area}, and $\delta^{13}C$, varied 1.03 to two-fold and P_D ranged from zero to 677 mm^{-2} from hypo-to amphistomatous taxa. The SD_t, and *N/P* varied 1.3- to two-fold from amphi- to hyperstomatous taxa.

Correlation of leaf surface traits with climate and habitat

Across the native taxa, there were many correlations between stomatal traits and environmental parameters. Numerous traits correlated positively with %*OS*, i.e., $\%SD_{ad}$, SD_{ad}, P_D, SPI_{ad}, and $\%SPI_{ad}$ (r_s and r_p = 0.46 - 0.66; $P < 0.05$). The $\%SD_{ad}$ declined with elevation and increased with *MAT* ($|r_s|$ and $|r_p|$ = 0.42-0.46). The SD_{ab} correlated positively with *MAP*, while $\%SD_{ad}$, SD_{ad}, $\%SPI_{ad}$, and $\%SD_{amphi}$ correlated negatively with *MAP* ($|r_s|$ and $|r_p|$ = 0.41- 0.78). The $\%SD_{ad}$, SD_{ad}, and P_{dia} correlated positively with *VPD* (r_s and r_p = 0.40-0.53). Thus, the $\%SD_{ad}$ correlated with four environmental variables, %*OS*, *MAT*, *MAP*, and *VPD*, themselves inter-correlated (Figure 1.6; see previous section). I examined the partial correlations of $\%SD_{ad}$ with each variable; the correlations were maintained in the same direction for %*OS*, *MAT*, and *MAP* (partial *r* values were 0.76, 0.54, and -0.70; P < 0.01-0.001, but for *VPD* the partial correlation changed direction to negative (partial *r* = -0.48; P <0.05). The SD_{ad} and $\%SPI_{ad}$, which

were tight correlates of %SD_{ad} (see following section) also correlated with multiple environmental traits; SD_{ad} correlated with *MAP*, %*OS*, and *VPD*, while %SPI_{ad} correlated with *MAP* and %*OS*.

Other leaf traits also correlated with environment. The N_{area} and $\delta^{13}C$ correlated positively with %*OS* and *LA* and *Chl:N* correlated negatively with %*OS* ($|r_s|$ and $|r_p|$ = 0.43-0.57; P <0.05). Height and *LA* correlated positively and *N/P* correlated negatively with elevation ($|r_s|$ and $|r_p|$ = 0.39 -0.58; $P < 0.05$); these traits correlated in the opposite direction with *MAT* ($|r_s|$ and $|r_p|$ = 0.40 - 0.56; $P < 0.05$). The *T* and *LA* correlated positively and *D* negatively with *MAP* ($|r_s|$ and $|r_p|$ = 0.42 - 0.65; $P < 0.05$), and *LA* correlated negatively with *VPD* (r_s = -0.01, r_p= -0.49; $P < 0.05$). Additionally, ECS_{ab} was positively correlated with *MAT* and *VPD* when weed data was included but not when weed data was not included (r_s = 0.46-0.57; r_p = 0.46-0.48; P <), though ECS_{ad} was not correlated with *MAT*, *MAP*, or *VPD*.

Correlations among stomatal traits and with other leaf traits

For the native taxa the abaxial and adaxial faces frequently showed substantially independent trait variation. Although ECS_{ad} and ECS_{ab} were positively correlated across taxa (r_s and r_p = 0.53-0.57; $P < 0.05$), no correlations were found between GL_{ab} and GL_{ad}, or PL_{ab} and PL_{ad}, or SPI_{ad} and SPI_{ab} (r_s and r_p = 0.38-.51; $P > 0.05$), and a negative correlation was found between SD_{ad} and SD_{ab} (r_s and r_p = -0.70 to -0.77; $P < 0.001$). Stomatal density was equally variable on both faces, and thus SD_t was not driven primarily by the stomatal density on either face, i.e., SD_t was not correlated with SD_{ad} or SD_{ab} (r_s and r_p = 0.08-0.20; $P > 0.05$).

For each face, I found significant stomatal trait correlations. I found positive relationships between stomatal size traits on each face, PL_{ab} with GL_{ab}, and PL_{ad} with GL_{ad} (r_s and r_p = 0.59-0.94; $P < 0.05$). Additionally, on each face, stomatal size correlated negatively with density; PL_{ab} and GL_{ab} correlated negatively with SD_{ab}; and GL_{ad} correlated negatively with SD_{ad} (r_s and r_p = -0.58 to -0.79; $P < 0.05$). Consequently, there was a negative relationship of SD_t with stomatal size traits GL_{ad}, GL_{ab} and PL_{ab} (r_s and r_p = -0.52 to -0.67; $P < 0.05$). There was a positive correlation between $\%SD_{ad}$ and g_m (r_s and r_p = 0.61 to 0.86; $P < 0.05$).

Notably, *SPI* is determined by stomatal density and by stomatal size ($SPI = SD \times PL^2$), but was principally driven by variation in stomatal density. Thus, the SPI_{ad} was positively driven by SD_{ad} (r_s and r_p = 0.96-0.98; $P < 0.001$), but not by GL_{ad} or PL_{ad} (r_s and r_p = -0.10 to -0.37; $P > 0.05$), and the SPI_{ab} was positively driven by SD_{ab} (r_s and r_p = 0.72-0.77; $P < 0.001$). The SPI_t was driven by the total stomatal pore area per leaf area of the adaxial and not the abaxial surface. Thus, the SPI_t correlated with SD_{ad} and with SPI_{ad} (r_s and r_p = 0.60-0.83; $P < 0.01$), and although SPI_t correlated with PL_{ab} (r_s and r_p = 0.45-0.64; $P < 0.05$), it was independent of SD_{ab} and SPI_{ab} ($|r_s|$ and $|r_p|$ = 0.15-0.39; $P > 0.05$). Consequently, SPI_t correlated with stomatal pore distribution, i.e., positively with $\%SPI_{ad}$ (r_s and r_p = 0.52-0.57; $P < 0.01$). The SPI_t was driven by SPI_{ad} due to its greater variation across species than SPI_{ab}, This pattern arose because although stomatal densities and sizes were equally variable on both faces, the negative correlation of stomatal density and size was much greater on the abaxial than on the adaxial face, leading to a lower variability in SPI_{ab} (Figure 1.3 and Figure 1.7).

Across taxa, several other epidermal traits correlated significantly with stomatal traits on the adaxial, but not the abaxial surface. Thus, the SD_{ad}, SPI_{ad}, $\%SD_{ad}$, and $\%SPI_{ad}$ all correlated negatively with ECS_{ad} (r_s and r_p = -0.59 to -0.68; $P < 0.05$), but there were no analogous correlations of abaxial stomatal traits with ECS_{ab} ($|r_s|$ and $|r_p|$ = 0.008-0.10; $P > 0.05$). Papillae traits were also correlated with adaxial stomatal traits. The P_D was positively correlated with SD_{ad} (Figure 1.8), and with $\%SD_{ad}$, SPI_{ad}, $\%SPI_{ad}$, and SPI_t (r_s and r_p = 0.48-0.93; P <0.05). P_{dia} correlated negatively with SD_{ad}, $SPI_{ad,}$ and SD_t (r_s and r_p = -0.82 to -0.94; P <0.05), and was not correlated with ECS_{ad} (r_s and r_p = 0.57-0.67; $P > 0.05$). The correlations were not significant for P_{dia} and ECS_{ad} ($|r_s|$ and $|r_p|$ = 0.05-0.64; $P > 0.05$).

Many stomatal and epidermal traits correlated with composition and morphological traits that shifted between sunny dry and moist shady habitats (Table 1.2). The ECS_{ab} was not correlated with P_{area} (r_s = -0.47 and r_p= -0.15; $P > 0.05$), which was higher in taxa of sunny, dry habitats, while ECS_{ad}, higher for taxa of moist, shady habitats, correlated positively with Chl_{mass} and *Chl:N* (r_s and r_p = 0.52-0.67; $P < 0.01$), and negatively with *LMA*, N_{area}, and P_{area} (r_s and r_p = -0.46 to -0.70; $P < 0.05$). The P_D was not correlated with *LMA*, *D*, and N_{area} (r_s and r_p= 0.44 – 0.58; $P > 0.05$), and negatively with *Chl:N* and Chl_{mass} (r_s and r_p = -0.51 to -0.58; P <). The $SD_{ad,}$ $\%SD_{ad}$, SPI_{ad}, and $\%SPI_{ad}$ also correlated with traits associated with sunny, dry habitats, i.e., positively with N_{area}, *D*, *LMA*, and/or P_{area} (r_s and r_p = 0.45-0.71; $P < 0.05$) and negatively with *LA*, *Chl:N*, and/or Chl_{mass} (r_s and r_p = -0.40 to-0.57; $P < 0.05$). The SD_{ab} correlated negatively with *D* (r_s and r_p = -0.49 to-0.51; $P < 0.05$). The $\%SD_{amphi}$ correlated positively with $\delta^{13}C$, P_{area}, and *D* (r_s and r_p = 0.43-0.58; $P < 0.05$), and negatively with *N/P*, *LA*, and

T (r_s and r_p = -0.43 to -0.73; $P < 0.05$). The SD_{ab} correlated negatively with *D* (r_s and r_p = -0.49 to -0.51; $P < 0.05$). The GL_{ab} correlated negatively with *LA* and *T* (r_s and r_p = -0.46 to -0.57; $P < 0.05$) and the PL_{ab} correlated negatively with *LA* (r_s and r_p = -0.60 to -0.67; $P < 0.01$), whereas the GL_{ad} correlated negatively with *D* (r_s and r_p = -0.56 to -0.58; $P < 0.05$), and the PL_{ad} correlated positively with *Chl:N* (r_s and r_p = 0.62-0.72; $P < 0.05$). The SPI_t correlated positively with P_{area} (r_s and r_p = 0.60-0.65) and with *Chl:N* (r_s and r_p = -0.50 to -0.55; $P < 0.05$). The SD_t was not correlated with *LA* and *T* (r_s and r_p = 0.44-0.52; $P < 0.05$).

Correlations of traits for field plants with traits previously studied in common garden seedlings

The data collected for field plants correlated significantly with gas exchange measurements made decades ago in a common garden study of seedlings for the 11 taxa measured in common (Pearcy et al., 1982). The A_{area} (assimilation rate per area, or photosynthetic rate per area of leaf) for common garden seedlings was higher for taxa of sunny, dry habitats, correlating negatively with *MARH* and *MAP* for field adults (r_s and r_p = -0.68 to -0.79; $P < 0.05$). Stomatal conductance measured for common garden seedlings correlated positively with SPI_t, *LMA*, and P_D for field adults (r_s and r_p = 0.71-0.93; $P < 0.05$). The A_{area} for common garden seedlings correlated positively with $\%SD_{ad}$, PL_{ab}, P_D, and *D* of field adults (r_s and r_p = 0.67-0.90; $P < 0.05$), and negatively with SD_{ab} of field adults (r_s and r_p = -0.89 to -0.79; $P < 0.05$). The water use efficiency of common garden seedlings correlated negatively with SPI_{ab}, SD_{ab}, and ECS_{ad} for field adults (r_s and r_p = -0.61 to -0.89; $P < 0.05$).

DISCUSSION

The 26 Hawaiian *Euphorbia* taxa showed dramatic variation in epidermal and stomatal traits (Table 1.1, Figure 1.3-Figure 1.9 & Figure 1.11) that is greater within a genus than has been reported for any other genera.. For instance, only up to maximum of a 2.5-fold variation in stomatal density was described across native Hawaiian *Plantago* taxa (Dunbar-Co et al., 2009). Further, species within genera typically are all hypostomatous, or range from hypo- to amphistomaty, but do not include hyperstomaty. The discovery of five hyperstomatic taxa in the Hawaiian *Euphorbia*, builds upon the observation of hyperstomaty in *E. mesembranthifolium* (Gaucher, 1898; Roth, 1992). Hyperstomaty as an anatomical feature is apparently unique for typical plagiotropic dicotyledon leaf types.

The wealth of data collected for this study allowed me to address 14 hypotheses between stomatal and epidermal traits, and the natural environments in which the plants occur. Leaf epidermal and stomatal traits related to vegetation type, and to open- vs. shade-establishment in support of the hypotheses based on previous studies of plants across environmental gradients. Thus, consistent with my hypotheses, *Euphorbia* taxa establishing in shade were usually hypostomatous, while amphistomaty was typically found in taxa establishing in higher irradiance. All hyperstomatous taxa occupy habitats of high irradiance and temperature, and of intermediate rainfall, or a bog. Notably, amphistomaty and hyperstomaty correlated with other leaf traits associated with high irradiance and/or xeric habitats, including small leaf size, high *LMA* and high *SPI*. I had hypothesized that (i) adaxial distribution (%SD_{ad}) and amphistomaty (%SD_{amphi}) would correlate negatively with mean annual precipitation (*MAP*) because it would be

disadvantageous to produce stomata on the surface that receives the most rain. Also, the %SD_{ad} and %SD_{amphi} would correlate positively with %*OS* and *MAT* to allow more effective gas exchange and cooling in the intervals when water is available in drought prone habitats (Salisbury, 1928; Parkhurst, 1978; Mott et al., 1982; Korner et al., 1989; Peat and Fitter, 1994). By contrast, a high abaxial stomatal distribution in shade would protect shade-adapted stomata from high irradiance, and reduce shading of mesophyll that would occur if stomata were on the adaxial face (Salisbury, 1928; Mott et al., 1982; Korner et al., 1989; Roth, 1992; Peat and Fitter, 1994; Cavender-Bares et al., 2007; Dunbar-Co et al., 2009). Indeed, SD_{ad} and %SD_{ad} were three-fold higher for open- than shade-establishing taxa. Further, %SD_{ad} was higher for taxa at lower *MAP* and higher *MAT* and %*OS*. Notably %SD_{ad} varied across vegetation types, with higher values for dry and bog vegetation than wet forest. Thus, hypostomaty was associated with moist, shady habitat, and amphi- and hyperstomaty with sunny, dry habitat. Notably the %SD_{amphi} was linked with vegetation type, being three-fold higher for open- than shade-establishing taxa, and %SD_{amphi} on average ranged from zero for the wet forest and bog taxa to 44% for dry forest taxa. There was no correlation between %SD_{amphi} and %*OS*, *VPD* or *MAT*, but %SD_{amphi} was significantly correlated with *MAP*, consistent with this distribution type being associated with the driest sites, with hyperstomatous species at intermediate sites, and hypostomatous taxa at moist shady sites. Contrary to expectations, I found that *Euphorbia* of higher elevation were not necessarily amphistomatous. The lack of a trend may reflect the horizontal leaf positioning of all taxa. Further, no association of amphistomaty with leaf thickness was found in this group.

To further assess the potential significance of unexpected and dramatic variation in stomatal distribution, I used two other methods for common garden plants of three taxa varying significantly in $\%SD_{ad}$ and $\%SPI_{ad}$. The results for stomatal distribution were supported, and corresponded with stomatal conductance under high irradiance, pointing to the functional importance of stomatal distribution. The lack of relationship of $\%SD_{ad}$ with total stomatal density, and its determination equally by both adaxial and abaxial stomatal densities indicates that stomatal development and evolution on the two leaf faces can be independent, consistent with developmental studies indicating different precursor tissues in the leaf primordium and thus different sensitivity to the signal for stomatal formation from meristematic cells (Maksymowych, 1973). The advantage of hyperstomaty in xeric environments is unknown, but I suspect that this feature may contribute to rapid leaf cooling and gas exchange during periods of high rainfall, given that other traits enable hyperstomaty (see discussion of papillae below).

What is the advantage of hyperstomaty? We found hyperstomatic species to occupy intermediate *MAP* sites on average. The five hyperstomatous taxa also occupy greatly differing habitat types: bog (*E. sparsiflora*); coastal strand (*E. celastroides* var. *stokesii* and *E. degeneri*); and mesic forest (*E. celastroides* var. *celastroides* and var. *hanapepensis*), thus are not all occupants of the driest habitat types. Indeed, these taxa were intermediate between hypostomatous taxa and amphistomatous taxa in their trait values for moisture-related characteristics, *LA*, *T*, and N_{area}. This pattern and the correlation of hyperstomaty with gas exchange rates (see below) support a role for hyperstomaty in allowing the leaves to carry out high rates of gas exchange per area between short rainfall events. Additionally, preliminary observations indicate that the

hyperstomatous taxa are highest in their degree of dense water storage tissue under the palisade (L. Sack, J. Pasquet-Kok and M.J. Sporck, pers. obs.), which should enable gas exchange to continue between rainfall events. Further, water storage tissue would obstruct gas diffusion from the abaxial surface, another reason for hyperstomaty to correlate with water storage across these taxa. Finally, hyperstomatous taxa had greatest development of papillae (see below). Papillae make it possible to keep adaxial stomata clean. These advantages of adaxial stomata must outweigh their cost, namely that the mesophyll shaded by the guard cells does not incur much of a cost. Amphistomatous distribution was more common in the most xeric habitats, and those taxa did not develop water storage tissue, presumably because dry conditions are too chronic for the leaf water storage tissue to play a key role. Without water storage tissue, having stomata on the bottom is not a design problem and the advantage of amphistomaty in increasing diffusion to the palisade would be significant. Taxa of the driest environments tend to be drought deciduous, thus investment in water storage tissue for those taxa would not be an efficient strategy.

I hypothesized that (ii) stomatal size (PL_{ad}, PL_{ab}, GL_{ad}, GL_{ab}) would be correlated negatively with *MAT*, and *VPD* and positively with *MAP*, and that stomata would be smaller in open- than shade-establishing taxa because smaller stomata may be able to close faster and/or more completely (Franks et al., 2009). Comparing taxa across vegetation types, these patterns were supported. The GL_{ab} and PL_{ab} varied two-fold from wet forest to coastal taxa while the PL_{ad} varied 1.25-fold from dry forest to coastal taxa. However, there were no consistent differences between open- and shade-establishing taxa. For GL_{ad}, shade-establishing taxa had higher values, whereas for GL_{ab} and PL_{ab},

open-establishing taxa had slightly higher values, and for PL_{ad} there was no significant difference. Further, PL_{ad}, PL_{ab}, GL_{ad}, and GL_{ab} did not have significant relationships with *MAT*, *VPD*, *MAP* or %*OS*. Stomatal size varied much less than stomatal density (5.2-fold vs. 10-fold). Obstacles, such as the fact that stomatal characteristics may be constrained by allometric properties (Franks et al., 2009) and have be reported to be highly conserved traits as in some aquatic species (*Nymphaea alba* "white lotus," *Nuphar lutea* "spatterdock," and, *Lemna minor* "duckweed") that retain nonfunctioning stomata under epidermal and cuticular layers or lacking a sub-stomatal cavity (Zeiger, 1987). These potential obstacles could prevent evolutionary shifts in stomatal size and may have reduced that ability for this trait to adapt to climate and habitat, and shifts in stomatal density were an apparently important mechanism to affect changes in overall total stomatal pore area.

I hypothesized that (iii and iv) total stomatal density and total stomatal pore (SPI_t) should be positively correlated with high irradiance, *MAT* and *VPD*, and low *MAP* to facilitate cooling and CO_2 assimilation in more productive and competitive environments (Sack et al., 2003; Cavender-Bares et al., 2007), though the degree to which such a trend should be expected in C_4 plants is not known. I found that SD_t was higher for open than shade-establishing taxa. However, contrary to my hypothesis, SD_t, was three-fold higher for wet than dry forest on average, and there was no significant relationship between SD_t and *MAT*, *VPD*, or *MAP*. Further, there was great variation in *SPI* across vegetation types, but in contrary directions for the two leaf faces. The SPI_{ad} increased from wet forest to coastal taxa, while SPI_{ab} showed the opposite trend, increasing from coastal to wet taxa, and there was a two-fold range in SPI_t from dry forest to mesic taxa, with wet

forest taxa intermediate. I found significant differences in SPI_t between open vs. shade establishing taxa with SPI_t increasing for open establishing taxa, However, SPI_t was not correlated with climate variables (%*OS*, elevation, *MAT*, *MAP*, *MFVPD*, or *VPD*). These findings indicate a more important role for irradiance than for soil moisture or temperature in determining the variation in SPI_t across taxa.

I had hypothesized a correlation of (v) stomatal density, $\%SD_{amphi}$, $\%SD_{ad}$, and SPI_t with other traits known to be correlated with sunny dry habitats (Table 1.2). Indeed, $\%SD_{ad}$ and/or $\%SPI_{ad}$ were positively correlated with N_{area}, *D*, *LMA*, and P_{area} and negatively with *LA*, *Chl:N*, and Chl_{mass}. I did not find a trend with thickness, presumably for the same reason that thickness was uncoupled with $\%SD_{amphi}$, as explained above. The SD_t was negatively correlated with *Chl:N*. The SPI_t was positively correlated with P_{area}, and SPI_{ad} was positively correlated with *D*, N_{area}, and P_{area}. However, contrary to my hypotheses, SPI_t was not significantly correlated with *T*, *LA* or *LMA*. It appeared that SPI_t was not selected by high irradiance, low *MAP* or high *VPD*, since there were no correlations between SPI_t and these climate traits. Although SD_t and SPI_t values were higher for open than for shade establishing taxa overall establishment irradiance is critical to plant success (Grubb, 1998), the lack of the expected correlation of stomatal pore with climate and leaf morphology could be due to the Hawaiian *Euphorbia* retaining C_4 biochemistry. I expected $\%SD_{amphi}$ to show associations with other traits known to be selected in other lineages by sunny, dry habitats and thus to correlate positively with smaller and thicker leaves of higher *LMA*, *D*, Chl_{area}, Chl_{mass}, N_{area}, N_{mass}, P_{area}, P_{mass} and $\delta^{13}C$ and lower *Chl:N* and *N:P* (Table 1.2; Givnish, 1988; Walters and Reich, 1999; Givnish et al., 2009). Indeed, the $\%SD_{amphi}$ was positively correlated with $\delta^{13}C$, P_{area}, and

D, and negatively with *N:P*, *LA*, and *T*. There was a lack of the expected positive correlation of $\%SD_{amphi}$ and *T* , which has been previously strongly supported as a major leaf design principle (Mott et al., 1982; Dunbar-Co et al., 2009). It is generally accepted that leaves greater than 500 μm in thickness are held to require amphistomaty for CO_2 to diffuse effectively from stomata to mesophyll chloroplasts (Mott et al., 1982). It is likely due to these being C_4 species with biochemical carbon concentration, and also to there being exceptional variation in leaf cross-sectional anatomy in these taxa, amounting to several types of thick *Euphorbia* leaves that this correlation was not present. For example, several open-establishing taxa have thick leaves full of mesophyll (*E. celastroides* var. *kaenana*), and lacking water storage tissue, whereas others have thick leaves with > 50% water storage tissue (*E. degeneri*), and some shade taxa have thick leaves dominated by airspace (*E. herbstii*) (Pearcy et al., 1982). It is apparent that the taxa with water storage tissue in the abaxial side of the leaf, and air space only in the adaxial side of the leaf, would not need stomatal pores on both sides for CO_2 to reach the mesophyll cells since CO_2 moves through air space, not water storage tissue (Figure 1.11). and those with airspace can be thick without needing stomata to reduce the diffusive resistance through cell walls.

I hypothesized that (vi) SPI_t would correlate with gas exchange rates, i.e., with photosynthesis and or stomatal conductance (*g* and A_{area}). Indeed, the *g* of common garden seedlings correlated positively with SPI_t and A_{area} for field adults. Also I hypothesized that $\%SD_{ad}$ would hold an advantage in photosynthetic rate (*g*, A_{area}) by reducing the CO_2 transfer path length. The A_{area} for common garden seedlings correlated positively with $\%SD_{ad}$ of field adults, due to a correlation of *g* and g_m for common garden

seedlings with %SD_{ad}. These correlations were especially striking given that the study of Pearcy et al. (1982) was conducted decades ago for greenhouse-grown, common garden seedlings, while our study was conducted recently on mature plants in their native habitats.

I hypothesized that (vii) papillar density and size would be greater in open-establishing species and in taxa of high *MAT* and *VPD*, and of low *MAP*, because smaller cells can be an adaptation to desiccating conditions (Cutler et al., 1977). Further, papillae may protect stomata from wind, rain and particles, and may reflect excess irradiance, and deter the intrusion of liquid water into the stomata, and thus may be especially selected in high irradiance and xeric habitats (Jordan et al., 2005). This is especially true where short rainfall events may provide a substantial part of the available precipitation and a plant may need to respond immediately with gas exchange (Wagner et al., 2003; Haworth and McElwain, 2008). Indeed, P_{dia} correlated positively with *VPD,* and P_D was higher for open- than shade-establishing taxa. The P_D ranged from zero for wet forest taxa to 1,750 per mm^{-2} for mesic taxa and up to 4,249 per mm^{-2} for the single bog taxon. The average P_{dia} varied two-fold from mesic to dry taxa and two-fold higher for bog taxon *E. sparsiflora*. Wet forest taxa lacked papillae. As predicted, P_D was positively correlated with %*OS*, but surprisingly, P_D was not significantly correlated with *MAP*, *MAT* or *VPD*. There was a significant correlation between papillae density and adaxial stomatal density, indicating a role for papillae in the protection of amphi- and hyperstomatous leaves from excessive transient water losses, photodamage, and/or particle blockage of the stomata, and facilitating rapid gas exchange of adaxial stomata after rainfall events.

I hypothesized that (vii), papillae traits would show associations with other traits known to be selected in other lineages in sunny, dry habitats (see Table 1.2). Indeed, the P_D correlated negatively with *Chl:N* and Chl_{mass}. I hypothesized that (viii) papillae density would be correlated with SD_{ad} Indeed, P_D was positively correlated with SD_{ad}, and with $\%SD_{ad}$, SPI_{ad}, $\%SPI_{ad}$, and SPI_t.

I hypothesized (ix) that smaller epidermal cells (ECS_{ad} and ECS_{ab}) would occur more frequently in open-establishing species and in taxa of high *MAT* and *VPD* and low *MAP*, because smaller cells can be an adaptation to desiccating conditions due to their ability to maintain a lower cellular osmotic potential and greater cell turgor (Cutler et al., 1977; Rahim and Fordham, 1991). Small epidermal cell sizes and stomatal sizes would be associated with other traits known to be selected in other lineages in sunny, dry habitats (Table 1.2). These expectations were not supported. Rather, across the native Hawaiian *Euphorbia,* ECS_{ad} was not correlated with *MAT*, *MAP*, or *VPD*, and ECS_{ab} was positively correlated with *MAT* and *VPD*. I posit two possible explanations for why the Hawaiian *Euphorbia ECS* does not hold true to Cutler's explanation of the relationship of cell size to plant water stress. First, it is possible that epidermal cell size did not decrease in water stressed habitats because of the allometric relationship of epidermal cell size and stomatal number. This means that there could be a trade-off between optimum stomatal densities and epidermal cell size that prevents cell size decline in water deficient habitats because the increase in stomatal density that comes with smaller epidermal cell size would be too detrimental to overall plant water balance. Another explanation is that the water storage tissue in the leaf might decouple cell size from dryness because there is stored water to pull from in the leaf, much like the anatomy of many succulents such as

cacti, which also do not have particularly small epidermal cells yet they grow in extremely water-stressed conditions Ogburn and Edwards, 2009. The ECS_{ad} correlated positively with Chl_{mass} and *Chl:N* and negatively with *LMA*, N_{area}, and P_{area}. The GL_{ad} correlated negatively with *D*, while the GL_{ab} correlated negatively with *LA* and *T*. P_{dia} correlated negatively with SD_{ad}. The P_D correlated positively with *LMA*, *D*, and $N_{area,}$ and negatively with *Chl:N* and Chl_{mass}.

I hypothesized that (x) cell size traits will be inter-correlated, and thus that ECS_{ab}, ECS_{ad}, GL_{ab}, GL_{ad}, PL_{ad}, PL_{ab}, and P_{dia} would be correlated. However, there were few significant correlations between cell size traits. There were positive correlations between ECS_{ab} and ECS_{ad}, indicating a constraint on the development and evolution of epidermal pavement cells and of guard cells on both leaf faces as well as positive correlations between GL_{ad} and PL_{ad}, and PL_{ab} and GL_{ab}, indicating a constraint on the geometry of guard cell and pore dimensions. The independence of epidermal cells from guard cell size highlights their independence in development and evolution in this lineage. Thus, the stomata of the upper and lower leaf surfaces can adapt to their environment in number and size independently of each other.

I hypothesized that (xi): larger leaves would have larger cells due to the rules of allometric constraints (Franks et al., 2009). This relationship was expected due to scaling principals that are known to exist in nature. However, no relationship between leaf size and epidermal cell size was found for the Hawaiian euphorbias.

I expected (xii) under allometric constraints that larger epidermal cells would drive lower *SD* and larger stomata, so larger ECS_{ab} should correlate with lower SD_{ab}, and higher PL_{ab} and GCL_{ab}, and larger ECS_{ad} should correlate with lower SD_{ad}, and higher

GCL_{ad} and PL_{ad}. Additionally, there should be a negative relationship between *SD* and *GCL* (and *PL*) on either leaf face (Salisbury, 1928; Grubb et al., 1975; Sack et al., 2003; Franks et al., 2009). Consequently, depending on the slopes of the relationship between *SD* and stomatal size on each leaf face, ECS_{ad} should correlate positively or negatively with SPI_{ad}, and the same should be true of and ECS_{ab} with SPI_{ab}. Indeed, these relationships were found for the adaxial face: SD_{ad} was negatively correlated with GL_{ad}, and the ECS_{ad} correlated negatively with SD_{ad}, SPI_{ad}, $\%SD_{ad}$, and $\%SPI_{ad}$. On the abaxial face there were negative correlations between SD_{ab} and GL_{ab}, as well as SD_{ab} and PL_{ab}. That relationship may differ between wet-adapted and dry-adapted species. Indeed, there was a negative relationship between SD_{ad} and GL_{ad} as well as a negative relationship between SD_{ab} and GL_{ab}. Also, there were negative relationships between SD_t and GL_{ad} and SD_t and GL_{ab}. These relationships suggest strong intrinsic controls during evolution and development in the Hawaiian *Euphorbia* lineage (Franks et al., 2009).

I hypothesized (xiii) that larger cells on the adaxial surface would result in larger and fewer papillae. Indeed, P_{dia} was negatively correlated with SD_{ad}, SPI_{ad}, and SD_t. Additionally, I hypothesized a negative trend between P_D and ECS_{ad} for the same reasons. This hypothesis was supported.

I hypothesized that given their adaptive radiation across habitats and life forms (xiv) the native species would show much wider variation than the weeds, putatively similar to the ancestral colonist (Morden and Motley, 2005; Yang and Berry, 2007). For 15 of 17 traits, the mean fell centrally within the range of values for the native species. For the two traits PL_{ad} and GL_{ad}, the weeds had values slightly lower than the native range. The weed species had no papillae.

These results have potential implications for new systematic or taxonomic treatments of the lineage. These possible implications are relevant to this study because varieties that comprise species among the Hawaiian *Euphorbia* are currently separated by taxonomic morphological observations. Leaf morphology, and growth habit or habitat preference (as in *remyi)*, are examples of these. The findings in the present study show some striking differences (and similarities) between the species and varieties that could support the need for taxonomic rearrangement.

The support for so many hypotheses for stomatal adaptation across species in this recent radiation, with tremendous diversity in epidermal features, points to their importance given the short evolutionary time. Our findings, by substantiating many fundamental expectations discovered in other lineages or across communities, identify the Hawaiian *Euphorbia* lineage as a possible model for future studies of stomatal function and evolution. The finding of this novel variation in a C_4 plant genus demonstrates the great capacity for evolution of anatomical and physiological diversity in light of Hawaii's isolation and large environmental gradients. The potential for gaining new scientific knowledge is one of the most important reasons for preserving rare and endangered species. These discoveries increase our respect for these taxa and excite us about their biology. Thus, this work contributes to the urgency to protect and restore them as many of these taxa are rare, and 10 are federally listed as endangered. The ecophysiological divergences implied by such contrasting stomatal distributions highlights the necessity to accurately characterize the habitat niches of taxa for most effective conservation.

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TABLES

Table 1.1. List of Hawaiian *Euphorbia* taxa included in study, island of sampled population, maximum height observed in the field, habitat type ("Wet", "Mesic" and "Dry" were determined according to moisture regimes and elevation bands for coastal taxa, following Wagner et al., 1999, with an additional "Bog" specification for SPAR, which occurs exclusively in Wahiawa bog on Kauai. Federal conservation status: ***Endangered, **Species of concern; *Recommended for candidacy as endangered. Island abbreviations: H, Hawaii, Kah, Kahoolawe, Kau, Kauai, L, Lanai, Ma, Maui, Mo, Molokai, O, Oahu, †Not included in study due to lack of current knowledge of accessible populations.

Taxon	Authors of authority	Code name	Location	Islands (sampled island pop. in bold)	Max ht. (m)	Habitat	Open or Shade
Hawaiian taxa							
E. arnottiana var. *arnottiana***	(Endl.) O. Deg. & I. Deg.	ARNO	Aina Haina,	**O**	0.91	Mesic	Shade
E. arnottiana var. *integrifolia*	Hillebrand	ARIN	Kapuuakea,	**Ma**	0.43	Mesic	Shade
E. atrococca	(A. Heller) Croizat & O. Deg.	ATRO	Makaha Ridge	**Kau**	3.17	Dry	Shade
E. celastroides var. *amplectens*	(Sherff) O. Deg. & I. Deg.	CEAM	Hawaii Loa Ridge	**O**, all main	1.62	Dry	Open
E. celastroides var. *celastroides*	(Boiss.) Croizat & O. Deg.	CECE	NTBG	**Kau**	1.95	Dry	Open
E. celastroides var. *hanapepensis*	(Sherff) O. Deg. & I. Deg.	CEHA	Halemanu Rd. Kokee	**Kau**	1.61	Wet	Shade
E. celastroides var. *kaenana****	(Sherff) O. Deg. & I. Deg.	CEKA	Makua Valley	**O**	1.70	Coast	Open
E. celastroides var. *laehiensis*	(O. Deg., I. Deg. & Sherff) Koutnik	CELA	Waiopai	**Ma,** L	0.10	Coast	Open
E. celastroides var. *lorifolia***	(A. Gray) O. Deg. & I. Deg.	CELO	NTBG	**Kau**, Ma, L	5.30	Dry	Open
E. celastroides var. *stokesii*	(C. N. Forbes) O. Deg. & I. Deg.	CEST	Kilauea Point	**Kau**, Mo	1.13	Coast	Open
E. celastroides var. *tomentella***	(Boiss.) Koutnik	CETO	Waianae Kai	**O**	0.99	Mesic	Shade
*E. clusiifolia***	(Hook. & Arn.) Arthur	CLUS	Poamoho trail	**O**	2.44	Wet	Shade
E. degeneri	(Sherff) Croizat & O. Deg.	DEGE	Secret Beach	H**, Kau,** Ma, Mo, O	0.19	Coast	Open
*E. deppeana****	(Boiss.) Millsp.	DEPP	Pali Lookout	**O**	0.24	Mesic	Open
*E. eleanoriae***†	Lorence & W. L. Wagner	ELEA	Napali	**Kau**			
*E. halemanui****	(Sherff) Croizat & O. Deg.	HALE	Halemanu Rd. Kokee	**Kau**	3.18	Wet	Shade
*E. herbstii****	W. L. Wagner	HERB	Makaha Valley	**O**	0.91	Wet	Shade
*E. kuwaleana****	(O. Deg. & Sherff) O. Deg. & I. Deg.	KUWA	Kauaopuu Ridge	**O**	0.50	Dry	Open
E. multiformis var. *microphylla*	(Boiss.) O. Deg. & I. Deg.	MUMI	PTA	**H,** Ma, Mo, O	2.01	Dry	Open
E. multiformis var. *multiformis*	(Hook. & Arn.) Croizat & O. Deg	MUMU	Pahole	Ma, **O**	1.14	Mesic	Shade
*E. olowaluana***	(Sherff) Croizat & O. Deg.	OLOW	PTA	**H**	6.15	Dry	Open
E. remyi var. *hanaleiensis*†	(Sherff) O. Deg. & I. Deg.	REHA	Hanalei	**Kau**		Wet	Shade
E. remyi var. *kuaiensis**	(O. Deg. & Sherff) O. Deg. & I. Deg.	REKU	Blue Hole	**Kau**	3.12	Wet	Shade
E. remyi var. *remyi**	(A. Gray ex Boiss.) Croizat & O. Deg.	RERE	Kokee	**Kau**		Wet	Shade
*E. rockii****	(C. N. Forbes) Croizat & O. Deg.	ROCK	Koolau Summit Trail	**O**	2.36	Wet	Shade

Taxon	Authors of authority	Code name	Location	Islands (sampled island pop. in bold)	Max ht. (m)	Habitat	Open or Shade
E. skottsbergii var. *audens*	(Sherff) O. Deg. & I. Deg.	SKAU	W.Molokai	**Mo**	0.04	Coast	Open
E. skottsbergii var. *skottsbergii***	(Sherff) Croizat & O. Deg.	SKSK	Ewa Plain	Mo, **O**	1.04	Dry	Open
E. skottsbergii var. *vaccinioides***	(Sherff) Koutnik	SKVA	Central Molokai	Kah, Ma, **Mo**	1.28	Dry	Open
*E. sparsiflora***	(A. Heller) Koutnik	SPAR	Kanaele Bog	**Kau**	0.91	Bog	Open
Weed species							
E. hirta	(L.) Millsp.	HIRT	Manoa	**O**, all main	0.37	Weed	Open
E. hypericifolia	(L.) Millsp.	HYPE	Manoa	H, Kau, **O**, Ma	0.29	Weed	Open
E. prostrata	(Aiton)	PROS	Manoa	H, Kah, Kau, **O**, Ma, L	0.11	Weed	Open

Table 1.2. Hypotheses for trait adaptation to environmental parameters based on previous studies of differences within species grown in different conditions, or comparisons of species or communities native to different environments (see *Introduction*).. Environmental traits are often autocorrelated, i.e., "sunny and dry environments" tend to have high irradiance, low mean annual precipitation (*MAP*), high mean annual temperature (*MAT*), and high vapor pressure deficit (*VPD*), and "shady wet environments" the opposite, though we note there is no strict coupling of these environmental parameters.

Leaf Traits	***"Sunny. dry environments": High Irradiance Low MAP High MAT High VPD***	***"Shady, wet environments": Low Irradiance High MAP Low MAT Low VPD***
Adaxial stomatal distribution *(*%SD_{ad})	higher	lower
Abaxial stomatal distribution (%SD_{ab},)	lower	higher
Amphistomaty (%SD_{amphi})	higher	lower
Stomatal density (SD_t, SD_{ab}, SD_{ad})	higher	lower
Papillae density (P_D)	higher	lower
Abaxial epidermal cell (ECS_{ab}), adaxial epidermal cell (ECS_{ad}), papillae (P_{dia}), and stomatal size (GL_{ad}, GL_{ab}, PL_{ab}, PL_{ad})	lower	higher
Leaf mass per area*(LMA)*	higher	lower
Leaf area *(LA)*	lower	higher
Density *(D)*	higher	lower
Chlorophyll per area *(*Chl_{area})	higher	lower
Chlorophyll per mass (Chl_{mass})	higher	lower
Nitrogen per area (N_{area})	higher	lower
Nitrogen per mass (N_{mass})	higher	lower
Phosphorus per area (P_{area})	higher	lower
Phosphorus per mass (P_{mass})	higher	lower
Stable isotope ratio ^{13}C:^{12}C, ($\delta^{13}C$)	higher	lower
Chlorophyll:Nitrogen (*Chl:N*)	lower	higher
Nitrogen:Phophorus (*N:P*)	lower	higher

FIGURES

Figure 1.1. Photograph of *E. celastroides* var. *celastroides* with rain droplet, illustrating the "lotus effect" caused by the presence of papillae on the upper leaf surface.

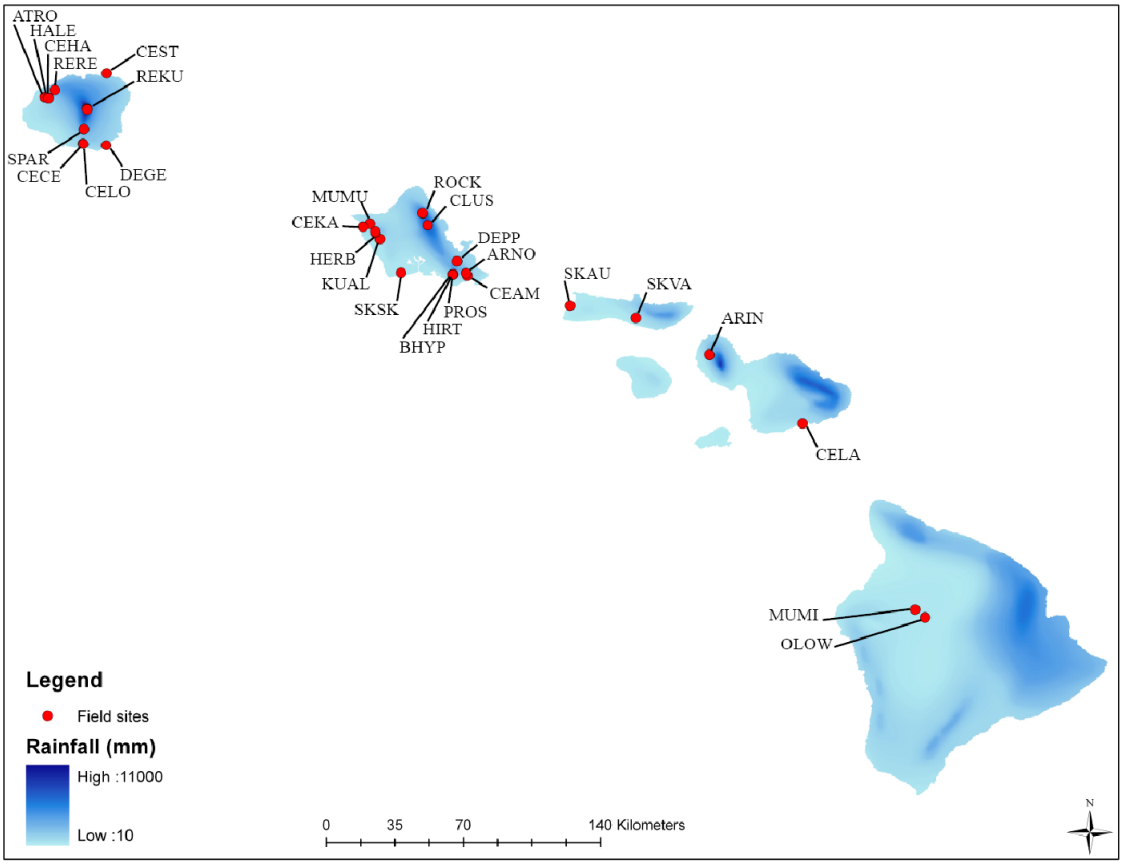

Figure 1.2. Map of the Hawaiian Islands indicating collection sites and rainfall gradient. Code names for species indicate field locations. Species names in Table 1.1.

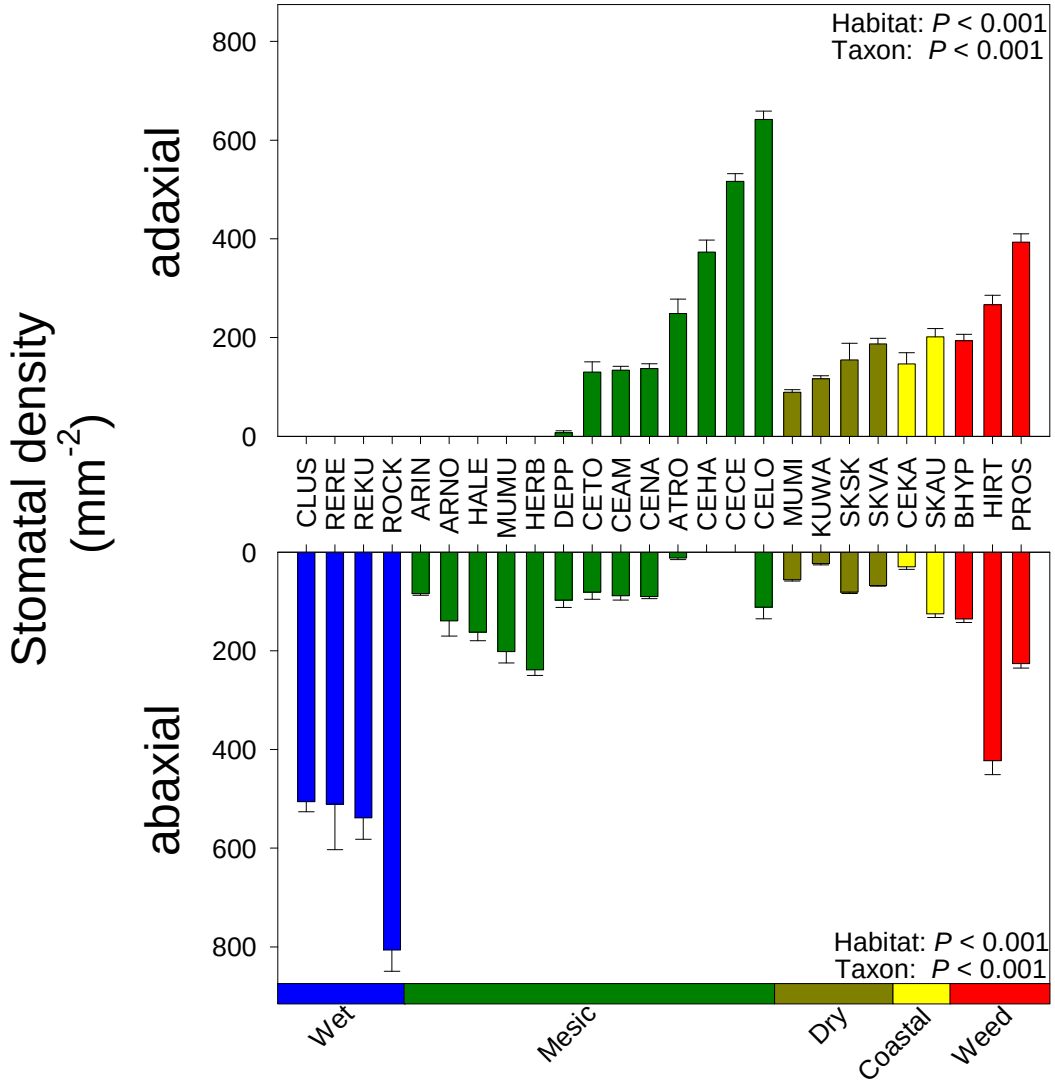

Figure 1.3. Mean values for stomatal density on adaxial and abaxial leaf surfaces for 22 native Hawaiian *Euphorbia* taxa, and three *Euphorbia* species that are weeds in Hawaii. Taxa are represented by a four letter code (Table 1.1), and arranged by habitat (Wet, Mesic, Dry, Dry Coastal, with weeds as a separate category). Bars indicate standard error (n=3). *E. celastroides* var. *stokesii; E. degeneri, E. olowaluana*, and *E. sparsiflora* were not included because dense papillae on the adaxial surface prevented measurement of stomatal density.

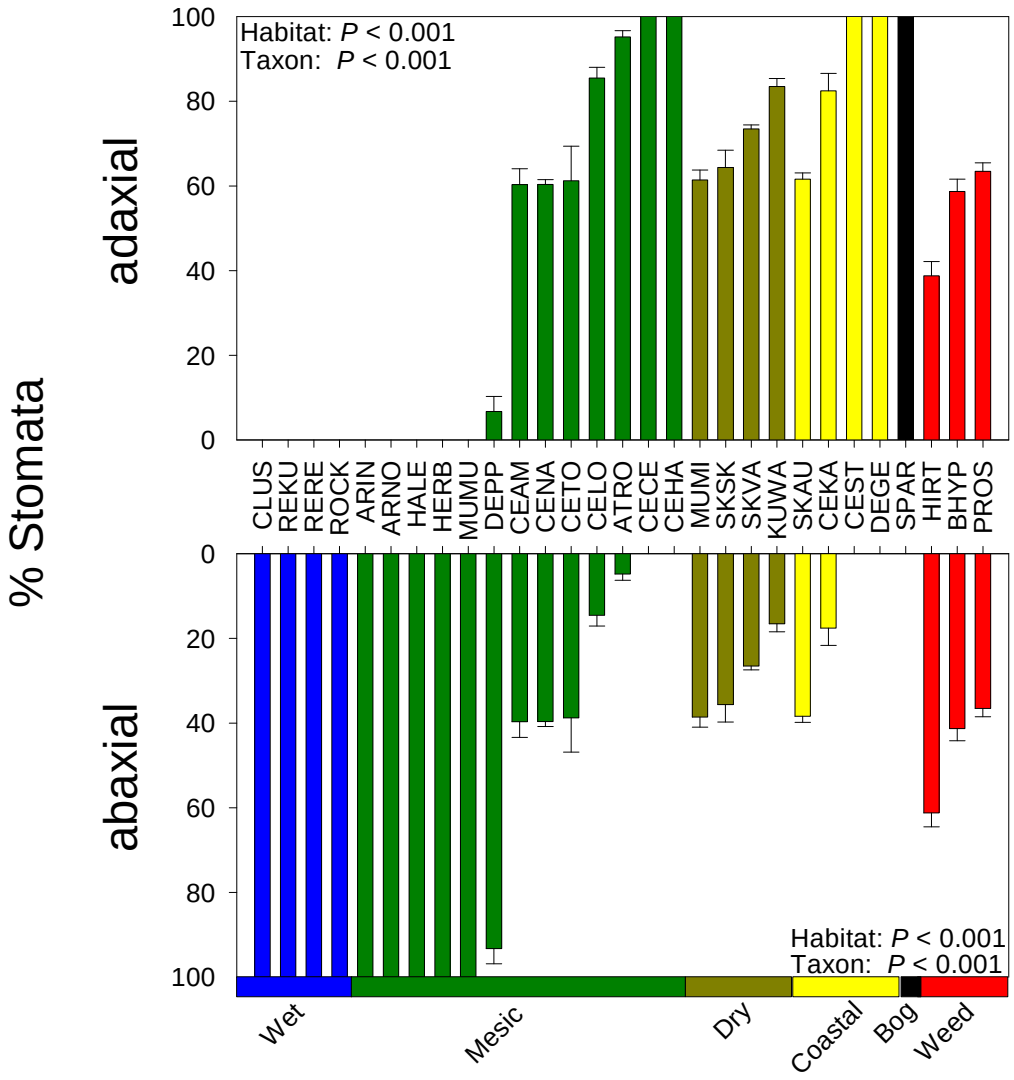

Figure 1.4. Mean values for percent stomata on adaxial and abaxial leaf surfaces for 25 native Hawaiian *Euphorbia* taxa, and three *Euphorbia* species that are weeds in Hawaii. Taxa are represented by a four letter code (Table 1.1), and arranged by habitat (Wet, Mesic, Dry, Coastal, with Weeds as a separate category). Bars indicate standard error (n=3). *E. olowaluana* was not included because the presence of dense papillae on the adaxial surface prevented measurement of stomates.

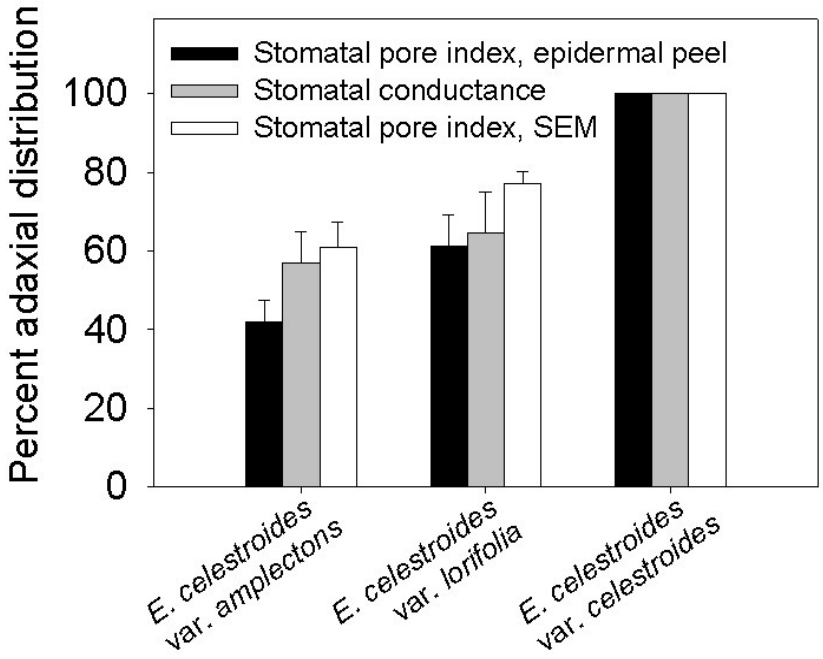

Figure 1.5. The adaxial distribution of stomatal pore assessed using light microscopy of epidermal peels, scanning electron microscopy of leaf surfaces, and porometry in a common garden field site for three taxa. All three methods yielded the same results, confirming the finding of hyperstomaty and its correlation with gas exchange (3-way ANOVA on log-transformed data, for species, $P < 0.001$; for method, $P = 0.25$; for species x method, $P = 0.60$; n = 3-5).

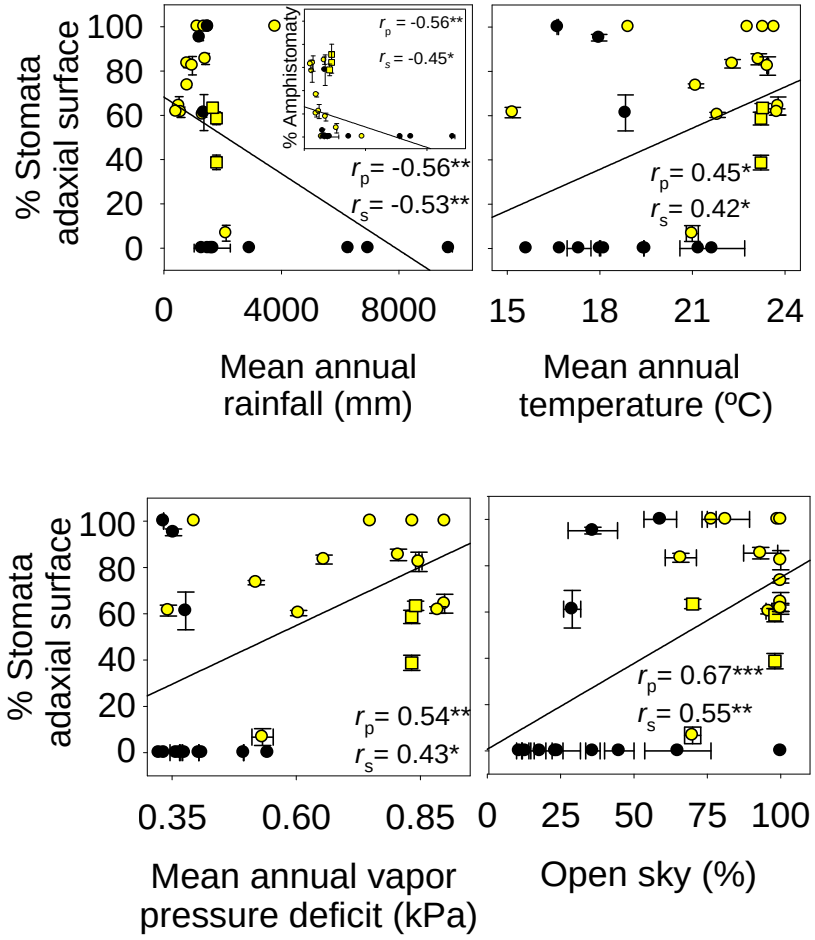

Figure 1.6. Correlation of percent stomatal pore index on adaxial surface with climate for 26 native Hawaiian *Euphorbia* taxa and three *Euphorbia* species that are weeds in Hawaii, for mean annual temperature (*MAT)*, the r_p and r_s values were 0.45 and 0.42 respectively, with $P < 0.05$ and < 0.05 respectively; for mean annual precipitation (*MAP)* the and r_s values were -0.56 and -0.53 respectively, with $P < 0.01$ and < 0.01 respectively; for vapor pressure deficit (*VPD)*, the r_p and r_s values were 0.54 and 0.43 respectively, with $P < 0.01$ and < 0.05 respectively; and for % open sky the and r_s values were 0.67 and 0.55 with P between <0.001 and < 0.01 respectively. Black filled circles indicate shade-establishing taxa, yellow filled circles open-establishing taxa.

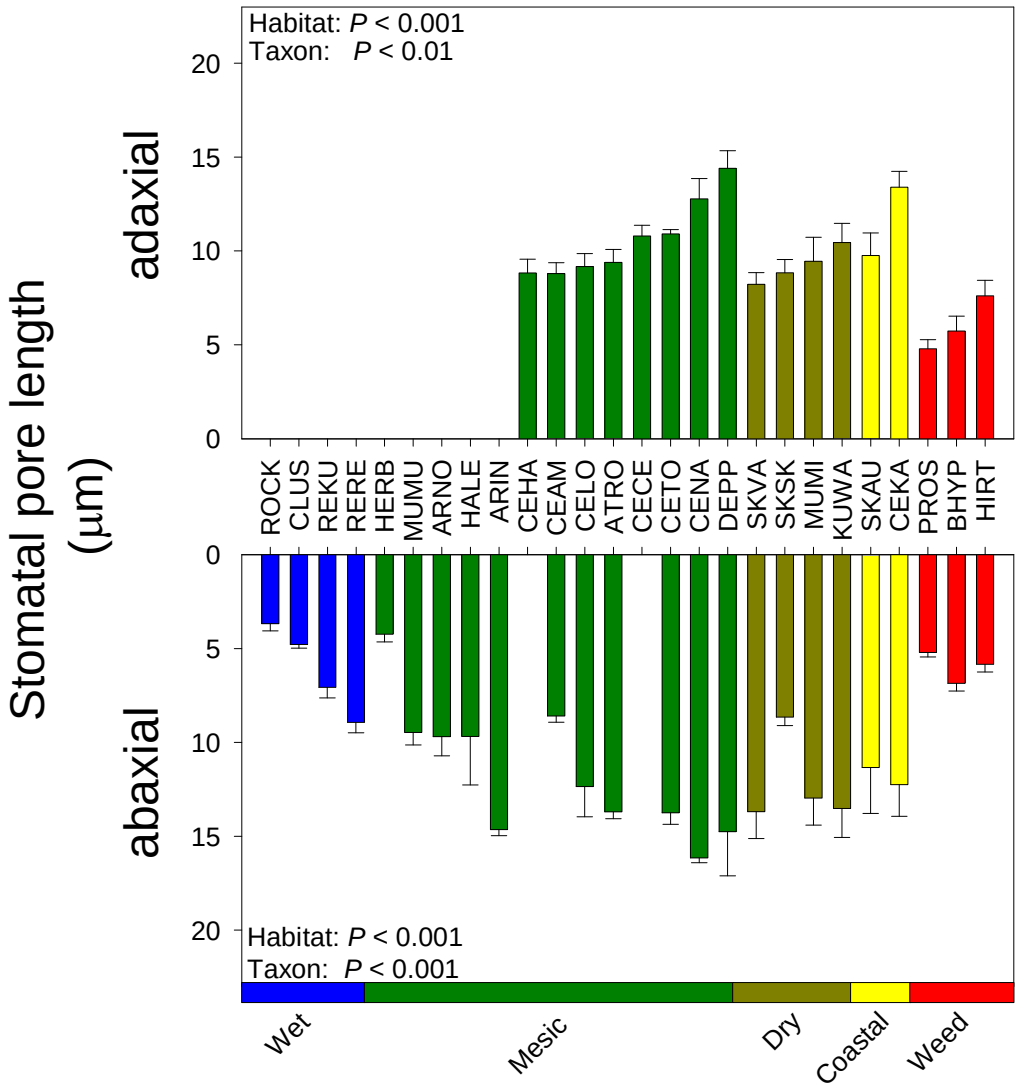

Figure 1.7. Mean values for stomatal pore length on adaxial and abaxial leaf surfaces for 22 native Hawaiian *Euphorbia* taxa and three *Euphorbia* species that are weeds in Hawaii. Taxa are represented by a four letter code (Table 1.1), and arranged by habitat (Wet, Mesic, Dry, Dry Coastal, with Weeds as a separate category). Bars indicate standard error (n=3). *E. celastroides* var. *stokesii; E. degeneri, E. olowaluana*, and *E. sparsiflora* were not included because the presence of dense papillae on the adaxial surface prevented the measurement of stomatal pore length.

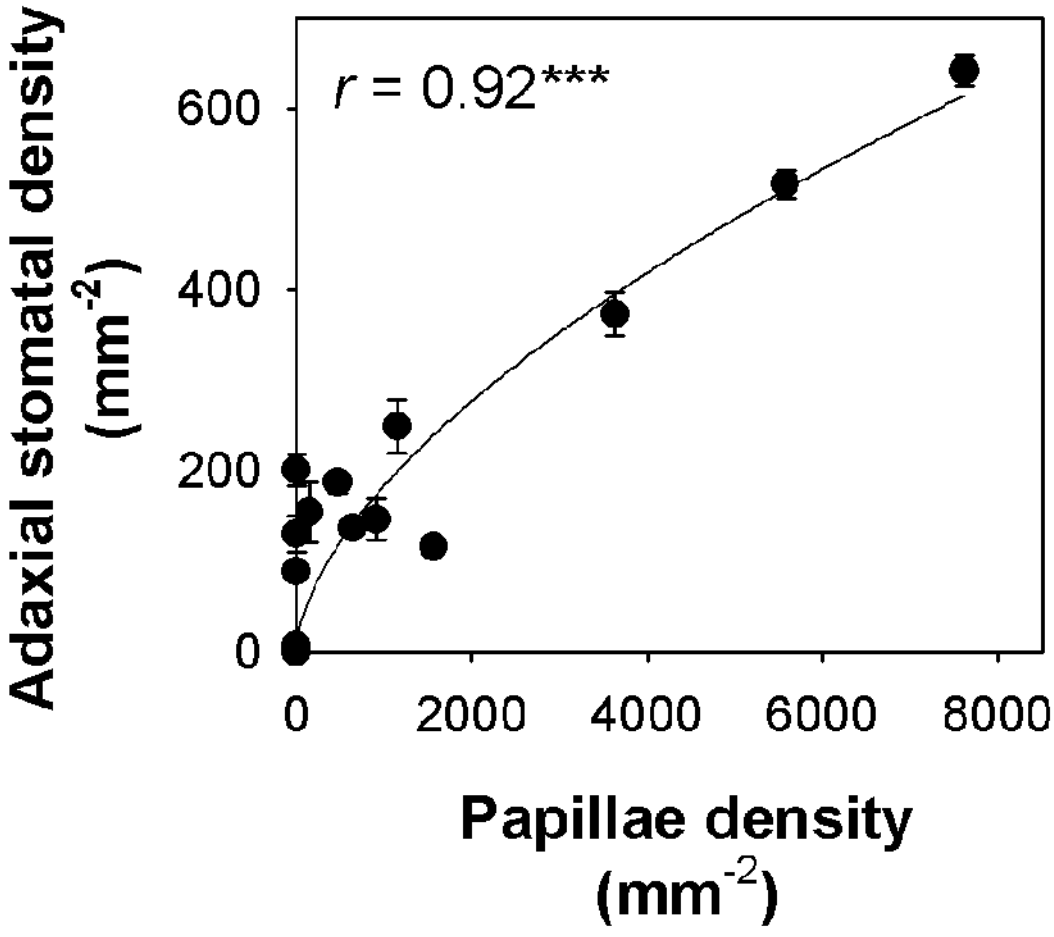

Figure 1.8. Correlation of papillar density with stomatal density on adaxial surface with climate for 13 native Hawaiian *Euphorbia* taxa.

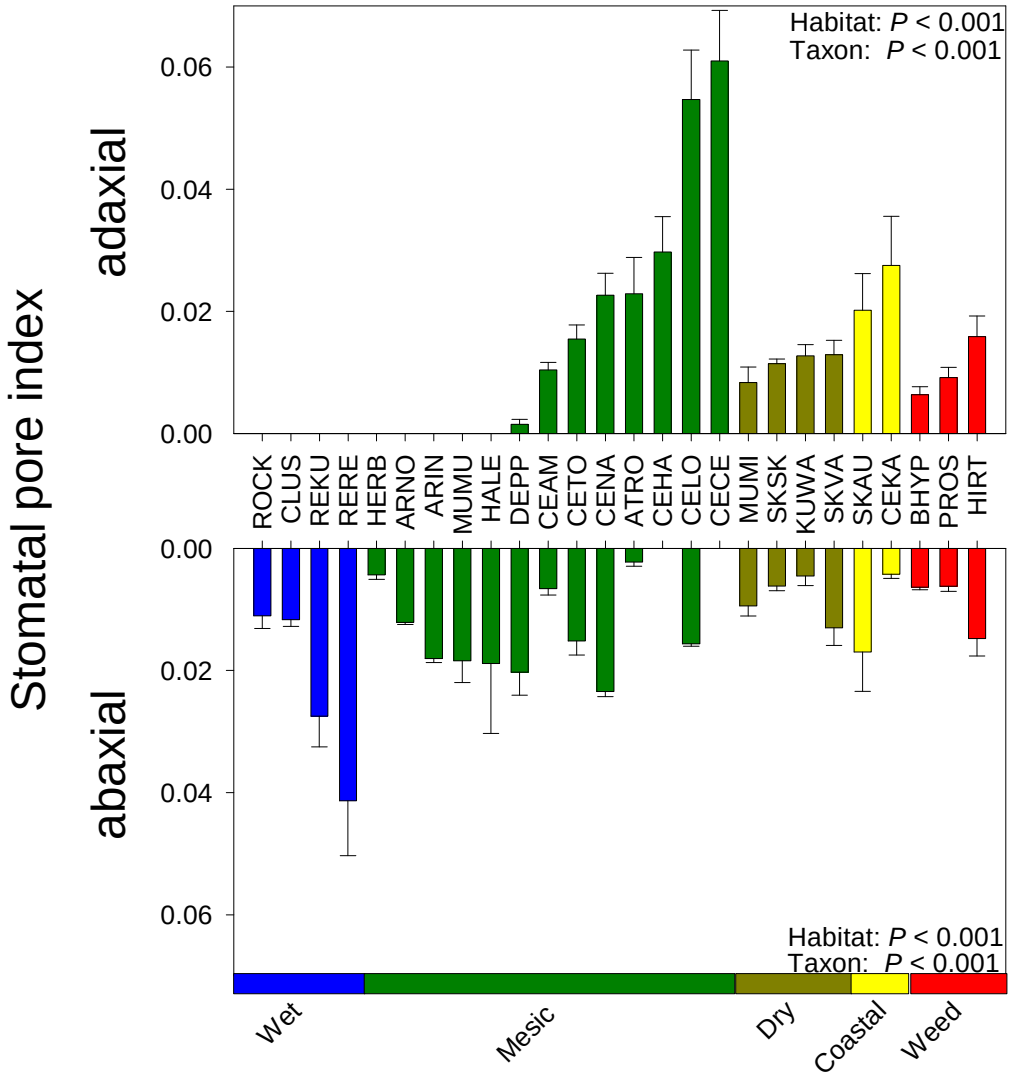

Figure 1.9. Mean values for stomatal pore index on adaxial and abaxial leaf surfaces for 22 native Hawaiian *Euphorbia* taxa and three *Euphorbia* species that are weeds in Hawaii. Taxa are represented by a four letter code (Table 1.1), and arranged by habitat (Wet, Mesic, Dry, Dry Coastal, with Weeds as a separate category). Bars indicate standard error (n=3). *E. celastroides* var. *stokesii; E. degeneri*, *E. olowaluana*, and *E. sparsiflora* were not included because the presence of dense papillae on the adaxial surface prevented the measurement of stomatal pore index.

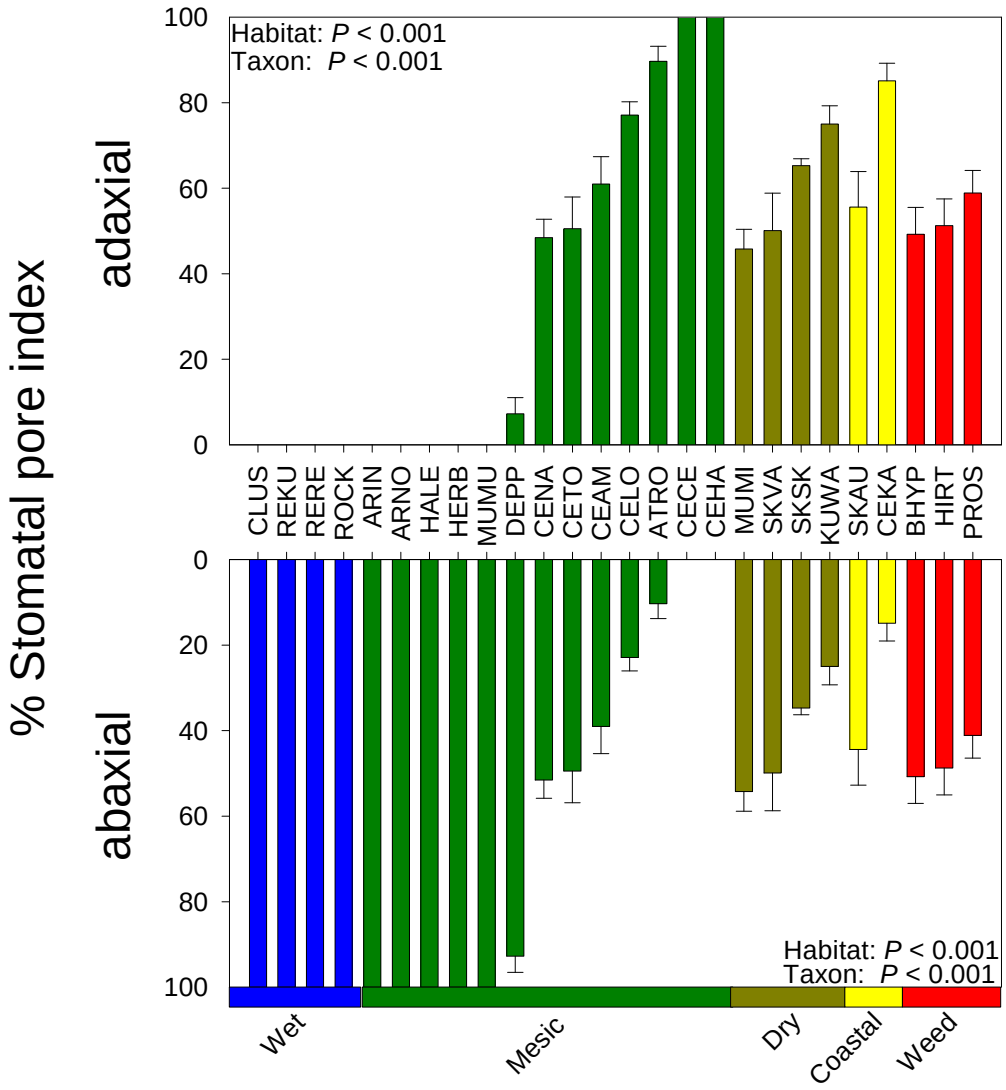

Figure 1.10. Mean values for percent stomatal pore index on adaxial and abaxial leaf surfaces for 22 native Hawaiian *Euphorbia* taxa and three *Euphorbia* species that are weeds in Hawaii. Taxa are represented by a four letter code (Table 1.1), and arranged by habitat (Wet, Mesic, Dry, Dry Coastal, with Weeds as a separate category). Bars indicate standard error (n=3). *E. celastroides* var. *stokesii; E. degeneri*, *E. olowaluana*, and *E. sparsiflora* were not included because the presence of dense papillae prevented measurement of % stomatal pore index.

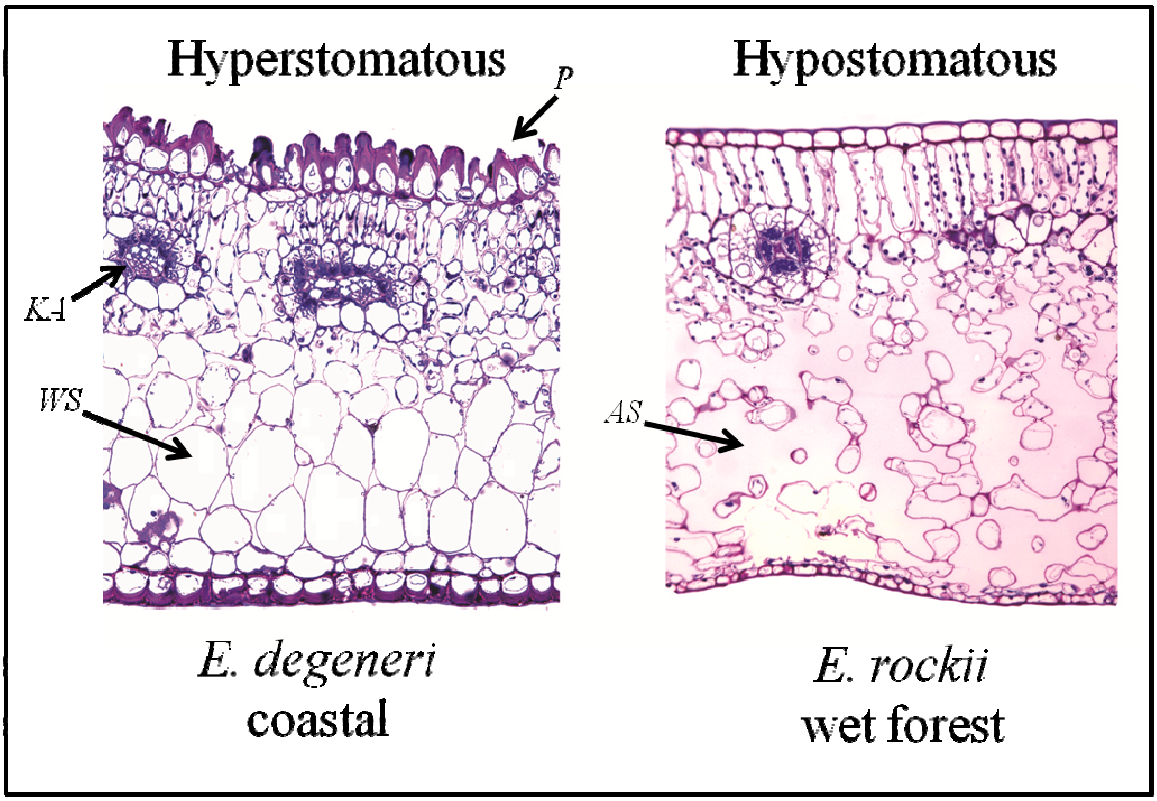

Figure 1.11. Cross sections for leaves of *E. degeneri* and *E. rockii* illustrating contrasting leaf anatomical characteristics. *E. degeneri* is a hyperstomatous species that occurs in coastal habitats on all of the main Hawaiian Islands. Presence of papillae; water storage tissue adjacent to the abaxial surface; Kranz anatomy; as well as relatively higher vein density are apparent in *E. degeneri* sections. *E. rockii* is a hypostomatous species that occurs only on Oahu. Large areas of air space adjacent to the abaxial surface; Kranz anatomy; and a relatively lower vein density are apparent in sections of *E. rockii*. Letter codes stand for: *WS*= water storage tissue; *AS*= air space; *P*=papillae; *KA*= Kranz anatomy (cross section images from L. Sack, J. Pasquet-Kok and M.J. Sporck, in prep).

APPENDICES

Appendix A1-1. Mean squares of analysis of variance for traits of *Euphorbia* taxa, testing for differences between vegetation types (e.g. coastal, dry forest, mesic forest, and wet forest), and among taxa nested with in vegetation type, with degrees of freedom in parentheses. When no differences were found between vegetation types (P>0.05), data and significance levels are reported for one-way ANOVAs testing for differences among taxa. Significance: *P<0.05; **P<0.01; ***P<0.001. We also ran the ANOVA test excluding the 3 non-native species. Any change in significance was appended after the asterisk symbols, separated by a comma. For example "***, **" indicates that P<0.001 when non-natives were included, and P<0.01 when non-natives were excluded. "NS" means that there was no significance. Data for all variables was log-transformed before testing to improve normality and heteroscedasticity. For adaxial stomatal density; abaxial stomatal density; adaxial % stomata; adaxial % stomata; adaxial *SPI*; abaxial *SPI*; adaxial *%SPI*; abaxial *%SPI*; and adaxial papillae density we added 1 before logging. For $\delta^{13}C$ we changed the values to negative numbers before logging. Abbreviations for traits: stomatal density adaxial (SD_{ad}), stomatal density abaxial (SD_{ab}) , stomatal density whole leaf (SD_{tot}), % stomatal density adaxial surface($\%SD_{ad}$), stomatal density abaxial surface ($\%SD_{ab}$), % amphistomatous (*%Amphi*), pore length adaxial surface (PL_{ad}), pore length abaxial surface (PL_{ab}), stomatal pore index adaxial surface (SPI_{ad}), stomatal pore index abaxial surface (SPI_{ab}), % stomatal pore index adaxial surface ($\%SPI_{ad}$), % stomatal pore index abaxial surface ($\%SPI_{ab}$), stomatal pore index whole leaf (SPI_{tot}), guard cell length adaxial surface (GCL_{ad}), guard cell length abaxial surface (GCL_{ab}), papillae diameter adaxial surface (Ad pap diameter), papillae density adaxial surface (Ad pap density), epidermal cell area adaxial surface (ECS_{ad}), epidermal cell area abaxial surface (ECS_{ab}), leaf mass per area (*LMA*), leaf density (*D*), chlorophyll per area (Chl_{area}), chlorophyll concentration per mass (Chl_{mass}), nitrogen concentration per area (N_{area}), nitrogen concentration per mass (N_{mass}), Chlorophyll: Nitrogen (*Chl:N*), carbon isotope discrimination ($\delta^{13}C$), phosphorous concentration per area (P_{area}), phosphorous concentration per mass (P_{mass}) , carbon concentration per mass (C_{mass}), nitrogen to phosphorous ratio (*N:P*), diameter 10 cm from base of plant (Diam.), height of plant in the field (*Ht*), % open sky (*%OS*), elevation at collection site (*E*), mean annual relative humidity (*MARH*), mean annual temperature at collection site (*MAT*), mean annual rainfall at collection site (*MAP*), leaf area (*LA*), and leaf thickness (*T*).

	Mean and range of trait values from taxa averages			Nested ANOVA with taxon nested within habitat	
Trait	**Units**	**Min/ mean/ max (natives)**	**Weed mean**	**Habitat**	**Taxon**
Leaf composition					
LMA	g· m^{-2}	15.5-**73.2**-127	44.4	0.236(5)***	0.203(23)***
D	g·cm^{-3}	0.0732-**0.231**-0.387	0.250	0.139(5)***	0.142(23)***
Chl_{area}	SPAD	23.2-**46.0**-61.2	49.4	0.0145(5)***	0.00226(23)***
Chl_{mass}	SPAD g·m^{-2}	0.313-**0.745**-1.64	1.49	0.314(5)***	0.172(23)***
N_{area}	g·m^{-2}	0.167-**1.22**-2.18	1.10	0.195(5)***	0.237(23)***
N_{mass}	%	0.738-**1.76**-2.98	2.38	0.0939(5)***	0.101(23)***
Chl: N		25.6-**45.1**-162.4	74.6	0.192(5)***	0.147(23)***
$\delta^{13}C$	‰	-14.6- **-13.3**- -12.0	-13.0	0.0000154(5)***·	0.0000530(23)***
P_{area}	g·m^{-2}	0.463-**0.117**-0.323	0.228	0.255(5)***	0.288(23)***
P_{mass}	%	0.0633-**0.177**-0.439	0.480	0.584(5)***	0.208(23)***
C_{mass}	%	39.7-**42.3**-44.8	42.3	$0.000(5)^{NS}$	0.001(23)***
N:P		5.55-**12.2**-22.9	6.11	0.330(5)***	0.120(23)***
Field					
Diameter	mm	1.7-**32.5**-133	2.6	3.40(5)***	1.06(22)***
Height	m	0.035-**1.37**-4.73	0.22	2.52(5)***	0.985(22)***
exposure	%	11.0-**67.0**-100	86.7	1.02(5)***	0.330(22)***
Elevation	masl	19.2-**582**-1695	71.2	5.27(5)***	0.930(23)***
Climate					
MARH	%	69.3-**77.7**-82.9	70.7	2.52(5)***	0.985(22)***
MAT	°C	13.3-**19.9**-23.8	23.2	1.02(5)***	0.330(22)***
MAP	mm	425-**2115**-9704	1746.3	5.27(5)***	0.930(23)***
VPD	kilopascals	0.324-**0.539**-0.899	0.835	0.443(5)***	0.069(23)***
Gross morphology					
LA	cm^2	0.449-**8.40**-36.9	0.877	4.79(5)***	0.737(23)***
T	µm	0.188-**0.331**-0.618	0.169	0.366(5)***	0.0594(23)***

Trait	Nested ANOVA with taxon nested within open vs. shade establishing		Nested ANOVA with taxon nested within stomatal distribution type	
	Open vs. shade establishing	Taxon	Stomatal distribution	Taxon
Field				
Diameter	0.291(1)**,*	1.54(26)***	0.0654(2)^{NS,}***	1.49(27)***
Height	4.08(1)***	1.14(26)***	0.481(2)***,**	1.20(27)***
exposure	7.67(1)***	0.179(26)***	2.02(2)***	0.238(27)***
Elevation	15.8(1)***	1.15(27)***	3.08(2)***	1.39(28)***
Climate				
MARH	4.08(1)***	1.14(26)***	0.481(2)***	1.20(27)***
MAT	7.67(1)***	0.0179(26)***	2.02(2)***	0.238(27)***
MAP	15.8(1)***	1.15(27)***	3.08(2)***	1.39(28)***
VPD	1.80(1)***	0.070(27)***	0.315(2)***	0.102(28)***
Gross morphology				
LA	20.9(1)***	0.727(27)***	7.46(2)***	0.702(28)***
T	0.254(1)***	0.110(27)***	0.275(2)***	0.0891(28)***

		Mean and range of trait values from taxa averages		Nested ANOVA with taxon nested within habitat	
Trait	**Units**	**Min/ mean/ max (natives)**	**Weed mean**	**Habitat**	**Taxon**
Stomatal Traits					
SD_{ad}	mm^{-2}	0-**134**-642	284	10.2(4)***	2.61(20)***
SD_{ab}	mm^{-2}	0-**155**-806	261	6.05(5)***	1.45(23)***
SD_{tot}	mm^{-2}	84.2-**314**-806	546	0.642(4)***	0.153(21)***
$\%SD_{ad}$	%	0-**49.4**-100	53.7	6.66(5)***	1.41(22)***
$\%SD_{ab}$	%	0-**50.6**-100	46.3	3.23(5)***	1.23(22)***
%Amphi	%	0-**16.6**-65.9	64.5	5.46(5)***	1.17(22)***
PL_{ad}	µm	8.22-**10.5**-14.4	6.05	0.160(3)***,**	0.0171(12)**
PL_{ab}	µm	3.68-**11.1**-19.0	5.97	0.344(4)***	0.0624(20)***
SPI_{ad}	×100	0-**0.0137**-0.0610	0.0105	0.000160(4)***	0.000144(20)***
SPI_{ab}	×100	0-**0.0117**-0.0413	0.00910	0.0000964(5)***	0.0000405(23)***
$\%SPI_{ad}$	%	0-**38.6**-100	53.1	6.44(4)***	1.49(20)***
$\%SPI_{ab}$	%	0-**61.4**-100	46.9	0.679(4)***	1.01(20)***
SPI_{tot}	×100	0.00433-**0.0271**-0.0703	0.0196	0.0825(4)*	0.233(20)***
GCL_{ad}	µm	15.49-**18.7**-23.4	12.0	0.102(3)***,NS	0.0183(12)***
GCL_{ab}	µm	9.38-**20.0**-35.6	11.0	0.295(4)***	0.0279(20)***
Papillae					
P_{dia}	µm	4.18-**6.74**-10.1	0	0.0999(3)***	0.0223(9)***
P_D	mm^{-2}	0-**1597**-7617	0	16.5(5)***	6.80(23)***
Epidermal cell					
ECS_{ad}	µm	252-**751**-2194	990	0.0569(4)***,**	0.164(18)***
ECS_{ab}	µm	389-**732**-1722	1181	0.209(4)***	0.0526(17)***

Trait	Nested ANOVA with taxon nested within open vs. shade establishing		Nested ANOVA with taxon nested within stomatal distribution type	
	Open vs. shade establishing	Taxon	Stomatal distribution	Taxon
Stomatal Traits				
SD_{ad}	46.1(1)***	2.04(23)***	40.7(2)***	0.195(23)*·***
SD_{ab}	7.55(1)***	2.07(27)***	26.1(2)***	0.320(27)***
SD_{tot}	0.00558(1)^{NS,}**	0.244(23)***	0.156(2)***	0.234(23)***
$\%SD_{ad}$	34.3(1)***	1.16(26)***	28.8(2)***	0.0859(26)*·***
$\%SD_{ab}$	6.70(1)***	1.40(26)***	19.2(2)***	0.137(26)***
%Amphi	18.2(1)***	1.32(26)***	23.3(2)***	0.171(26)***
PL_{ad}	0.00344(1)^{NS}	0.0474(14)***·**	0.00344(1)^{NS}	0.0474(14)***·**
PL_{ab}	0.282(1)***	0.101(23)***	0.235(2)***	0.0864(23)***
SPI_{ad}	0.000717(1)***	0.000123(23)***	0.00101(2)***	0.0000607(23)***
SPI_{ab}	0.000154(1)***	0.0000467(27)***	0.000287(2)***	0.0000306(27)***
$\%SPI_{ad}$	27.3(1)***	1.23(23)***	24.9(2)***	0.0858(23)^{NS,}***
$\%SPI_{ab}$	1.43(1)***	0.930(23)***	9.77(2)***	0.119(23)**·***
SPI_{tot}	0.684(1)***	0.187(23)***	0.637(2)***	0.154(23)***
GCL_{ad}	0.000641(1)^{NS,}*	0.0373(14)***	0.000361(1)^{NS,}*	0.0374(14)***
GCL_{ab}	0.0949(1)***	0.072(23)***	0.132(2)***	0.0605(23)***
Papillae				
P_{dia}	0.0338(1)**	0.0424(11)***	0.00612(1)^{NS}	0.0449(11)***
P_D	49.4(1)***	7.02(27)***	63.7(2)***	3.88(27)***
Epidermal cell				
ECS_{ad}	0.00860(1)^{NS}	0.151(21)***	0.480(2)***	0.112(21)***
ECS_{ab}	0.232(1)***·**	0.0731(20)***	0.0118(2)^{NS}	0.0833(20)***
Leaf composition				
LMA	0.136(1)***	0.210(27)***	0.398(2)***	0.174(28)***
D	0.761(1)***	0.123(27)***	0.780(2)***	0.0846(28)***
Chl_{area}	0.0556(1)***·^{NS}	0.00246(27)***	0.00899(2)**·*	0.00349(28)***
Chl_{mass}	0.110(1)**·***	0.199(27)***	0.228(2)***	0.178(28)***
N_{area}	0.443(1)***	0.220(27)***	0.481(2)***	0.183(28)***
N_{mass}	0.0880(1)***·**	0.100(27)***	0.0454(2)***·**	0.0961(28)***
Chl: N	0.395(1)***·^{NS}	0.146(27)***	0.290(2)***·*	0.128(28)***
$\delta^{13}C$	0.000159(1)***	0.0000420(27)***	0.0000616(2)***	0.0000381(28)***
P_{area}	1.87(1)***	0.222(27)***	1.09(2)***	0.199(28)***
P_{mass}	0.998(1)***	0.247(27)***	0.451(2)***	0.233(28)***
C_{mass}	0.000(1)^{NA,}*	0.001(27)***	0.001(2)***	0.001(28)***
N:P	0.493(1)***·**	0.141(27)***	0.214(2)***·*	0.134(28)***

Appendix A1-2. Correlation matrix of mean traits for *Euphorbia* taxa, with traits organized by category: stomatal traits, epidermal traits, and composition traits. These data are intended to show the inter-correlative structure of the traits, rather than to derive conclusions about non-hypothesized relationships (see Methods, "*Statistics*"). Values presented are Pearson correlation coefficients (r_p) for all taxa, below the diagonal and for only native taxa (excluding non-native weed species) above the diagonal. Values in bold face are significant at $P < 0.05$ for both r_p and for Spearman correlations (r_s); values in italics are r_p for log-transformed data, when for significant correlations these were higher than for untransformed data. Abbreviations t: Leaf area (*LA*), thickness (*T*), leaf mass per area (LMA), leaf density (*D*), carbon per mass (C_{mass}), chlorophyll per area (Chl_{area}), chlorophyll per mass (Chl_{mass})nitrogen per mass (N_{mass}), nitrogen per area (N_{area}), phosphorus per mass (P_{mass}), phosphorus per area (P_{area}), abaxial guard cell length (GL_{ab}), adaxial guard cell length (GL_{ad}), abaxial stomatal pore length (PL_{ab}), adaxial stomatal pore length (PL_{ad}), total stomatal density (SD_t), percentage of stomatal density on adaxial surface ($\%SD_{ad}$), percentage of stomatal density on abaxial surface ($\%SD_{ab}$), adaxial stomatal density (SD_{ad}), abaxial stomatal density (SD_{ab}), total stomatal pore index (SPI_t), adaxial stomatal pore index (SPI_{ad}), abaxial stomatal pore index (SPI_{ab}), percentage adaxial stomatal pore index ($\%SPI_{ad}$), percentage abaxial stomatal pore index ($\%SPI_{ab}$), papillae diameter (P_{dia}), adaxial epidermal cell size (ECS_{ad}), abaxial epidermal cell size (ECS_{ab}), and *Chl:N*.

	Stomatal Traits												
	$\%SD_{ad}$	*%Amphi*	SD_{ad}	SD_{ab}	SD_t	$\%SPI_{ad}$	SPI_{ad}	SPI_{ab}	SPI_t	GL_{ad}	GL_{ab}	PL_{ad}	PL_{ab}
$\%SD_{ad}$		0.26	***0.88***	***-0.71***	0.004	1.0	**0.82**	**-0.65**	0.54	-0.44	***0.58***	-0.54	***0.66***
%Amphi	0.23		***0.70***	-0.27	-0.27	0.35	0.17	0.10	0.18	0.17	0.19	-0.08	***0.49***
SD_{ad}	***0.99***	***0.74***		***-0.70***	0.38	***0.99***	**0.96**	***-0.47***	**0.79**	**-0.57**	0.26	-0.40	***0.61***
SD_{ab}	**-0.68**	-0.13	***-0.56***		0.64	***-0.70***	***-0.70***	***0.72***	-0.25	0.12	***-0.79***	0.11	**-0.77**
SD_t	0.03	-0.21	0.45	0.65		0.03	0.32	0.13	0.42	***-0.58***	***-0.66***	-0.38	***-0.56***
$\%SPI_{ad}$	***1.0***	***0.75***	***0.99***	**-0.60**	0.08		**0.83**	**-0.60**	***0.57***	-0.45	0.43	-0.40	***0.63***
SPI_{ad}	**0.79**	0.10	**0.88**	***-0.68***	0.28	**0.81**		-0.41	**0.83**	-0.35	0.32	-0.14	**0.45**
SPI_{ab}	**-0.65**	0.04	-0.42	***0.68***	0.08	***-0.61***	-0.38		0.16	0.36	-0.21	0.37	-0.12
SPI_t	***0.49***	0.06	**0.68**	-0.22	0.35	**0.49**	**0.83**	0.20		-0.19	0.27	0.03	***0.64***
GL_{ad}	-0.06	-0.25	***-0.78***	-0.39	**-0.63**	-0.11	0.23	0.32	0.42		0.51	**0.69**	0.44
GL_{ab}	0.43	-0.10	-0.15	***-0.78***	**-0.81**	0.19	0.29	-0.07	0.34	***0.78***		0.28	***0.94***
PL_{ad}	-0.16	-0.39	***-0.51***	-0.44	***-0.59***	-0.13	0.15	0.33	0.29	***0.86***	0.75		0.49
PL_{ab}	**0.52**	0.032	0.12	***-0.76***	***-0.63***	0.37	0.40	-0.03	***0.60***	***0.76***	***0.94***	***0.83***	
ECS_{ad}	**-0.45**	0.06	-0.29	***0.55***	-0.17	***0.63***	***-0.57***	0.27	-0.33	-0.20	-0.08	0.11	-0.09
ECS_{ab}	-0.02	0.37	0.10	0.14	0.19	0.01	-0.11	-0.08	-0.16	-0.58	-0.37	-0.32	-0.34
P_D	***0.68***	-0.32	**0.82**	***-0.77***	0.36	***0.65***	**0.88**	**-0.45**	**0.76**	0.09	0.50	0.06	***0.53***
P_{dia}	0.09	-0.05	***-0.93***	0.13	***-0.89***	-0.58	***-0.94***	0.02	-0.61	0.49	0.15	0.10	0.07
LA	-0.52	***-0.73***	***-0.61***	0.54	0.20	***-0.62***	-0.31	0.29	-0.17	0.27	-0.32	0.23	-0.48
T	-0.20	***-0.56***	***-0.47***	0.49	0.32	-0.35	-0.18	0.05	-0.08	***0.80***	-0.20	***0.84***	-0.26
LMA	0.45	-0.09	0.16	-0.13	0.11	0.36	0.32	-0.12	0.37	0.37	0.30	0.36	0.36
D	**0.60**	**0.46**	***0.65***	**-0.42**	-0.06	***0.67***	***0.45***	-0.18	0.38	0.01	0.34	-0.04	0.43
Chl_{area}	0.25	0.09	0.14	-0.04	0.11	0.26	0.20	-0.16	0.14	-0.16	-0.16	0.00	-0.24
Chl_{mass}	-0.20	0.20	0.07	0.02	-0.00	-0.11	-0.19	0.00	-0.27	-0.60	-0.25	-0.47	-0.28
N_{area}	**0.50**	0.10	0.30	-0.12	0.21	**0.47**	**0.41**	-0.23	0.35	0.16	0.04	0.06	0.02
N_{mass}	0.17	0.38	0.22	-0.04	0.08	0.25	0.15	-0.14	0.03	-0.25	-0.19	-0.31	-0.28
P_{area}	***0.49***	**0.58**	0.35	-0.11	0.15	***0.52***	***0.42***	-0.02	0.27	-0.27	-0.06	-0.33	0.01
P_{mass}	0.07	**0.62**	0.26	-0.07	0.06	0.15	0.08	0.01	0.02	-0.55	-0.17	-0.55	-0.12
Chl:N	***-0.39***	-0.02	-0.09	0.01	-0.12	-0.26	-0.28	0.09	-0.29	-0.51	-0.10	-0.37	-0.07
N:P	-0.05	**-0.55**	-0.34	0.06	-0.14	-0.14	-0.21	-0.17	-0.26	0.40	0.19	0.33	-0.07
$\delta^{13}C$	0.17	***0.48***	0.04	-0.07	-0.09	0.17	0.01	0.11	0.06	0.24	0.13	0.28	0.30

	Epidermis Traits				Leaf Composition Traits											
	ECS_{ad}	ECS_{ab}	P_D	P_{dia}	*LA*	*T*	LMA	*D*	Chl_{area}	Chl_{mass}	N_{area}	N_{mass}	P_{area}	P_{mass}	*Chl:N*	*N:P*	$\delta^{13}C$
$\%SD_{ad}$	**-0.55**	-0.06	***0.79***	0.01	***-0.57***	-0.20	**0.52**	***0.70***	0.25	**-0.40**	**0.58**	0.07	***0.55***	0.13	***-0.55***	-0.03	0.16
%Amphi	-0.02	0.27	-0.26	-0.05	***-0.67***	***-0.44***	0.10	**0.50**	0.02	-0.08	0.17	0.24	**0.54**	0.49	-0.22	***-0.42***	***0.49***
SD_{ad}	***-0.53***	-0.12	**0.93**	***-0.93***	***-0.55***	-0.27	0.32	***0.70***	0.13	-0.28	***0.49***	0.18	***0.62***	0.25	***-0.53***	-0.26	0.30
SD_{ab}	***0.55***	-0.10	***-0.75***	-0.13	0.61	0.64	-0.11	**-0.49**	-0.06	-0.05	-0.14	-0.10	-0.21	-0.19	-0.02	0.14	-0.002
SD_t	-0.36	-0.20	0.47	***-0.89***	***0.44***	***0.52***	0.28	-0.08	0.09	-0.40	0.34	0.01	0.19	-0.8	***-0.55***	-.003	-0.22
$\%SPI_{ad}$	**-0.54**	-0.06	***0.77***	-0.62	-0.49	-0.33	0.45	***0.71***	0.25	-0.33	**0.56**	0.24	***0.58***	0.14	***-0.52***	-0.10	0.34
SPI_{ad}	***-0.61***	-0.08	**0.89**	***-0.94***	-0.34	-0.25	0.32	***0.48***	0.22	-0.24	**0.45**	0.19	***0.56***	0.23	-0.33	-0.28	0.03
SPI_{ab}	0.37	-0.02	***-0.60***	-0.14	0.28	0.00	-0.18	-0.21	-0.16	0.15	-0.30	-0.13	0.03	0.14	0.20	-0.24	0.13
SPI_t	-0.33	-0.10	**0.76**	-0.80	-0.23	-0.19	0.33	0.40	0.18	-0.23	0.36	0.09	***0.60***	0.27	-0.27	-0.40	0.23
GL_{ad}	0.33	0.07	-0.49	0.41	-0.29	0.24	-0.32	***-0.59***	0.03	0.36	-0.43	0.13	-0.22	-0.02	0.44	-0.15	0.53
GL_{ab}	-0.03	-0.06	0.34	0.32	***-0.53***	***-0.57***	0.19	0.45	-0.07	0.003	0.02	-0.05	0.15	0.14	0.07	-0.08	0.25
PL_{ad}	0.53	0.09	-0.23	0.10	-0.06	0.55	-0.09	-0.38	0.15	0.19	-0.43	-0.20	-0.27	-0.25	***0.63***	-0.06	0.41
PL_{ab}	-0.004	-0.07	0.35	0.16	**-0.70**	-0.57	0.27	***0.53***	-0.18	-0.08	-0.02	-0.18	0.25	0.20	0.10	-0.31	0.38
ECS_{ad}		***0.57***	***-0.74***	0.64	-0.04	-0.10	***-0.53***	-0.38	-0.23	***0.52***	**-0.51**	-0.01	***-0.46***	-0.00	***0.52***	-0.01	0.13
ECS_{ab}	***0.64***		-0.27	0.48	-0.06	-0.15	-0.35	-0.19	-0.08	0.45	-0.21	0.35	***-0.15***	0.31	0.11	0.03	0.11
P_D	0.62	-0.29		-0.01	-0.24	0.10	***0.53***	***0.52***	0.11	***-0.54***	**0.44**	-0.09	0.33	-0.05	***-0.51***	-0.11	-0.06
P_{dia}	***-0.70***	0.48	0.52		-0.54	0.34	0.13	-0.12	-0.04	-0.14	-0.20	-0.25	-0.44	-0.45	0.19	0.25	-0.05
LA	-0.08	***-0.49***	-0.36	-0.42		***0.63***	-0.10	**-0.48**	0.17	**0.73**	0.06	0.10	-0.23	-0.25	-0.09	0.36	***-0.41***
T	-0.20	-0.37	0.18	0.19	***0.69***		***0.46***	-0.33	0.08	***-0.45***	0.25	-0.17	-0.12	-0.42	-0.32	0.24	-0.11
LMA	***-0.61***	-0.48	***0.54***	-0.00	-0.02	***0.58***		**0.74**	0.17	**-0.90**	***0.77***	-0.38	0.27	-0.43	***-0.80***	0.06	0.004
D	***-0.47***	***-0.53***	***0.46***	-0.15	**-0.46**	-0.28	***0.69***		0.14	**-0.64**	0.41	-0.18	***0.41***	-0.12	***-0.61***	-0.09	0.10
Chl_{area}	-0.22	-0.03	0.08	-0.03	0.14	0.02	0.14	0.17		-0.05	***0.75***	***0.53***	0.07	-0.19	-0.48	**0.47**	-0.006
Chl_{mass}	***0.59***	0.68	***-0.55***	-0.08	-0.10	***-0.60***	***-0.93***	***-0.59***	-0.02		-0.50	***0.48***	-0.27	0.47	**0.73**	-0.03	0.02
N_{area}	**-0.60**	-0.36	**0.41**	-0.32	0.07	0.27	***0.79***	***0.62***	***-0.94***	-0.57		***0.49***	**0.54**	0.01	***-0.93***	0.20	0.09
N_{mass}	-0.03	0.28	-0.15	-0.30	0.07	-0.27	-.39	-0.08	0.52	***0.46***	***0.44***		0.30	0.48	-0.33	0.15	0.15
P_{area}	***-0.47***	-0.19	0.13	-0.34	-0.25	-0.30	0.13	***0.48***	***-0.57***	-0.18	***0.54***	0.45		**0.67**	***-0.57***	***-0.65***	0.37
P_{mass}	-0.02	0.22	-0.17	-0.31	-0.30	***-0.57***	***-0.49***	0.05	0.00	***0.52***	0.05	***0.57***	***0.80***		0.24	***-0.72***	0.36
Chl:N	***0.61***	0.43	***-0.47***	0.39	-0.14	-0.39	***-0.84***	***-0.61***	-0.39	**0.83**	-0.79	-0.24	-0.35	0.16		-0.22	-0.10
N:P	-0.08	-0.16	0.00	0.15	***0.47***	***0.50***	0.19	-0.11	0.35	-0.24	0.20	-0.03	***-0.62***	***-0.81***	-0.28		-0.33
$\delta^{13}C$	0.29	0.22	-0.210	-0.31	***-0.43***	-0.18	0.45	0.05	-0.02	0.17	0.03	0.23	0.23	0.28	0.06	-0.36	

Appendix A1-3. Plant height and diameter of main stem. average values for plant height and diameter of main stem/trunk, 10cm from the bass of the plant (*indicates plants were shorter than 10cm and measurement was taken 2-4 cm from base). Italic text indicates weed species.

Taxon	average height (m)	stem diameter (mm)
ARNO	0.7774	7.32
ARIN	0.4	3.55
ATRO	2.962	38.92
CEAM	1.147	33.21
CECE	1.496	58.36
CEHA	1.288	25.52
CEKA	1.51	110.82
CELA	0.08	17.7
CELO	3.328	70
CEST	1.059	7.9
CETO	0.7268	18
CLUS	1.536	17
DEGE*	0.1204	1.7
DEPP*	0.1428	7.2
ELEA	no data	no data
HALE	2.332	20.24
HERB	0.7778	9.66
KUWA	0.4	48
MUMI	1.508	27.26
MUMU	0.909	6.12
OLOW	4.73	133.04
REHA	no data	no data
REKU	2.752	71.28
RERE	no data	no data
ROCK	1.87	53.36
SKAU*	0.035	4.2
SKSK	0.781	12.46
SKVA	0.9588	13.84
SPAR	0.655	14.12
HIRT	*0.297*	*3.08*
HYPE	*0.2742*	*3.84*
PROS	*0.096*	*0.88*

CHAPTER 2

THE ADAPTIVE RADIATION OF THE LEAF VENATION IN HAWAIIAN C_4 *EUPHORBIA*: "SHEDDING" VEINS IN SHADE REDUCES COST

ABSTRACT

The diversity of leaf venation architecture within and across lineages is gaining increasing interest as a source of functional adaptation to contrasting environments. Because of Hawaii's geographic isolation and diversity of biomes represented across the islands, it provides a unique opportunity to investigate biological relationships in a way that is not possible elsewhere. The C_4 Hawaiian *Euphorbia* (Euphorbiaceae) radiated from one colonizing species into nearly 30 taxa. This group includes a variety of life forms, from creeping woody sub-shrubs to trees over 6 m tall, with taxa adapted to diverse habitats, from rain forest to dry forest to coastal strand. The leaves of the taxa in this group differ strongly, with 80-fold variation in leaf size and 8-fold variation in leaf mass per area. One study (Herbst, 1971), pointed out a qualitative trait unique to this group, "disjunct minor veins," unattached to the rest of the vein network and surrounded by mesophyll cells. No study has quantified the venation architecture or its relationship to environment for this scientifically important radiation. For 27 of 29 (there are two additional known Hawaiian taxa, *E eleanoriae* and *E. remyi* var. *hanaleiensis*, that were not included in the study due to lack of information and rarity) native *Euphorbia* taxa, I chemically cleared leaves and quantified 40 traits relating to venation architecture, including densities of all vein orders (i.e., length/area) and of vein islands. I tested for correlation of venation traits with climate and habitat, and with other aspects of leaf structure and composition. I hypothesized that leaves of taxa distributed at higher temperatures and establishing at higher irradiance would have greater vein density, and that venation architecture would depend on leaf size. I hypothesized that vein island

formation would be associated with moist rainforest habitats, as in shaded habitat these C_4 species might not suffer from the loss of vein length and even might benefit from reduced construction cost. I found strong support for these hypotheses. Hawaii's isolated location and strong climatic gradients have led to strong diversification and apparent adaptation of venation characteristics, providing a model for understanding these traits in other lineages.

INTRODUCTION

Leaf venation architecture is extremely diverse across plant species (Roth-Nebelsick et al., 2001) and recent work has focused intensively on its functional implications. For instance, veins act in biomechanical support as well as plumbing for the leaf, supplying water and moving photosynthates (Niklas, 1999; Tyree and Zimmermann, 2002; Ellis et al., 2009). The leaf vasculature plays a key role in the overall ability of the plant to perform photosynthesis and veins themselves make up a significant fraction of the leaf mass per area (LMA), an important determinant of plant relative growth rate (Sack and Holbrook, 2006; Brodribb et al., 2007; Niinemets et al., 2007; Niinemets et al., 2007). Recent work has focused on the functional significance of venation architecture for the leaves of different species within a community (Sack and Frole, 2006) and across very diverse lineages (Nardini et al., 2005; Brodribb et al., 2007), but only a few studies have focused on closely-related species that diversified within adaptive radiations (Edwards, 2006; Dunbar-Co et al., 2009).

The Hawaiian C_4 *Euphorbia* (Euphorbiaceae) is one of the most noteworthy examples of adaptive radiation among the angiosperms known to science. The *Euphorbia* radiation in Hawaii includes 29 currently recognized taxa (Table 1.1), most likely descended from a single species colonizer probably within the last five million years (Price and Clague, 2002). *Euphorbia* taxa are found across the Hawaiian Islands with many single-island endemics. These taxa occupy an extreme range of habitats and range widely in vegetative form and height (Table 2.1). There have been several influential studies of physiology and morphology in the Hawaiian *Euphorbia* (Herbst, 1971, 1972;

Pearcy and Troughton, 1975; Robichaux and Pearcy, 1980, 1980; Pearcy et al., 1982; Robichaux and Pearcy, 1984; Pearcy et al., 1985), but none has quantified the variation in venation architecture. Given the lack of research using a comparative plant approach to anatomy, coupled with the unique radiation of Euphorbias, my overall goal was to determine how venation traits might adapt to contrasting environments. The Hawaiian *Euphorbia* could be a leading model for leaf evolution given their exceptional range of habitats and plant and leaf morphologies.

I determined how leaf vein densities (length/area) differed among habitat types and among taxa. With my research goal of aiming to understand relationships between venation characteristics and environmental factors in mind, I hypothesized that 1) greater minor vein densities and total vein densities would be associated with high irradiance and warmer habitats, which should require greater hydraulic supply and faster photosynthetic rates. I also quantified shifts in venation architecture with leaf size and hypothesized that 2) major vein densities would decline with leaf size, because these veins would be spaced further apart in larger leaves given a constrained developmental template during leaf expansion (Sack et al., in prep). I also investigated the correlation of venation traits with other leaf traits. Given the importance of venation to photosynthetic capacity, due to the importance of hydraulic supply, and, more directly, because sugar production occurs in the bundle sheath around veins in these C_4 plants, I hypothesized that 3) total vein density would correlate positively with stomatal pore area per leaf area and with photosynthetic rate.

Additionally, I focused on an exceptional characteristic of the Hawaiian *Euphorbia*. The presence of vein islands in several species was discovered by Herbst in

1971.. While vein islands or “islands of vascular tissue” have been observed in *Arabidopsis* mutants with dysfunctional hormone pathways (Pullen et al., , they occur very rarely in wild type individuals of any species (Herbst 1971, 1972). The vein islands of the Hawaiian *Euphorbia* were found to be tracheids, and thus distinctly different in form from the well-known idioblasts, or tracheid-like cells dispersed in the leaf mesophyll in certain tissues of plants (Foster, 1956), and which may function in water storage and/or transport (Mauseth, 1988; Brodribb et al., 2010). Herbst described the leaf and vein development in *E. herbstii* as similar to the typical eudicot leaf. Vein development in that species begins with the midrib during the primordium stage, then, as the lamina expands, the secondary veins form, followed by the rapid or simultaneous development of the minor vein network and vein islands. Herbst described these vein fragments as idioblastic veinlets representing a single or small cluster of tracheary elements surrounded by enlarged Kranz bundle sheath cells. The vein fragments become isolated early in the histogenesis of the minor venation and they arise from procambial cells that have become isolated from the reticulum of procambial tissue; vein islands thus do not arise independently of the procambial reticulum, but result from intervening procambial cells failing to develop into vascular tissue. Herbst examined the vein islands in 14 Hawaiian *Euphorbia* taxa, and rated their commonness in subjective categories.

I quantified the numbers and lengths of vein islands in leaves of 27 taxa and determined their correlation with environment, leaf size and other leaf traits. I hypothesized that 4) vein islands would be associated with shaded and wet environments. I predicted that a decrease in vein length would not be as deleterious for shade species due to their reduced hydraulic demand, and possibly, a reduced need for bundle sheath

cells running photosynthetic carbon reduction reactions (Ogle, 2003). Additionally, the deletion of the intervening veins between the vein islands might reduce the cost of xylem and bundle sheath construction, and reduce the shading or displacement of leaf mesophyll. The evolution of vein islands might thus represent the fixation of a mutation selected for a benefit. This mutation might have been associated with the very large leaves of a few taxa that evolved in deep shade. The vein islands might have their origin in leaf expansion rates increased beyond the capacity of the leaf to elongate existing vein orders or initiate new vein orders. I thus tested for 5) the association of disjunct venation with large leaf size. I attempted to clarify the environmental association of venation architecture across the striking environmental range of this intrageneric radiation.

METHODS

Taxa, sites and collection of material

Leaf samples were collected from 30 populations (27 of 29 endemic taxa, plus three cosmopolitan weedy taxa) on the five high islands, of Hawaii, Kauai, Maui, Molokai, and Oahu (Figure 2.1). Habitat types were categorized as coastal, dry, mesic, wet, bog, or weed (Wagner et al., 1999; Table 2.1). For taxa with multiple populations, I sampled from one population of characteristic habitat and sampled from healthy and reproductively mature plants were selected of representative size based on average size for the given natural population. Several taxa exist only in remote locations with difficult access, including rare and endangered species (Table 2.1). I sampled plants of *E. celastroides* var. *celastroides* and var. *lorifolia* in cultivation at the National Tropical Botanical Garden (NTBG) Kauai, and for *E. herbstii* we sampled out-plantings on Oahu.

I recorded plant height, the diameter of the main stem 10 cm from the base, leaf number, soil type, and the surrounding vegetation. The percent open sky (equivalent to 100% minus the "canopy closure" *sensu* (Jennings et al., 1999), was visually assessed to the nearest 5%. Visual canopy cover estimates correlate with measurements using a densiometer or hemispherical photography Korhonen et al., 2006; Paletto and Tosi, 2009. I recorded the elevation and coordinates of each sampling location using a Global Positioning System (Garmin 60CSx Garmin, Kansas City). For each sampled population, I determined modeled values for mean annual relative humidity, temperature and rainfall (MARH, MAT, and MAP, respectively) using the Hawaii Digital Climate Map System in ArcGIS (Giambelluca and Cuo, in prep). I calculated vapor pressure (VPD) by the formula: $VPD = VP_{sat}-(MARH \times VP_{sat})$, where saturation vapor pressure in kPa (*Prometheus Wiki* and contributors, 2010), or(VP_{sat}) was determined for the given MAT (Campbell and Norman, 1998). The vapor pressure deficit (VPD) is a measure of atmospheric drought, the driving force for evaporation, and can be quantified as an absolute pressure difference (in kPa), or as a mole fraction normalized by atmospheric pressure. Across the study populations, the two VPD measures were highly correlated (R^2=0.998; $P < 0.001$); I present results for both but discuss in the text correlations with absolute VPD.

I collected leaves from five individuals of each taxon (except three for *E. arnottiana* var. *integrifolia*, and *E. remyi* var. *remyi*). Fully exposed leaves were selected from the most recent mature cohort, several nodes distal from the apex. Leaves were transported in plastic bags to the laboratory for processing.

Leaf traits: dimensions and composition

I measured mean leaf area (*LA*) for three leaves from three plants per species. Measurements were made using Image J (Image J software, http://rsbweb.nih.gov/ij/). Leaves were oven dried for over 48 hrs at > 70ºC before measuring dry mass for calculation of leaf mass per area (LMA; leaf area / dry mass).

I measured chlorophyll concentration per area on fresh leaves (Chl_{area}) using a SPAD-502 meter (Minolta Co., Japan), averaging two measurements for each of three to five leaves per individual. For three to 15 leaves per taxon, dried leaves were ground into a fine powder in a Wiley mill with mesh size 20. Leaves were analyzed for concentrations of nitrogen and phosphorus per mass (N_{mass} and P_{mass}, respectively), and for carbon isotope ratio ($\delta^{13}C$) using high temperature combustion in an elemental analyzer (Costech ECS 4010; Valencia, CA, USA), with effluent passed into a continuous flow isotope ratio mass spectrometer (ThermoFinnigan Delta V Advantage with a Conflo III interface; ThermoFisher Scientific; Waltham, MA, USA; Fry et al., 1996). Samples were dry ashed in glass vials (Miller, 1998), dissolved in 1N HCL and analyzed for P_{mass} using inductively-coupled plasma-optical emission spectrometry (Varian Vista MPX Instrument, Varian InE., Palo Alto, CA USA; Porder et al., 2005). Chlorophyll per mass (Chl_{mass}) was calculated as Chl_{area} divided by LMA; concentrations of nitrogen and phosphorus per area (N_{area} and P_{area} respectively) were determined respectively N_{mass} and P_{mass} multiplied by *LMA*. Chlorophyll: nitrogen ratio (*Chl:N*) was calculated as Chl_{area}/N_{area}.

Five leaves per taxon were stored in FAA (37% formaldehyde, glacial acidic acid, 95% ethanol, and deionized water in a 10:5:50:35 mixture), from which I measured leaf

thickness (*T*) midway along the leaf between midrib and margin (using digital calipers; model 14-648-17, Fisher Scientific).

Measurement of venation architecture

In *Euphorbia*, as for the typical dicotyledonous leaf, veins are arranged in a hierarchical system of vein orders defined by size and branching, with the number of vein orders varying across species. I chemically cleared three leaves per taxon, each from a unique individual; leaves were cleared using 5% (wt/vol) NaOH in ethanol in glass Petri dishes, followed by 50% aqueous bleach solution, then rinsed and stained with Safranin and fast green (5% wt/vol in ethanol). Cleared and stained leaves were scanned (Epson Perfection V100 Photo; Epson; Long Beach, California, USA) on overhead projector transparency film and digital images were analyzed for leaf shape and major vein traits with ImageJ software (Rasband, 1997-2008). After scanning, leaves were placed on microscope slides for imaging at 40× and 100× with a light microscope and digital camera (microscope: SM-LUX Leitz; Wetzlar, Germany, and camera: Nikon Coolpix 4500; Nikon; Tokyo, Japan) and images were analyzed for minor vein traits using ImageJ.

From the scanned leaves I determined leaf size, perimeter, maximum length and width, and vein densities (length per leaf area) and central vein diameters (excluding bundle sheath) for 1°, 2°, and 3° veins, using ImageJ. For the 2° veins I measured half of each leaf and then doubled the measurements. For 3° veins and minor veins, I measured micrographs taken at three fields of view on each leaf, in the top, middle, and bottom thirds of the leaf; the free ending veinlets were also measured in these micrographs. Vein diameters were measured once for each vein order for one leaf for three leaves per taxon, and the diameters for 4° and 5° (when present) were averaged as minor vein diameter.

Additionally, micrographs were made to determine vein island numbers and length. For species with few enough veins to count all vein islands, all were imaged, using 1 to 60 images per leaf. For species with too many vein islands to count, I averaged from 3-10 images to determine vein island number per area. From these images I also determined the length of "missing vein" in the spaces between vein islands, by connecting the attached and vein islands to complete the vein network.

The values for 43 traits were calculated from the above measurements (Table 2.2). Two indices of leaf shape were determined: leaf lamina length: leaf width ratio and perimeter^2: area ratio (a dimensionless index of edge relative to leaf size). Vein densities for each vein order were determined as vein length divided by leaf area; minor vein and total vein density did not include vein islands. The major vein density was calculated as the sum of the 1°, 2°, and 3° vein densities. The minor vein density was calculated as the sum of 4^o and 5^o order vein densities. The total vein density was determined as the sum of major and minor vein densities. The number of free ending veinlets per leaf area was also determined. Vein cross-sectional surface area per leaf area (SAPA), projected area per leaf area (PAPA), and volume per leaf area (VPA) were estimated by idealizing the veins as round in cross-section, as $\pi \times$ the vein diameter $\times$ vein density, vein diameter $\times$ vein density, and $\pi \times (\text{vein diameter}/2)^2 \times$ vein density respectively. These were determined for 1° veins (1° SAPA, 1°PAPA, and 1°VPA), 2° veins (2°SAPA, 2°PAPA, and 2°VPA) and 3° veins (3°SAPA, 3°PAPA, and 3°VPA), the major vein system (MajVSAPA, MajVPAPA, and MajVVPA), and the minor vein system (MinVSAPA, MinVPAPA, and MinVVPA) and summing the values of the major and minor vein systems, for the total vein system (TotVSAPA, TotVPAPA, and TotVVPA).

The vein island number per area (VINA) and length per area (VILA) were calculated. The percent of the vein system made up by vein islands (%VI) was calculated by dividing VILA by the sum of VILA and total vein density. I also calculated the average length of the "missing" vein segments (MVL) and missing vein length per area (MVLA). The percent of the total vein length, volume, and projected area that were missing (%LM, %VM, and %PAM respectively) were calculated by dividing the missing vein length, volume, and projected area by the sums of those for the total vein system, vein islands and missing veins.

Statistical Analysis

All statistical analysis were conducted using Minitab Release 14 (Minitab, State College, Pennsylvania, USA) and SigmaPlot (SigmaPlot, ver. 8.02; Systat Software, San Jose, California, USA). Data were log transformed to achieve normality and homoscedasicity (Zar, 1999). For traits with zero values I added one before transformation. I tested differences in leaf traits using two (ANOVAs), first nesting taxa within habitat type (bog, coastal, dry forest, mesic forest, and wet forest) and second, nesting taxa within establishment habitat type (open- versus shade-establishing) To examine trait-environment and trait-trait relationships, I tested hypothesized correlations using Spearman and Pearson correlation coefficients (Sokal and Rohlf, 1995). Cut offs for significance were P values of 0.05-0.01*, 0.01-0.001**, and < 0.001***.

RESULTS

Variation in leaf venation among taxa, and between open- and shade-establishing taxa

For the native Hawaiian taxa, 53 of 57 measured traits varied significantly among taxa (ANOVA; $P < 0.012$), and 52 of the 57 traits varied significantly between open and shade-establishing taxa (ANOVA; $P < 0.017$; Table 2.2 and Table 2.3, and Figure 2.2 - Figure 2.8). Numerous leaf traits varied dramatically between open- and shade-establishing taxa. On average, shade leaves had 5.8-fold greater leaf area, and thus had higher values for traits that tended to be greater for larger leaves, including 11% greater lamina length/width, threefold greater perimeter^2/area, 2.3-fold greater 1° vein diameter, and 1.1-1.4 fold greater 2°, 3° and minor vein diameters. Shade leaves had significantly more vein orders than sun-establishing species, on average 4.6 versus to 4.1.

Open-establishing taxa had on average 3.1-fold higher 1° vein density, corresponding to their smaller leaves, and 1.6- and 1.3-fold higher 2° and 3° vein density, resulting in 1.3-fold higher major vein density. Additionally, open-establishing taxa had 1.2- and 1.3-fold higher minor and total vein density, and 1.3-fold higher number of free ending veinlets per area (Figure 2.2 - Figure 2.4).

The allocation of vein length across vein orders varied strongly between open- and shade-establishing species, with 1° veins accounting for 9% of the major vein length in open-establishing species, relative to 4% of vein length in shade-establishing species. Open and shade-establishing species were similar in the allocation of 2° and 3° veins to the major vein system and of major and minor vein length to total vein length.

Sun and shade species had similar 1° and 2° vein surface area per leaf area (the smaller veins of sun-establishing species were compensated for by their higher densities).

However, sun-establishing species had 1.9-fold higher 3° vein surface area per leaf area (SAPA), and 1.3-fold and 1.9-fold higher major and minor vein surface area per leaf area, resulting in a fourfold higher total vein surface area per leaf area. The sun and shade species were rather similar in the allocation of vein orders to vein surface area. The shade-establishing species had 13% higher allocation of 1° vein surface area to major vein surface area, while the sun establishing species had 1.1% and 14% higher allocation of 2° and 3° surface areas to major vein surface area. Overall, the shade-establishing species had 12% higher allocation of major veins to total vein surface area while the sun-establishing species had 12% higher allocation of minor veins to total vein surface area.

Shade-establishing species tended to have lower values for 2°, 3°, and minor vein volume per leaf area. However, shade-establishing species had higher 1° vein volume per leaf area, and because the 1° vein accounted for the majority of major vein volume, shade-establishing species had 1.6-fold higher major vein volume per leaf area (Table 2.2). Although major vein volume accounted for the bulk of total vein volume, shade-establishing species had much lower minor vein volume per area, and thus sun- and shade-establishing species had similar total vein volumes per area. The open and shade-establishing taxa differed strongly in the allocation of vein volume by different vein orders. The shade-establishing taxa had 15% higher allocation of 1° veins to major vein volume, but the sun-establishing species had 1.7-fold and 4.3-fold higher allocation of 2° and 3° veins, respectively, to major vein volume. Shade-establishing species had on average 12% greater contribution of major veins to total vein volume, whereas sun-establishing species had 33% greater allocation of minor veins to vein volume.

Open-establishing species had 2.5-fold larger major vein surface area: volume ratio, but shade-establishing species had 1.5-fold higher minor vein surface area: volume ratio. Open-establishing species had 2.2-fold higher total vein surface area: volume. The same trends were found for vein length: volume (Table 2.2). Shade-establishing species had 2.4-fold lower total vein projected surface area per leaf area.

Vein islands and "missing vein" traits

Of the 27 native and three non-native taxa examined for vein islands, only two taxa lacked vein islands completely (CECE and CEST), though several taxa had very few. All three weedy taxa surveyed had vein islands. I quantified four vein island traits: vein island average length (VIL), vein island number per area (VINA), vein island length per area (VILA), and percent of the vein system that was vein island (%VI). All four vein island traits varied significantly among taxa ($P < 0.001$; Table 2.2 & Table 2.4, and Figure 2.2, Figure 2.3, Figure 2.5, Figure 2.7, & Figure 2.8); VIL varied 8-fold, from 0.033 for MUMI to 0.26 mm for CELA. The VINA ranged from 0.0014 mm^{-2} for weed species HYPE and 0.0021 mm^{-2} for CEHA to 9.35 mm^{-2} for ROCK VILA ranged from 0.000058 mm mm^{-2} for weed species HYPE and 0.00023 for CEHA to 0.74 mm mm^{-2} for ROCK; and %VI ranged from zero for CECE and CEST to 13.6% for ROCK.

Three out of the four vein island traits varied dramatically between open- and shade-establishing taxa. The shade taxa had 37- to 62-fold higher vein island number and vein length per area, and 59-fold higher % vein island. The open- and shade-establishing taxa did not differ significantly in average length of vein islands.

I quantified five "missing vein" traits measured: missing vein average length (MVL), missing vein length per area (MVLA); percent length missing (%LM); percent

volume missing (%VM); and percent projected surface area missing (%PSAM). All five missing vein traits varied significantly among taxa (; $P < 0.001$ for five traits; Table 2.2 and Table 2.4; Figure 2.8). Missing vein average length ranged from 0.0010 mm to 0.18 mm; missing vein length density ranged from no vein islands to 0.54 cm cm^{-2}; the % length missing ranged from none to 12.6%, and the % volume missing ranged from none to 2.8%; and the % projected surface area missing ranged from none to 7.68.

All five missing vein traits differed significantly between open- and shade-establishing taxa. For all five missing vein traits, the larger value was found in the shade-establishing taxa. The length of missing vein per leaf area was 7.2-fold higher for the shade-establishing taxa, and the % vein length that was missing was 51-fold higher; the % of the vein volume and projected area missing were 8.4- to eleven fold. The average length of missing veins was 18% higher in shade-establishing taxa.

Comparison of native taxa with weeds

The values for leaf size, shape, and venation traits for the weeds tended to be intermediate within the range of Hawaiian native species, emphasizing the exceptional diversification among the native species to higher and lower trait values when compared to herbaceous non native taxa that may be similar in growth form to the colonizing taxon. Thus, for 54 of 57 venation traits, the average weed values fell within the range of native values. Exceptions were *E. prostrata*, which had the smallest leaf, and the lowest 1° vein diameter and TotVPA, and *E. hirta* which had the lowest value of all taxa for MajV%TD. Weedy species tended to have similar numbers of vein orders, and similar major vein densities, but 1.4, 1.3, and 1.5-fold higher values for minor, total and free ending vein densities than the average for native species. The weedy species *E. hypericifolia*, had on

average, the lowest values of all 29 taxa for all vein island and "missing vein" traits (except MVL and VIL), even lower than sun-establishing native species. Thus, relative to native species (and even sun-establishing native species), this weedy species had low values for vein island number and length per leaf area and % of the vein system that was disjunct, and lower missing vein length density as well as % of the vein length, volume, and projected area that was missing. Across weedy taxa there was a similar length of the average vein island and missing vein as native species.

Correlation of key traits with environment

Across taxa, many venation traits were correlated with environmental variables. The percent of the vein system that was disjunct, the percent that was missing, and the percent of surface area that were missing were higher in the shade, and thus, negatively correlated with % open sky ($|r_s|$ and $|r_p| = 0.42 - 0.54$; $P < 0.05$). Traits negatively related to leaf area, including the 1°, 2° and 3° vein densities and the major vein density were negatively correlated with (MAP), which correlated positively with leaf size. Minor vein density and number of freely ending veinlets, which related to shady habitat, also correlated negatively with MAP($|r_s|$ and $|r_p|$ range from 0.43 - 0.71; $P < 0.05$). Thus, the major, minor, and total vein densities were positively correlated with % open sky ($|r_s|$ and $|r_p| = 0.40 - 0.59$; $P < 0.05$; Figure 2.6). As expected from their relationship with shady habitat, discussed above, the VILA, VINA, %VI, and %MVL correlated negatively with % open sky ($|r_s|$ and $|r_p| = 0.39 - 0.49$; $P < 0.05$). Consistent with shady habitat occurring in moist climates, VILA, VINA, MVLA, and %LM correlated positively with MAP ($r_s|$ and $|r_p| = 0.43 - 0.72$; $p < 0.05$), whereas major, minor, and total vein density, and the number of freely ending veinlets per leaf area all were correlated negatively with MAP ($|r_s|$ and $|r_p| =$

0.38 - 0.70; $P < 0.05$). No significant correlations were found among venation traits and the other environmental variables, MAT, MARH, VPD, MFVPD, or elevation ($P > 0.05$).

Leaf gross morphology correlated with other environmental factors. Lamina area: width correlated positively with elevation and MARH ($|r_s|$ and $|r_p| = 0.41 - 0.51$; $P < 0.05$) and negatively with VPD and MFVPD ($|r_s|$ and $|r_p| = 0.42 - 0.46$; $P < 0.05$). Leaf area correlated with all environmental factors in the study, increasing with elevation, MAP and MARH ($|r_s|$ and $|r_p| = 0.43 - 0.67$; $P < 0.05$), and declining with % open sky, MAT, VPD, and MFVPD ($|r_s|$ and $|r_p| = 0.41 - 0.55$; $P < 0.05$).

Correlation of disjunct and missing vein traits with other vein and leaf traits

I found significant correlations across taxa between vein island traits and other venation traits. These relationships were primarily mediated by leaf size. Vein island number per area, which was higher in larger leaves, correlated negatively with 1° vein density, 2° vein density, major vein density, minor vein density, total vein density, and the number of freely ending veinlets per area, and correlated positively with 3° diameter and minor vein diameter ($|r_s|$ and $|r_p| = 0.52 - 0.81$; $P < 0.05$). Further, as predicted, vein island traits and missing vein traits were significantly inter-correlated. The vein island number per area (VINA) was positively correlated with vein island length per area, % vein island, missing vein length density, % Length missing, %volume missing, and %PSA missing ($|r_s|$ and $|r_p| = 0.61 - 0.98$ $P < 0.05$). The missing vein length density was positively correlated with vein island number density, vein island length density, % vein island, % length missing, %volume missing, % projected surface area missing, ($r_s|$ and $|r_p| = 0.57 - 0.99$ $P < 0.05$), and negatively correlated with 1° vein density, minor vein density, total vein density, free-ending veinlets, 3° vein width, minor vein width, and total vein surface area

per leaf area ($|r_s|$ and $|r_p|$ = 0.40 - 0.81 $P < 0.05$; Figure 2.8, a and b). Notably, the average length of missing veins did not correlate with VINA.

Many leaf venation traits were correlated with leaf area, including vein island number and length per area, % vein island, missing vein length density, % length missing, 1°, 2°, and 3° vein density, major, minor, and total vein density, number of free ending veinlets per leaf area, and 1° and 2° vein diameter ($|r_s|$ and $|r_p|$ = 0.41 - 0.92; $P < 0.05$). However, the average length of missing veins, % volume missing, %PSA missing, number of vein orders, 3° and minor vein diameters were not correlated with leaf area ($P > 0.05$).

Disjunct and missing vein traits were also correlated with other leaf traits that differed between open- and shade-establishing taxa. The VINA was positively correlated with leaf area, as discussed above (Figure 2.3). Both VINA and VIVD correlated positively with distribution of stomatal density on the abaxial surface ($\%SD_{ab}$) and with leaf thickness (r = 0.40-0.77; $P < 0.001$ to $P = 0.029$), both of which were higher for shade-establishing taxa (data not shown). Both VINA and VIVD were negatively correlated with total stomatal pore area per leaf area and with foliar nitrogen and phosphorus per area (r = -0.43 to -0.48; P = 0.027-0.029), which were lower for shade-establishing taxa (Figure 2.8).

DISCUSSION

String significant correlations existed between venation architecture, habitat and climate in the Hawaiian *Euphorbia*, including important relationships with the major vein system, minor vein system and vein islands. These findings extend recent research indicating that

leaf venation architecture is adaptive to habitat, including specific climate variables and irradiance (Roth-Nebelsick et al., 2001; Sack and Holbrook, 2006; Brodribb et al., 2007; Brodribb et al., 2010; McKown et al., 2010). Moreover, considering the relatively recent arrival of *Euphorbia* to Hawaii, my findings supports the hypothesis that venation architecture can adapt rapidly following isolation and possibly in response to a strong climatic gradient, providing a likely case of adaptation within a single, highly-diversified lineage.

I found that major vein densities were significantly negatively correlated to leaf area. This suggests that habitat and climate factors that probably influence leaf area. These findings support the view that the major vein system is tightly developmentally linked to leaf size, and thus show parallel evolution with leaf size across environments (Dunbar-Co et al., 2009; McKown et al., 2010; Sack et al., in prep).

The minor vein system was also associated with habitat. Leaf minor veins hydraulically supply water to allow gas exchange, a critical need, even for C_4 plants that have lower water requirements than C_3 species (Sage, 2004). I predicted that I would find higher minor vein densities in environments that entail a higher evaporation load, and I found higher minor vein densities for sun than for shade species. A higher minor vein density and smaller leaves are both adaptive for high irradiance environments (Givnish, 1987; Sack and Frole, 2006).

I found that vein traits were independent of many other leaf traits, including leaf morphology (aside from leaf size), mass-based nutrient concentrations, and many stomatal traits. The densities of 1° and minor veins were negatively correlated with lamina thickness, probably because many of the large-leafed, shade-tolerant species have

thicker leaves, potentially conferring a long lifespan (Lusk et al., 2008). Thus, the vein system can evolve independently of numerous other leaf features.

Our study clarified the potential benefits of vein islands. Notably, I cannot exclude a functional capacity of vein islands in photosynthesis. These veins might be locally functional within the leaf in C_4 photosynthesis, as they do have Kranz anatomy, though they could not export photosynthate to the attached vein network or outside of the leaf. Such a function would, however, not confer an advantage in itself over leaves with all veins attached. Indeed, the missing vein length would entail a net loss of photosynthetic capacity. Our findings suggest several avenues of explanation for a functional role of vein islands. Vein islands were strongly associated with wet and shaded environments. The first advantage of vein islands in shaded habitats may be in decreasing the total vein density, thus saving the energy and materials that would have been used for constructing those veins. Notably, in shade, the leaves would have lower evaporative demand, and could thus tolerate a lower hydraulic supply; also, the increasing light limitation would mean adaptation to lower photosynthetic rates and the reduction of bundle sheath photosynthetic tissue is consistent with such adaptation (Ogle, 2003). Shade leaves did have lower vein density overall and the vein islands contributed to this lower investment in vein length and in bundle sheath tissue. Vein islands did not seem to reduce the volume cost of the vein system, the vein islands did not appear to reduce total vein system volume per leaf area, and shade taxa did not have lower vein system volume per leaf area than sun-establishing taxa. However, the vein islands did substantially reduce the *vein projected area* compared to those taxa that lacked vein islands, and shade-establishing taxa had an importantly lower value than sun-establishing taxa. Thus,

in addition to reducing vein and bundle sheath length, a second advantage of vein islands may be to increase mesophyll exposure to light, a benefit for shaded leaves. In C_4 plants the light reactions in chloroplasts are important as part of the PEP cycling reactions (Sage, 2004) and a loss of vein projected area would result in greater area for light capture. The significant correlation of VIVD and hypostomaty may indicate the co-selection of both traits that reduce mesophyll shading, as stomatal distribution on the abaxial rather than adaxial face may reduce light absorption by guard cells.

I also suggest that vein islands might not provide an adaptive advantage, and could be a case of a "neutral" retained mutation that could pose no disadvantage, for C_4 plants that establish in shaded, wet forests and that are not limited by water. Such a mutation has been found in *Arabidopsis* (Pullen et al.). However, the best available phylogenetic information indicates multiple origins of high vein island numbers per area. A phylogenetic analysis based on plastid DNA sequences (Yang and Berry, 2007) indicates that *E. remyi kauaiensis*, and *E. arnottiana* arise in distinct clades from each other, and from the clade that holds *E. clusiifolia*, *E. herbstii*, *E. multiformis* var. *multiformis* and *E. rockii*; all these taxa have very numerous vein islands, i.e., 1-2 orders of magnitudes higher than those of the taxa that they are nested among. One way such a mutation might arise is if the evolution of very large leaves in shade proceeded beyond the capacity of the expanding leaves to develop additional vein orders in the interstices of the expanding vein system, and additional vein orders did not evolve. Indeed I found a very conservative 4-5 vein orders across all the taxa, indicating a rather tight regulation of the number of vein orders. The evolution of a leaf to larger sizes could thus involve interruption of vein development in the spaces between previously developed veins. Such

a pattern would seem consistent with a study of development in *E. herbstii* (Herbst, 1972) in which the missing veins were visible as procambial tissue, but did not develop, as leaves expanded leading to vein island formation. Thus, disjunct (and missing) veins might arise as a side effect of the evolution of large leaf size for benefit in shade. However, the strong association of vein islands with environment and establishment habitat (even in a small leafed species such as *E. arnottiana*), and the considerable savings of cost in vein length and mesophyll shading suggest an advantage in shade.

The broad diversification of venation architecture in Hawaiian *Euphorbia,* and its correlation with environment, highlight principles that could be general in other lineages, i.e., the increase of vein density in high light environments, and the "shedding" of veins, i.e., the loss of density of veins within the attached, continuous vein system, as well as the loss of additional vein density through vein island formation. These patterns point to the leaf venation as an important, consistent locus of environmental adaptation.

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TABLES

Table 2.1. List of Hawaiian *Euphorbia* taxa included in study, island of sampled population, maximum height observed in the field, habitat type ("Wet", "Mesic" and "Dry" were determined according to moisture regimes and elevation bands for coastal taxa, following Wagner et al., 1999, with an additional "Bog" specification for SPAR, which occurs exclusively in Wahiawa bog on Kauai. Federal conservation status: ***Endangered, **Species of concern; *Recommended for candidacy as endangered. Island abbreviations: H, Hawaii, Kah, Kahoolawe, Kau, Kauai, L, Lanai, Ma, Maui, Mo, Molokai, O, Oahu, †Not included in study due to lack of current knowledge of accessible populations.

Taxon	Authors of authority	Code name	Location	Islands (sampled island pop. in bold)	Max ht. (m)	Habitat	Open or Shade
Hawaiian taxa							
E. arnottiana var. *arnottiana***	(Endl.) O. Deg. & I. Deg.	ARNO	Aina Haina,	**O**	0.91	Mesic	Shade
E. arnottiana var. *integrifolia*	Hillebrand	ARIN	Kapuuakea,	**Ma**	0.43	Mesic	Shade
E. atrococca	(A. Heller) Croizat & O. Deg.	ATRO	Makaha Ridge	**Kau**	3.17	Dry	Shade
E. celastroides var. *amplectens*	(Sherff) O. Deg. & I. Deg.	CEAM	Hawaii Loa Ridge	**O**, all main	1.62	Dry	Open
E. celastroides var. *celastroides*	(Boiss.) Croizat & O. Deg.	CECE	NTBG	**Kau**	1.95	Dry	Open
E. celastroides var. *hanapepensis*	(Sherff) O. Deg. & I. Deg.	CEHA	Halemanu Rd. Kokee	**Kau**	1.61	Wet	Shade
E. celastroides var. *kaenana****	(Sherff) O. Deg. & I. Deg.	CEKA	Makua Valley	**O**	1.70	Coast	Open
E. celastroides var. *laehiensis*	(O. Deg., I. Deg. & Sherff) Koutnik	CELA	Waiopai	**Ma,** L	0.10	Coast	Open
E. celastroides var. *lorifolia***	(A. Gray) O. Deg. & I. Deg.	CELO	NTBG	**Kau**, Ma, L	5.30	Dry	Open
E. celastroides var. *stokesii*	(C. N. Forbes) O. Deg. & I. Deg.	CEST	Kilauea Point	**Kau**, Mo	1.13	Coast	Open
E. celastroides var. *tomentella***	(Boiss.) Koutnik	CETO	Waianae Kai	**O**	0.99	Mesic	Shade
*E. clusiifolia***	(Hook. & Arn.) Arthur	CLUS	Poamoho trail	**O**	2.44	Wet	Shade
E. degeneri	(Sherff) Croizat & O. Deg.	DEGE	Secret Beach	H**, Kau,** Ma, Mo, O	0.19	Coast	Open
*E. deppeana****	(Boiss.) Millsp.	DEPP	Pali Lookout	**O**	0.24	Mesic	Open
*E. eleanoriae***†	Lorence & W. L. Wagner	ELEA	Napali	**Kau**			
*E. halemanui****	(Sherff) Croizat & O. Deg.	HALE	Halemanu Rd. Kokee	**Kau**	3.18	Wet	Shade
*E. herbstii****	W. L. Wagner	HERB	Makaha Valley	**O**	0.91	Wet	Shade
*E. kuwaleana****	(O. Deg. & Sherff) O. Deg. & I. Deg.	KUWA	Kauaopuu Ridge	**O**	0.50	Dry	Open
E. multiformis var. *microphylla*	(Boiss.) O. Deg. & I. Deg.	MUMI	PTA	**H,** Ma, Mo, O	2.01	Dry	Open
E. multiformis var. *multiformis*	(Hook. & Arn.) Croizat & O. Deg	MUMU	Pahole	Ma, **O**	1.14	Mesic	Shade
*E. olowaluana***	(Sherff) Croizat & O. Deg.	OLOW	PTA	**H**	6.15	Dry	Open
E. remyi var. *hanaleiensis*†	(Sherff) O. Deg. & I. Deg.	REHA	Hanalei	**Kau**		Wet	Shade
E. remyi var. *kuaiensis**	(O. Deg. & Sherff) O. Deg. & I. Deg.	REKU	Blue Hole	**Kau**	3.12	Wet	Shade
E. remyi var. *remyi**	(A. Gray ex Boiss.) Croizat & O. Deg.	RERE	Kokee	**Kau**		Wet	Shade
*E. rockii****	(C. N. Forbes) Croizat & O. Deg.	ROCK	Koolau Summit Trail	**O**	2.36	Wet	Shade

Taxon	Authors of authority	Code name	Location	Islands (sampled island pop. in bold)	Max ht. (m)	Habitat	Open or Shade
E. skottsbergii var. *audens*	(Sherff) O. Deg. & I. Deg.	SKAU	W.Molokai	**Mo**	0.04	Coast	Open
E. skottsbergii var. *skottsbergii***	(Sherff) Croizat & O. Deg.	SKSK	Ewa Plain	Mo, **O**	1.04	Dry	Open
E. skottsbergii var. *vaccinioides***	(Sherff) Koutnik	SKVA	Central Molokai	Kah, Ma, **Mo**	1.28	Dry	Open
*E. sparsiflora***	(A. Heller) Koutnik	SPAR	Kanaele Bog	**Kau**	0.91	Bog	Open
Weed species							
E. hirta	(L.) Millsp.	HIRT	Manoa	**O**, all main	0.37	Weed	Open
E. hypericifolia	(L.) Millsp.	HYPE	Manoa	H, Kau, **O**, Ma	0.29	Weed	Open
E. prostrata	(Aiton)	PROS	Manoa	H, Kah, Kau, **O**, Ma, L	0.11	Weed	Open

Table 2.2. Venation traits within categories, symbols, units, ranges of species mean values for non-native (weed) species, and for native taxa, and means for open- and shade-establishing native taxa. Nested ANOVA results are presented, for taxon nested within open vs. shade-establishing, for native taxa; *P*-values for the taxon and open vs shade comparisons in the fifth and sixth columns respectively. *$P < 0.05$, **P=0.01 – 0.001, ***P = <0.001.

Trait category/ trait	**Trait**	**Units**	Min-**mean**-max (non-native taxa)
Gross morphology			
Leaf area	LA	cm^2	0.42-**1.67**-2.74
Lamina L/Lamina W	LaMVL/LamW	cm	1.41-**1.89**-2.26
Perimeter squared/area	P^2/A	cm	13.35-**19.67**-25.28
Vein islands and "missing veins"			
Vein island average length	VIL	mm	0.041-**0.080**-0.10
Vein island number per area	VINA	mm^{-2}	0.0014-**0.012**-0.030
Vein island length per area	VILA	mm mm^{-2}	0.000058-**0.0011**-0.0030
% Vein island	%VI	%	0.00063-**0.013**-0.035
Missing vein average length	MVL	mm	0.027-**0.043**-0.053
Missing vein length density	MVLA	cm cm^{-2}	0.000039-**0.00040**-0.00098
% length missing	%LM	%	0.00045-**0.0045**-0.010
% volume missing	%VM	%	0.00012-**0.0012**-0.0032
% projected surface area missing	%PSAM	%	0.00032-**0.0031**-0.0072
Vein densities			
Number of vein orders	#Vorders	#	4-**4**-4
1^o vein density	1^oD	cm cm^{-2}	1.052-**1.47**-2.064
2^o vein density	2^oD	cm cm^{-2}	5.13-**8.46**-11.064
3^o vein density	3^oD	cm cm^{-2}	9.60-**11.050**-13.26
Major vein density	MajVD	cm cm^{-2}	15.78-**20.98**-26.38
Minor vein density	MinVD	mm mm^{-2}	6.47-**6.51**-6.56
Total vein density	TotVD	mm mm^{-2}	8.13-**8.60**-9.13
Free ending veinlets	FEV	mm^{-2}	13.04-**15.28**-16.58
Vein diameters			
1^o vein diameter	1^oW	mm	0.096-**0.15**-0.24
2^o vein diameter	2^oW	mm	0.055-**0.078**-0.093
3^o vein diameter	3^oW	mm	0.018-**0.025**-0.030
Minor vein diameter	MinW	mm	0.014-**0.017**-0.021
Vein volume per leaf area			
1^o vein	1^oVPA	mm	0.0015-**0.0029**-0.0054
2^o veins	2^oVPA	mm	0.0026-**0.0041**-0.0061
3^o veins	3^oVPA	mm	0.00035-**0.00056**-0.00074
Major veins	MajV VPA	mm	0.0045-**0.0076**-0.0097
Minor veins	MinV VPA	mm	0.0011-**0.0016**-0.0022
Total veins	TotVPA	mm	0.0056-**0.0092**-0.012
Vein surface area per leaf area			
1^o vein	1^oSAPA	mm mm^{-2}	0.054-**0.066**-0.081
2^o veins	2^oSAPA	mm mm^{-22}	0.15-**0.20**-0.25
3^o veins	3^oSAPA	mm mm^{-2}	0.076-**0.085**-0.093

Trait category/ trait	**Trait**	**Units**	Min-**mean**-max (non-native taxa)
Major veins	MajVSAPA	mm mm^{-2}	0.32-**0.35**-0.39
Minor veins	MinVSAPA	mm mm^{-2}	0.30-**0.36**-0.42
Total veins	TotVSAPA	mm mm^{-2}	0.62-**0.70**-0.74
Vein surface area: volume			
Major veins	MajVSAVOL	mm^{-1}	34.25-**54.69**-78.081
Minor veins	MinVSAVOL	mm^{-1}	197.18-**239.49**-284.96
Total veins	TotVSAVOL	mm^{-1}	63.78-**88.73**-118.79
Vein length per volume			
Major veins	MajVLVOL	mm^{-2}	169.18-**387.55**-684.72
Minor veins	MinVLVOL	mm^{-2}	313.079-**478.20**-671.26
Total veins	TotVLVOL	mm^{-2}	704.53-**1208.098**-1867.98
Vein projected surface area per area			
Total veins	TotVPSAPA	mm^{-2}	0.20-**0.22**-0.24

Trait category/ trait	Min-**mean**-max (native taxa)	Mean± SE, open-establishing	Mean ± SE, shade-establishing
Gross morphology			
Leaf area	0.31-**8.21**-34.68***	2.60 ± 0.56, ***	15.00± 3.50
Lamina L/Lamina W	1.09-**2.88**-10.91***	2.48 ± 0.33***	2.82±0.18
Perimeter squared/area	2.82-**39.27**-526.97*	20.43 ± 2.55***	63.00±42.20
Vein islands and "missing veins"			
Vein island average length	0.033-**0.093**-0.26^{ns}	0.073 ± 0.015***	0.083 ± 0.011
Vein island number per area	0.0014-**1.39**-11.29***	0.047 ± 0.016***	2.89 ± 0.82
Vein island length per area	0.000058-**0.11**-0.76***	0.0062 ± 0.0024***	0.023 ± 0.069
% Vein island	0.00-**3.41**-13.6***	2.29 ± 2.24***	4.65 ± 1.48
Missing vein average length	0.0010-**0.080**-0.18**	0.070± 0.013***	0.083 ± 0.011
Missing vein length density	0.000039-0.**092**-0.54***	0.037 ± 0.031***	0.26 ± 0.074
% length missing	0.00-**2.14**-12.62***	0.092 ± 0.040***	4.72 ± 1.40
% volume missing	0.00045-**0.17**-2.076***	0.043 ± 0.022***	0.36 ± 0.17
% projected surface area missing	0.00-**1.19**-7.68***	0.24 ± 0.19***	2.62 ± 0.80
Vein densities			
Number of vein orders	4.0-**4.48**-5.0***	4.12 ± 0.27***	4.58 ± 0.15
1^o vein density	0.31-**0.98**-2.22***	1.98 ± 0.71***	0.65 ± 0.084
2^o vein density	3.24-**8.37**-16.3***	9.86 ± 0.81***	6.32 ± 0.52
3^o vein density	5.86-**11.0**-18.6***	12.93 ± 1.033***	9.61 ± 0.68
Major vein density	9.41-**20.3**-35.4***	22.24 ± 2.01***	16.58 ± 1.18
Minor vein density	2.28-**4.71**-6.26***	5.27 ± 0.25***	4.20± 0.36
Total vein density	3.59-**6.75**-9.30***	7.50 ± 0.32***	5.85 ± 0.42
Free ending veinlets	3.60-**10.2**-19.0**	10.91 ± 1.16***	8.57 ± 1.10

Vein diameters			
1^o vein diameter	0.12-**0.59**-2.24***	0.36 ± 0.067***	0.83 ± 0.16
2^o vein diameter	0.054-**0.089**-0.16*	0.083 ± 0.0079***	0.094 ± 0.0096
3^o vein diameter	0.018-**0.045**-0.087***	0.050 ± 0.0044***	0.037 ± 0.0041
Minor vein diameter	0.014-**0.026**-0.044***	0.029 ± 0.0024***	0.021 ± 0.002
Vein volume per leaf area			
1^o vein	0.038-**0.14**-0.30^{ns}	0.15 ± 0.026***	0.14 ± 0.011
2^o veins	0.080-**0.22**-0.43***	0.24 ± 0.020***	0.18 ± 0.026
3^o veins	0.054-**0.15**-0.27***	0.22 ± 0.033***	0.11 ± 0.016
Major veins	0.25-**0.51**-0.94***	0.57 ± 0.042***	0.43 ± 0.046
Minor veins	0.11-**0.40**-0.80***	0.54 ± 0.064***	0.28 ± 0.044
Total veins	0.36-**0.91**-1.65***	2.68 ± 1.62***	0.72 ± 0.088
Vein surface area: volume			
Major veins	4.45-**28.54**-70.76***	43.47 ± 7.51***	17.40 ± 2.56

Table 2.3. Allocation of vein length, volume, surface area and projected area from given vein orders to the major vein system, and of the major and minor vein systems to the total venation, symbols, units, ranges of species mean values for non-native (weed) species, and for native taxa, and means for open- and shade-establishing native taxa. Nested ANOVA, results are presented, for taxon nested within open vs. shade-establishing for native taxa. *P* values are given for the taxon and open vs shade comparisons in the fifth and sixth columns respectively. **P* < 0.05, ***P*=0.01 – 0.001, ****P* = <0.001.

Trait	**Trait**	**Units**	Min-**mean**-max (non-native taxa)	Min-**mean**-max (native taxa)	Mean± SE, open-est.	Mean ± SE, shade-est.
Allocation of vein length						
1^{o} veins, % of major vein length	1^{o} %MajD	%	6.25-**6.94**-7.94	2.11-**4.70**-7.40***	8.66 ± 3.23***	3.88 ± 0.35
2^{o} veins, % of major vein length	2^{o} %MajD	%	32.4-**39.4**-43.8	29.8-**40.6**-53.7**	41.8 ± 1.39**	38.1 ± 1.36
3^{o} veins, % of major vein length	3^{o} %MajD	%	50.0-**53.7**-61.0	42.4-**54.8**-64.7***	51.3 ± 2.01**	58.0 ± 1.40
Major veins, % of total vein length	MajV%TD	%	20.0-**24.4**-28.9	20.1-**30.6**-45.2^{ns}	34.1 ± 2.82**	29.8 ± 2.27
Minor veins, % of total vein length	MinV%TD	%	71.1-**75.6**-80.0	54.8-**69.4**-79.9^{ns}	67.7 ± 1.38*	70.2 ± 2.27
Allocation of vein volume						
1^{o} veins, % of major vein volume	1^{o} %MajVol	%	27.8-**37.3**-50.9	26.3-**64.4**-91.5***	51.8 ± 5.57***	78.2 ± 4.40
2^{o} veins, % of major vein volume	2^{o} %MajVol	%	41.9-**54.5**-63.3	6.26-**25.2**-55.5***	30.0 ± 3.98***	17.2 ± 3.50
3^{o} veins, % of major vein volume	3^{o} %MajVol	%	7.27-**8.22**-8.88	1.20-**10.5**-28.3***	20.0 ± 4.99***	4.66± 1.07
Major veins, % of total vein volume	MajV%TVol	%	80.3-**81.6**-82.8	72.9-**87.7**-98.4***	77.7 ± 4.73**	94.5 ± 1.16
Minor veins, % of total vein volume	MinV%TVol	%	17.2-**18.4**-19.7	1.61-**12.3**-27.1***	17.9 ± 1.75***	5.48 ± 1.16
Allocation of vein surface area						
1^{o} veins, % of major vein surface area	1^{o} %MajSA	%	14.9-**19.5**-24.7	11.3-**27.2**-46.5***	25.1 ± 3.32***	32.8 ± 2.72
2^{o} veins, % of major vein surface area	2^{o} %MajSA	%	47.1-**55.0**-60.6	29.6-**43.1**-57.2^{ns}	42.3 ± 2.18***	41.7 ± 1.68
3^{o} veins, % of major vein surface area	3^{o} %MajSA	%	23.8-**25.5**-28.2	16.6-**29.8**-41.6***	35.0 ± 1.83**	25.5 ± 1.84
Major veins, % of total vein surface area	MajV%TSA	%	43.9-**49.6**-52.6	42.1-**57.9**-71.7***	53.9 ± 1.40^{ns}	62.2 ± 1.82
Minor veins, % of total vein surface area	MinV%TSA	%	47.41-**50.4**-56.1	28.3-**42.1**-57.9**	46.1 ± 1.40^{ns}	37.7 ± 1.82
Allocation of vein projected area						
Major veins, % of total vein projected area	Maj%TPSA	%	43.9-**49.6**-52.6	42.1-**57.9**-71.7***	53.9 ± 1.40^{ns}	62.3 ± 1.82
Minor veins, % of total vein projected area	Min%TPSA	%	47.4-**50.4**-56.1	28.3-**42.1**-57.9***	42.5 ± 3.30^{ns}	37.7 ± 1.82

Table 2.4. Correlation of Hawaiian *Euphorbia* leaf size, shape and venation traits with environmental variables. Correlation coefficients presented: Pearson correlation calculated with untransformed data, Pearson correlation calculated with log-transformed data, Spearman correlation. Significance of P values displayed with * symbol: <0.000***, 0.01-.001**, 0.05-.01*, < 0.06 ^{X}. P values in bold are those that were significant for the Spearman correlation and Pearson correlation with untransformed and/or log-transformed data.

Leaf Traits	%Open Sky	Elevation	Mean annual temp.	Mean annual precipitation	Mean annual relative humidity	Vapor pressure deficit	Mole fraction vapor pressure deficit
Leaf area	**-0.41*,-0.50**, -0.51****	0.35, **0.47***, **0.43***	**-0.45***,**-0.45***, **-0.45***	**0.58****,**0.67*****, **0.61****	**0.47***,**0.53****, **0.56****	**-0.47***,**-0.55****, **-0.51****	**-0.48***,**-0.56****, **-0.57****
Lamina length/lamina width	-0.06,-0.14,-0.14	0.32,**0.50****, **0.52****	-0.32**,-0.43*, -0.48***	-0.097,0.22, 0.16	0.22,**0.38**[X],**0.50****	-0.28,**-0.43***, **-0.47***	-0.269,**-0.426***, **-0.452***
Perimeter^2/A	-0.34,-0.43*,-0.27	0.052,0.30,0.21	-0.038,-0.10,-0.15	-0.074,0.039, -0.011	0.18,0.22,0.18	-0.13,-0.18,-0.16	-0.138,-0.186,-0.147
Vein island length	0.12,0.044,0.030	-0.42*, -0.33, -0.26	0.41*,0.40[X],0.25	-0.13,-0.003,0.060	-0.34,-0.26, -0.18	0.39[X],0.36, 0.22	0.383[X],0.343, 0.193
Vein island # per area	**-0.38**[X],**-0.50****, **-0.47***	0.15,0.36,0.21	-0.28,-0.27, -0.21	**0.72*****,**0.70*****, **0.43***	0.45*,0.51**,0.27	-0.40*,-0.44*, -0.22	-0.425*,-0.480*, -0.267
Vein island length per area	**-0.40*,-0.40*, -0.48***	0.13,0.30,0.15	-0.24,-0.21,-0.15	0.65***,0.66***, 0.43[*]	0.43*,0.44*,0.24	-0.38[X],-0.38[X], -0.18	-0.396*,-0.408*, -0.230
% Vein island	**-0.41***, **-0.51****, **-0.50****	0.14, 0.34,0.15	-0.24,-0.25,-0.16	0.62**,0.68***, 0.44[*]	0.42*, 0.48*,0.24	-0.37[X],-0.42*, -0.18	-0.387*,-0.448*, -0.235
Missing vein length	-0.20,-0.26,-0.18	-0.15, 0.39[X], 0.019	0.14,-0.13,0.034	0.033,0.13,0.12	-0.020,0.25,-0.034	0.055,-0.205, 0.054	0.051, -0.210,0.039
Missing vein length density	**-0.38**[X],**-0.39*, -0.41***	0.13,0.30, 0.13	-0.26,-0.22,-0.17	**0.72*****,**0.70*****, **0.50****	0.44*,0.45*,0.25	-0.39*,-0.39*, -0.18	-0.414*,-0.426*, -0.243
% Missing vein length	**-0.40*,-0.51****, **-0.43***	0.14,0.33,0.14	-0.25,-0.25,-0.18	**0.67*****,**0.70*****, **0.52****	0.44*,0.49*,0.26	-0.38*,-0.42*, -0.197	-0.405*,-0.458*, -0.259
# vein orders	-0.068,-0.11,-0.055	0.15,-0.048, 0.032	-0.21,-0.22,-0.14	0.20,0.18,0.13	0.041,0.034,0.054	-0.096,-0.142, -0.130	-0.097,-0.138,-0.130
Major vein density	**0.59****,**0.59****, **0.58****	-0.17,-0.34,-0.27	0.23,0.23,0.27	**-0.38***,**-0.59****, **-0.66*****	-0.38[X],-0.37[X], -0.36	0.338,0.344, 0.324	0.352,0.361,0.381[X]
Minor vein density	**0.40***,0.31,**0.42***	-0.022,-0.20, -0.081	0.11,0.11,0.13	**-0.55****,**-0.62****, **-0.60****	-0.25,-0.28,-0.12	0.208,0.225, 0.140	0.224,0.250,0.180
Total vein density	**0.53****,**0.45***, **0.55****	-0.084,-0.28, -0.17	0.17,0.16,0.20	**-0.54****,**-0.68*****, **-0.71*****	-0.33,-0.34,-0.26	0.286,0.289, 0.225	0.303,0.314,0.277
Free ending veinlets	0.35,0.32,0.35	-0.075,-0.25, -0.11	0.16,0.16,0.18	**-0.52****, **-0.70*****,**0.74*****	-0.34,-0.34,-0.25	0.286,0.285,0.231	0.304,0.310,0.291

FIGURES

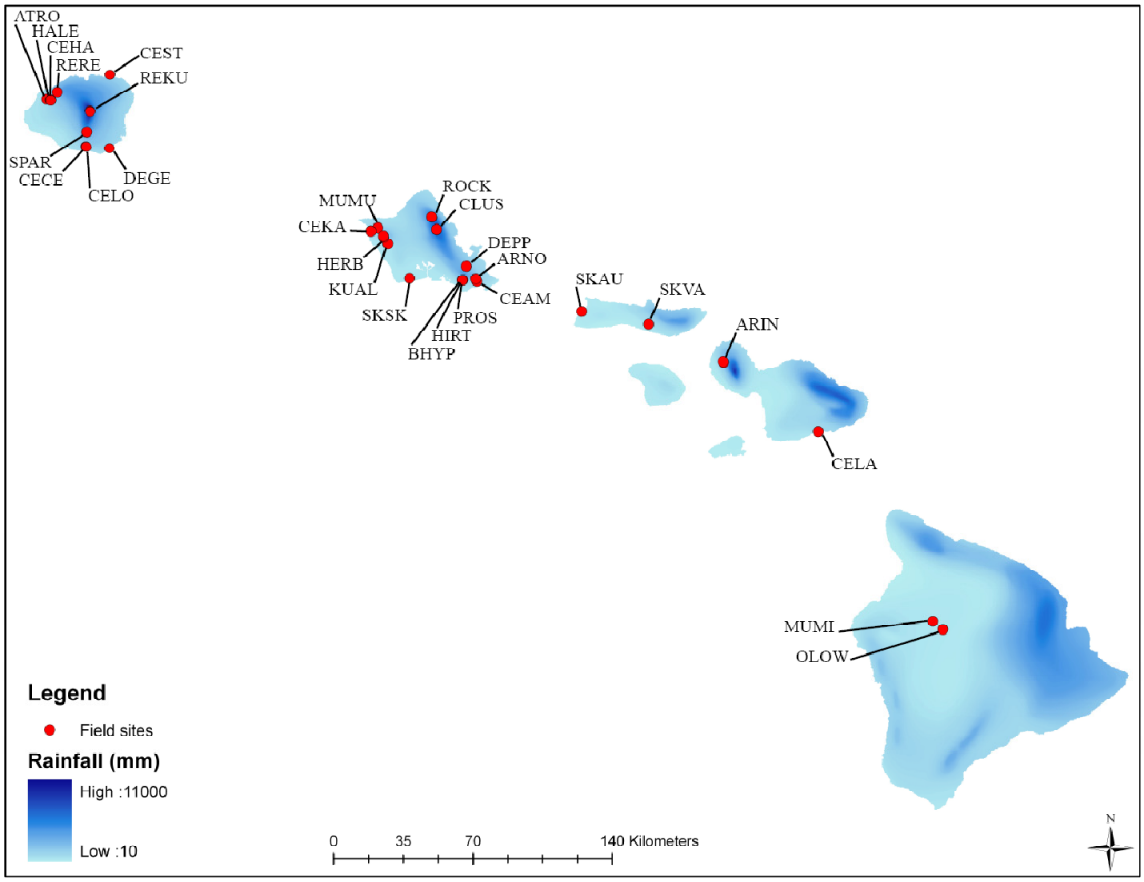

Figure 2.1. Map of the Hawaiian Islands, representing mean annual rainfall by the darkness of blue. Red symbols indicate population collection sites for 27 native Hawaiian *Euphorbia* taxa, and three weedy species of *Euphorbia* (codes in Table 2.1).

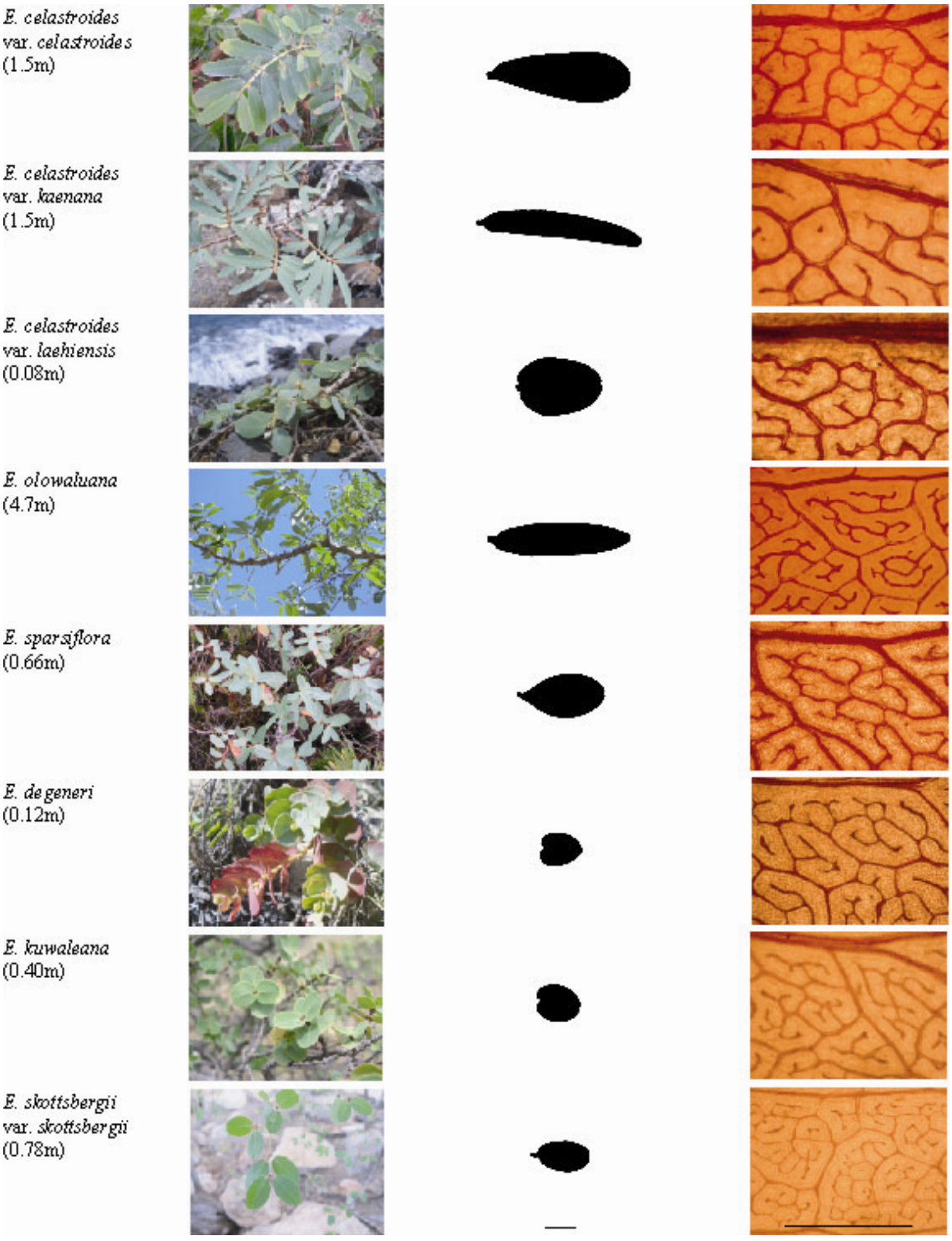

Figure 2.2. Plate showing eight of the 15 open-establishing taxa in the study. From left to right: taxon names and the maximum height of the taxon in parenthesis, an image of the taxon in the field, a silhouette of the leaf (scale bar = 1 cm), and a light micrograph of the leaf veins of a chemically cleared and stained leaf (scale bar = 100 μm). Taxa are arranged in order of declining leaf size from top to bottom.

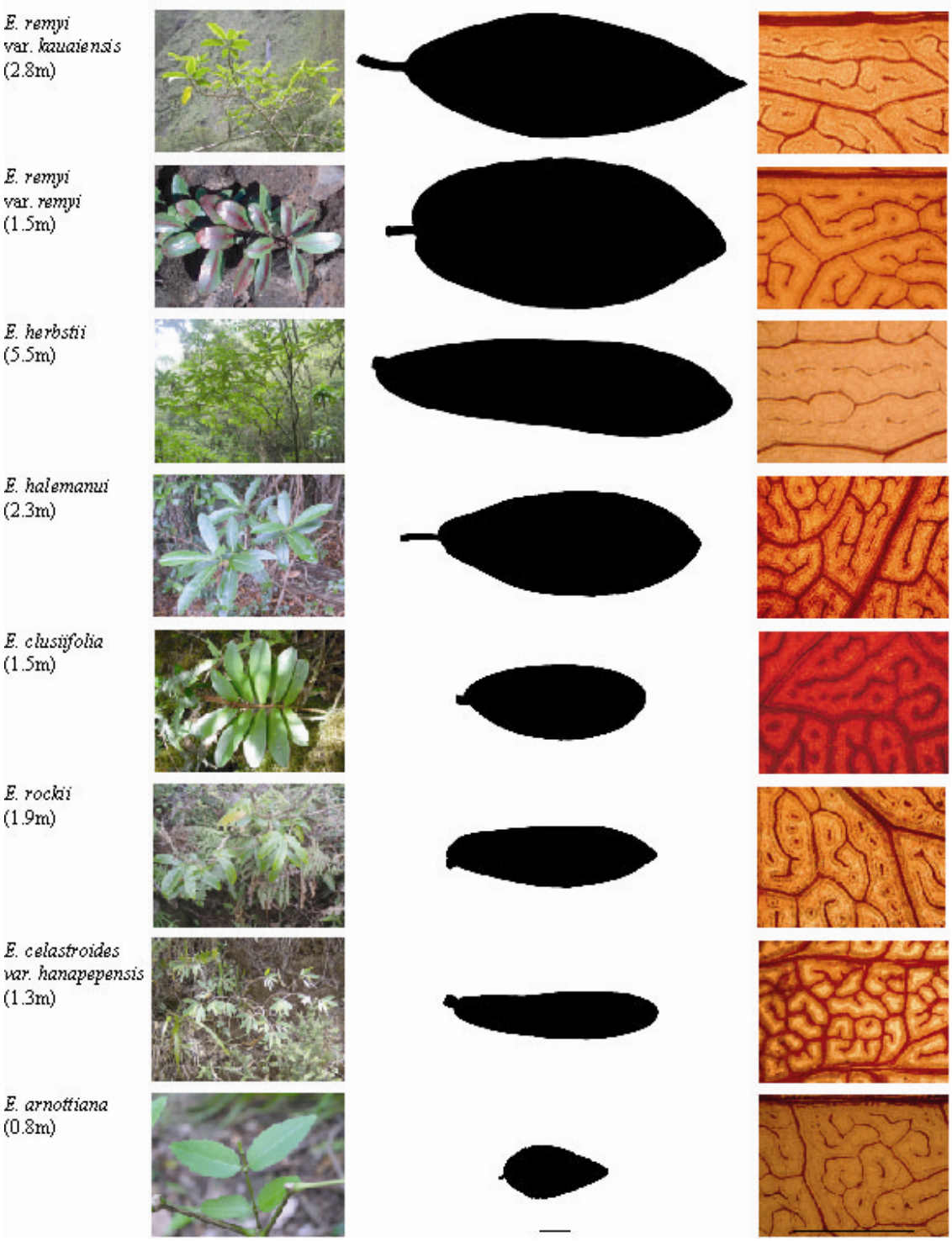

Figure 2.3. Plate showing eight of the 12 shade-establishing taxa in the study. From left to right: taxon names and the maximum height of the taxon in parenthesis, an image of the taxon in the field, a silhouette of the leaf (scale bar = 1 cm), and a light micrograph of the leaf veins of a chemically cleared and stained leaf (scale bar = 100 μm). Taxa are arranged in order of declining leaf size from top to bottom

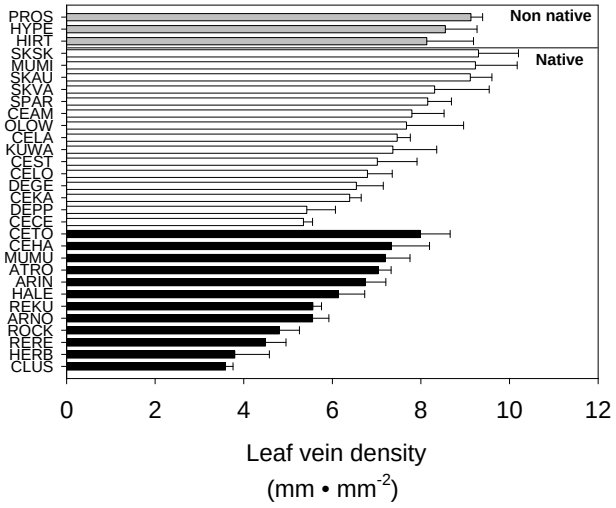

Figure 2.4. Leaf vein density for 27 open- and shade-establishing taxa of Hawaiian *Euphorbia* and three non-native weedy taxa (white, black and grey bars respectively; taxon codes as in Table 2.1). Means ± standard errors are shown; three individuals per species.

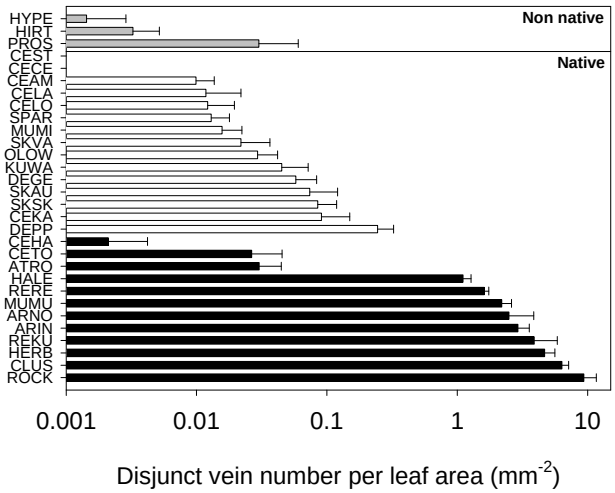

Figure 2.5. For 27 open- and shade-establishing taxa of Hawaiian *Euphorbia* and three non-native weedy taxa (white, black and grey bars respectively; taxon codes as in Table 2.1). Means ± standard errors are shown; three individuals per species. Native taxa CEST and CEAM had zero vein islands reported.

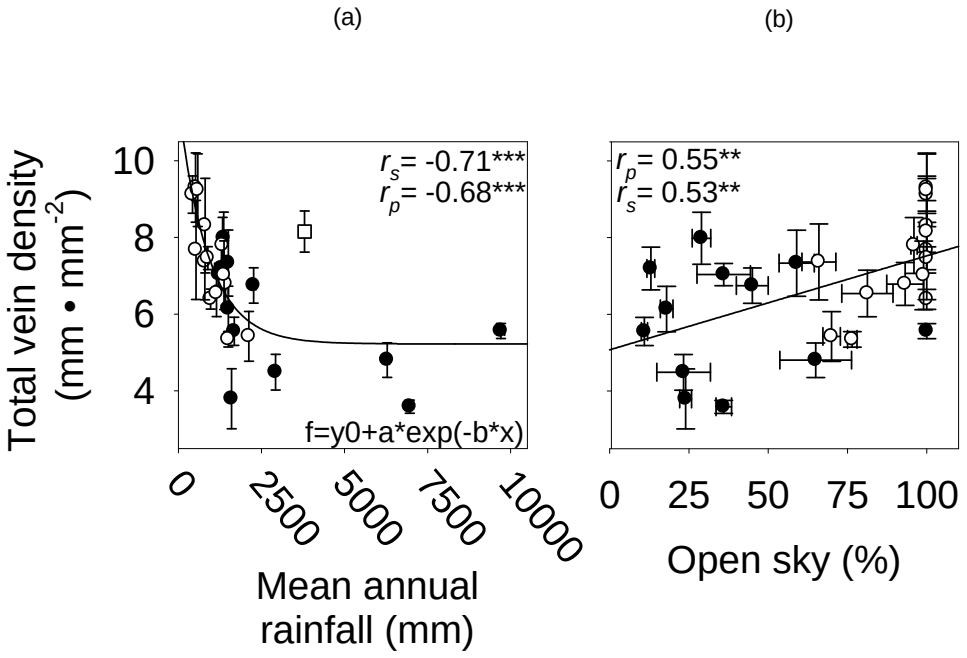

Figure 2.6. Relationships of total vein density, with native population mean annual precipitation (a) and % open sky (b) for 27 Hawaiian *Euphorbia* taxa. Symbols: closed, shade-establishing taxa; open, open-establishing taxa. In panel (a), fitted line is an exponential decline: $y = y_o + a \times e^{(-bx)}$. Parameters yo, a and b and r and P-values for fitted lines in panel (a): 5.23 ± 0.53, 6.27 ± 1.66, 0.0011 ± 0.0004 (r = 0.73; P = 0.0001). In panel (b), the fitted line is polynomial, linear: $y=y_0+ax$. Parameters yo, and a as well as r and P-values for fitted lines in panel 5.08 ± 0.60, 0.02 ± 0.008, (r = 0.53; P = 0.0049). Means ± standard errors are shown; three individuals per species.

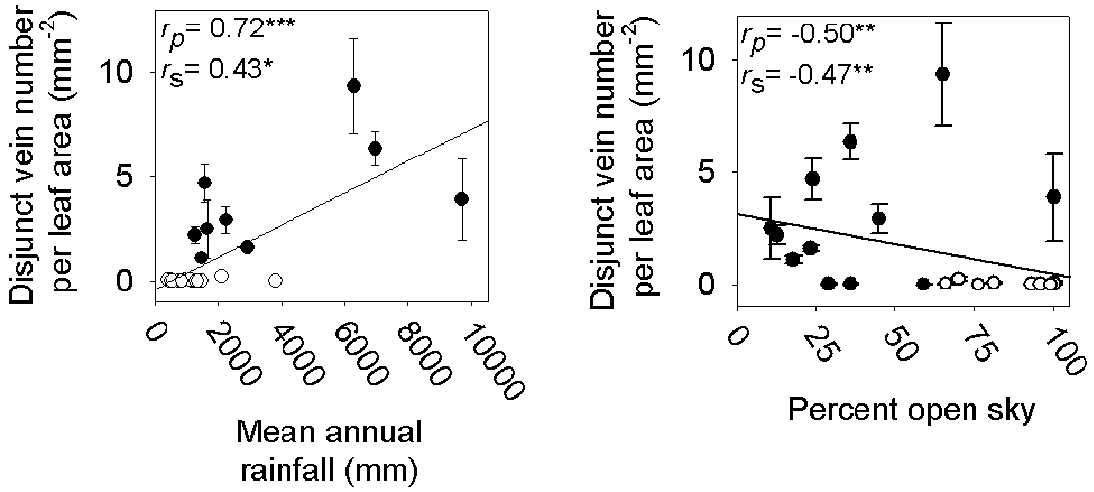

Figure 2.7. Relationships of vein island number per leaf area with mean annual precipitation for 27 Hawaiian *Euphorbia* taxa. Symbols: closed, shade-establishing taxa; open, open-establishing taxa. Means ± standard errors are shown; three individuals per species. Data indicates that the number of vein islands increases with rainfall. The fitted line is a power law: $y=ax^b$. Parameters a and b and r and P-values for fitted lines in the panel, 1.18 ± 2.64, 2.31 ± 0.54 (r = 0.000; P = 1.000). Means ± standard errors are shown; three individuals per species.

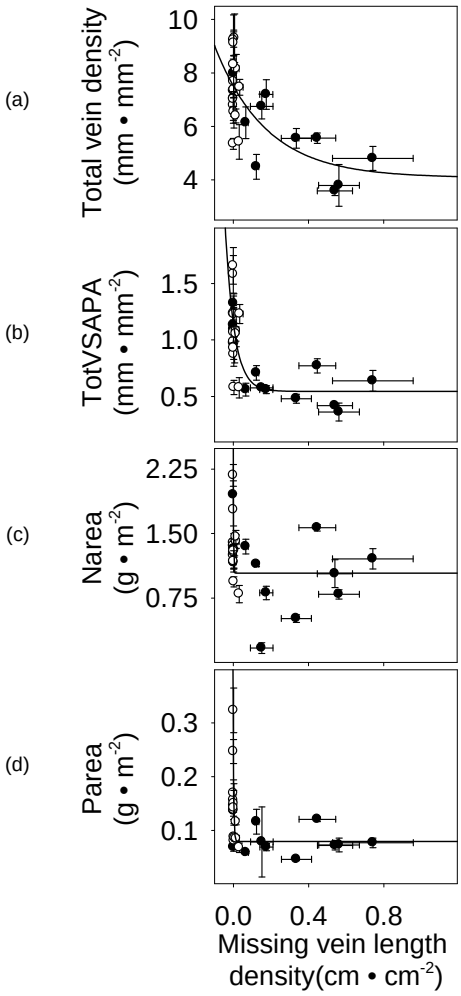

Figure 2.8. Relationships of missing vein length per leaf area with other leaf traits, total vein density (a); total vein surface area per leaf area (b); foliar nitrogen concentration per leaf area (N_{area})(c); and foliar phosphorus concentration per leaf area (P_{area}) (d) n = 27 Hawaiian *Euphorbia* taxa for total vein surface area per leaf area and total vein density and n=26 for N_{area} and P_{area} (data not available for CELA). Symbols: closed, shade-establishing taxa; open, open-establishing taxa. Declines of variables associated with leaf cost in leaves with greater abundance of vein islands in the Hawaiian *Euphorbia*. Fitted lines are exponential declines: $y = y_o + a \times e^{(-bx)}$. Parameters yo, a and b and r and P-values for fitted lines in panel (a): 4.0 ± 1.33, 3.4 ± 1.29, 3.65 ± 3.38 (r = 0.72; P = 0.0001); panel (b): 0.55 ± 0.094, 0.60 ± 0.11, 20.52 ± 13.95 (r = 0.75; P < 0.0001); panel (c) 1.04 ± 0.093, 0.76 ± 0.21, 750.82 ± 460.93 (r = 0.62; P = 0.0039); panel (d): 0.080 ± 0.014, 0.11 ± 0.023, 291 ± 198 (r = 0.66; P = 0.0013). Means ± standard errors are shown; three individuals per species.

CHAPTER 3

DETERMINANTS OF HIGHLY VARIABLE LEAF COMPOSITION IN THE HAWAIIAN *EUPHORBIA* LINEAGE

ABSTRACT

Leaf composition and anatomical traits can provide important information of plant adaptation to the environment. The native *Euphorbia* subgenus *Chamaesyce* of Hawaii are a group of C_4 eudicots that have radiated across dramatic habitat gradients from one herbaceous colonizing species into 29 endemic woody taxa within the last five million years. This lineage includes a variety of life forms, ranging from sub-shrubs a few centimeters in height, to trees over six meters tall. Members of the radiation are adapted to diverse habitats, including wet, mesic, and dry forests, bogs, and coastal zones. The leaves of the taxa in this group vary 80-fold in leaf area and eight-fold in leaf mass per area. I investigated leaf composition traits in 26 of the 29 native extant *Euphorbia* taxa. I tested for correlations across taxa of leaf composition with climate and habitat, and with leaf structural traits. I formulated multiple explicit hypotheses for how leaf trait variation should be driven by climate gradients and habitat, and found support for the majority of hypotheses. Environmental characteristics apparently play an important role in influencing leaf composition diversification. The most significant environmental factor driving leaf composition traits was irradiance level. Traits such as nitrogen isotope ratio, leaf mass per area, stem diameter, leaf density, and nitrogen (N) and phosphorus (P) concentrations per area and per mass were higher in open-establishing habitat types while chlorophyll concentration per mass and ratios of chlorophyll to N; N to P; and carbon (C) to P and height were higher in shade-establishing habitats. These findings indicate that the wide range of habitats occupied by members of the *Euphorbia* lineage were a selective factor for the diversification in leaf composition in this Hawaiian lineage.

INTRODUCTION

Leaf composition, structure, and tissue anatomy are important determinants of whole plant function because they fundamentally impact physiological processes including photosynthetic rate, relative growth rate, water balance and storage, and osmotic potential (Reich et al., 1998; Reich et al., 1998; Niinemets et al., 1999; Niinemets, 2001; Niinemets and Sack, 2006; Niinemets et al., 2007). Most recent studies pertaining to leaf dimensions and composition have focused on the ways in which composition and structure relate to plant function across diverse species (Givnish, 1987; Niinemets et al., 1999; Niinemets and Sack, 2006). Relatively few studies have focused on dramatic variation in leaf form and composition within closely related groups of species (Robichaux et al., 1990; Monasterio and Sarmiento, 1991; Dunbar-Co et al., 2009; Givnish et al., 2009; Santiago and Kim, 2009).

The Hawaiian C_4 *Euphorbia* subgenus *Chamaesyce* (family Euphorbiaceae) is one of the most noteworthy examples of adaptive radiation that is a part of the remarkable evolutionary history of the Hawaiian Islands due to chance long-distance dispersal events (Carlquist, 1967; Zimmerman, 1970; Baldwin and Wagner, 2010). The *Euphorbia* radiation in Hawaii includes 29 currently recognized taxa (Table 3.1), descended within the last five million years from a single colonizing species (Price and Clague, 2002). The seeds likely arrived by bird, as most species in this genus have small seeds form a mucous film that becomes sticky when wet and can stick to the feathers of birds (Carlquist, 1967; Koutnik, 1987; Carlquist, 1992; Jordan and Hayden, 1992; Ziegler, 2002). The taxa of *Euphorbia* subgenus *Chamaesyce* are found across the

Hawaiian Islands, and 20 of the 29 taxa are single island endemics of diverse ecology, as in other adaptive radiations in Hawaii, notably the lobeliads and the silverswords (Robichaux et al., 1990; Givnish et al., 2009; Givnish, 2010). These taxa occupy an extreme range of habitats (Table 3.1) and range widely in vegetative form and in height. There have been several studies of the Hawaiian *Euphorbia* (Herbst, 1971, 1972; Pearcy and Troughton, 1975; Robichaux and Pearcy, 1980, 1980; Pearcy et al., 1982; Robichaux and Pearcy, 1984; Pearcy et al., 1985; Koutnik, 1987; Morden and Gregoritza, 2005), as well as recent systematic study of the entire *Chamaesyce* clade, with special attention to the Hawaiian lineage (Yang and Berry, 2011), but none has quantified the variation in foliar composition across the radiation and its relationship with climate and habitat. My objective was to determine how leaf dimensions and composition have diversified across habitats, and to infer the potential importance of this diversification for plant function.

In light of previous work establishing a wide range of habitats in this lineage (Herbst, 1971, 1972; Pearcy and Troughton, 1975; Robichaux and Pearcy, 1980; Pearcy et al., 1982; Pearcy et al., 1985; Wagner et al., 1999; Morden and Gregoritza, 2005; Morden and Motley, 2005), I expected that (i) I would find large variation in elevation, (ii) irradiance, and (iii) gross plant morphological characteristics such as plant height and diameter as well as leaf area (Richardson, 2004). I hypothesized (iv) that all five of the climate traits measured (mean annual precipitation, mean annual temperature, mean annual relative humidity, vapor pressure deficit, and mole fraction vapor pressure deficit) for these 26 populations would vary substantially. Because Hawaiian forests tend to occur in cooler rainy areas, I expected (v) that open- versus shade-establishing taxa would vary in their mean climate variables. Further, because many plant composition

traits differ between open- and shade-establishing taxa (Givnish, 1988), I hypothesized that (vi) many of the leaf composition traits from this study would correlate with climate, and specifically, light.

I expected that (vii) the carbon isotope ratio ($\delta^{13}C$) would show only narrow variation because all of the Hawaiian *Euphorbia*, the *Chamaesyce* clade exhibits C_4 photosynthesis, with the NADP- malic enzyme (NADP-ME) C_4 pathway (Pearcy and Troughton, 1975; Sage et al., 1999). Thus all taxa should have values at the high end of the range for $\delta^{13}C$ in their leaf tissue (the expected range for C_4 plants is -10 to -14‰; Cerling, 1999), and that (viii) the $\delta^{13}C$ will be highest (least negative) in taxa under higher irradiance because under shade, the CO_2 concentrated by the C_4 system in the bundle sheath may leak out into the mesophyll tissue at a higher rate. While the enzyme phosphoenolpyruvate carboxylase (PEP carboxylase), that drives C_4 photosynthesis selects for greater assimilation of ^{13}C isotope by ribulose-1,5-bisphosphate carboxylase / oxygenase (Rubisco) in the bundle sheath, where greater bundle sheath leakage occurs, as is expected under shade, the enrichment of ^{13}C would be lower, leading to higher values of the ratio $\delta^{13}C$, which quantifies discrimination against ^{13}C (Farquhar, 1983; Farquhar et al., 1989; Hatch et al., 1995; Kromdijk et al., 2008). An additional explanation for variation in $\delta^{13}C$ is the "source air effect," in which plants under in shade due to natural canopies may have a lower $\delta^{13}C$ than plants in sun which are not shaded by natural canopies, if they fix CO_2 during photosynthesis that is already enriched in ^{13}C by containing a greater fraction of CO_2 that was respired by the thicker organic forest substrate (Buchmann et al., 1997; Buchmann et al., 2002; Waite and Sack, 2010; Waite and Sack, 2011).

I hypothesized that (ix) taxa with higher leaf mass per area (*LMA*), (x) higher density (*D*), and greater leaf thickness (*T*) values would be associated with exposed, drier areas receiving less rainfall. Previous studies have shown that *LMA* (= thickness × density) tends to be higher in low rainfall habitats due to the need to maintain longer-lived leaves (Castro-Diez et al., 1997, 1998; Poorter and De Jong, 1999; Hultine and Marshall, 2000; Poorter et al., 2009).

Investigation of foliar $\delta^{15}N$ can highlight sources of N, though the signal is confounded by many factors including presence of multiple potential N sources with differing isotopic composition, mycorrhizal associations (which can be difficult to quantify), variation in N availability, and temporal variability in plant N requirements (Dawson et al., 2002). Thus, foliar $\delta^{15}N$ values do not always provide clear answers about the environments in which plants occur. Based on the results of previous work, I hypothesized that (xi) $\delta^{15}N$ will be higher in habitats that have lower rainfall and higher temperature (Craine et al., 2009), however, there are many components in this process that still need further study to clearly understand the $\delta^{15}N$ accumulation in leaves. Factors such as dependence on mycorrhizal fungi, changes in N cycling within plants, and gaseous N loss are among the many variables that can influence foliar N isotopic signature (Dawson et al., 2002; Craine et al., 2009).

I hypothesized that (xii) N_{area} would be higher for taxa in habitats of higher irradiance, associated with lower rainfall, since N_{area} is aligned with *LMA* and higher photosynthetic rate per leaf area (Chapin et al., 1987; Ellsworth and Reich, 1992), and that (xiii) N_{mass} would also increase with increasing irradiance given it contributes to higher photosynthetic rate per mass and relative growth rate, typically selected in high

irradiance environments and that N_{mass} would be negatively correlated with *LMA*. I hypothesized (xiv) that P_{area} and (xv) P_{mass} would show these same trends (Givnish, 1988; Cornelissen et al., 1997; Wright et al., 2005). I hypothesized (xvi) that C_{mass}, *C:N*, and *C:P* would show the opposite trend as N_{mass} and P_{mass} because these nutrients have been shown to be inversely correlated to carbon (C) allocation, as more C usually means greater dilution by cell wall of protein-rich photosynthetic components. Further, having a lower leaf C relative to N would give plants an advantage in relative growth rate (Williams et al., 1989; Niinemets, 1997; Chang et al., 2011).

I also expected (xvii) that Chl_{area} would correlate positively with higher irradiance across habitats due to the higher potential for light capture in such an environment, and the advantage of thicker leaves with more layers of mesophyll cells and thus higher Chl_{area} (Dunbar-Co et al., 2009). By contrast, I expected (xviii) taxa of low irradiances to have higher Chl_{mass} to contribute to greater light harvesting efficiency per tissue mass allocation (Givnish, 1988; Monasterio and Sarmiento; Minotta and Pinzauti, 1996; Dunbar-Co et al., 2009). I hypothesized (xix) that foliar *Chl:N* would be higher in habitats of low irradiance since it is a shade adaptive trait, representing greater allocation to light than to carbon reactions for photosynthesis (Givnish, 1988). I hypothesized (xx) that *N:P* would correlate negatively with rainfall and irradiance because in productive environments, plants may assimilate more P relative to N due to greater RNA production to achieve faster growth rates (Elser et al., 2000; Pasquet-Kok et al., 2010). I hypothesized (xxi) that multiple leaf composition traits important to plant function would show significant differences between open- and shade-establishing taxa as has been the case in previous studies (Givnish, 1988). I hypothesized (xxii) that plant height would be

correlated with environmental factors, and in particular, to be positively correlated with rainfall (Fonseca et al., 2000).

METHODS

Taxa, sites and collection of material

Leaf samples were collected from 26 native taxa (of 29), each from a unique population, on the five high islands of Hawaii, Kauai, Maui, Molokai, and Oahu (Figure 3.1). Habitat types were categorized as coastal, dry, mesic, wet, or bog, and non-native weeds were considered in a separate category (Table 3.1). For taxa that occur in multiple populations, I sampled from one population of characteristic habitat, and selected healthy and reproductively mature plants of typical size for the population. Several taxa exist only in remote locations with difficult access, including rare and endangered taxa and single island endemics (Table 3.1). I sampled leaves from *E. celastroides* vars. *celastroides* and *lorifolia* growing in cultivation at the National Tropical Botanical Garden (NTBG) on Kauai, and for *E. herbstii* I sampled out-plantings on Oahu. I recorded plant height, diameter of the main stem 10 cm from the base, approximate leaf number, as well as the soil type, the surrounding vegetation, and a visual estimate of percent open sky. The percent open sky (equivalent to 100% minus the "canopy closure" *sensu*; Jennings et al., 1999), was assessed to the nearest 5%. Visual canopy cover estimates correlate with measurements using a densiometer or hemispherical photography (Korhonen et al., 2006; Paletto and Tosi, 2009). I recorded the elevation and coordinates of each sampling location using a Global Positioning System (Garmin 60CSx Garmin, Kansas City). For each sampled population, I determined modeled values for mean annual relative

humidity, temperature, and rainfall (*MARH*, *MAT*, and *MAP*, respectively) using the Hawaii Digital Climate Map System in ArcGIS (Giambelluca and Cuo, unpublished work). The vapor pressure deficit (*VPD*), is a measure of atmospheric drought, the driving force for evaporation, and can be quantified as an absolute pressure difference (*VPD*, in kPa), or as a mole fraction normalized by atmospheric pressure (*MFVPD*). I calculated *VPD* as VP_{sat} - (*MARH* × VP_{sat}), where VP_{sat} was determined from published tables for the given *MAT* (Campbell and Norman, 1998; *Prometheus Wiki* contributors, 2008). Across the study populations, the two *VPD* measures (*VPD* and *MFVPD*) were highly correlated (R^2=0.998; $P < 0.001$); I present results for both but discuss in the text correlations with absolute *VPD*.

Access and collection permits were obtained from the Department of Land and Natural Resources Division of Forestry and Wildlife, including threatened and endangered species research permits for seven taxa (Table 3.1) or I sampled with permit holders, in the case of Army and The Nature Conservancy managed lands. I collected leaves from five individuals of each taxon, three in the cases of *E. arnottiana* var. *integrifolia*, and *E. remyi* var. *remyi*. Fully exposed leaves were selected from the most recent mature flush, several nodes proximal to the apex. Leaves were transported in plastic bags to the laboratory for processing.

Leaf traits: dimensions and composition

I measured mean leaf area (*LA*) for three leaves from three plants per taxa. Measurements were made using Image J (Image J software, http://rsbweb.nih.gov/ij/). Leaves were oven dried for over 48 hrs at > 70ºC before measuring dry mass for calculation of leaf mass per area (*LMA*; leaf area / dry mass).

I measured chlorophyll concentration per area on fresh leaves (Chl_{area}) using a SPAD-502 meter (Minolta Co., Japan), averaging two measurements for each of three to five leaves per taxon. Nutrient composition was determined for three to 15 leaves per taxon. Dried leaves were ground into a fine powder in a Wiley mill with mesh size 20, and analyzed for concentrations of carbon, nitrogen and phosphorus per mass (C_{mass}, N_{mass} and P_{mass}, respectively), and for carbon isotope ratio ($\delta^{13}C$) and nitrogen isotope ratio ($\delta^{15}N$) using high temperature combustion in an elemental analyzer (Costech ECS 4010; Valencia, CA, USA), with effluent passed into a continuous flow isotope ratio mass spectrometer (ThermoFinnigan Delta V Advantage with a Conflo III interface; Thermo Fisher Scientific; Waltham, MA, USA; Fry et al., 1996) Samples were dry ashed in glass vials (Miller, 1998), dissolved in 1M HCL and analyzed for P_{mass} using inductively-coupled plasma-optical emission spectrometry (Varian Vista MPX Instrument, Varian InE., Palo Alto, CA USA; Porder et al., 2005). Chlorophyll per mass (Chl_{mass}) was calculated as Chl_{area} divided by *LMA*; concentrations of nitrogen and phosphorus per area (N_{area} and P_{area} respectively) were determined as N_{mass} and P_{mass} multiplied by *LMA*. Chlorophyll: nitrogen ratio (*Chl:N*) was calculated as Chl_{area}/N_{area}.

Five leaves per taxon were stored in FAA (37% formaldehyde, glacial acidic acid, 95% ethanol, and deionized water in a 10:5:50:35 mixture). I measured leaf thickness (*T*) for these leaves midway along the leaf between midrib and margin (using digital calipers; model 14-648-17, Fisher Scientific).

Note: Measurement methods for stomatal and epidermal anatomy are described in Chapter 1, and for leaf venation traits in Chapter 2.

Statistical Analysis

For each trait, I used nested analyses of variance (ANOVAs) to test differences among taxa, vegetation type categories, and open- versus shade-establishing taxa (Table 3.2). I used two nested ANOVAs, firstly nesting taxon within vegetation type category, and secondly nesting taxon within establishment category (open versus shade). All analyses were performed using Minitab Release 15 (Minitab, State College, PA). I performed each ANOVA with and without including the three naturalized weed species. Data were log-transformed before ANOVAs to increase normality and homoscedasticity (Sokal and Rohlf, 1995; Zar, 1999). Evaluation of a priori hypothesized trait-trait and trait-environment relationships was done using Pearson and Spearman coefficients (r_p and r_s respectively). I additionally prepared a correlation matrix to reveal the inter-correlative structure of the traits (Appendix A3-1; Givnish et al., 2004; Edwards, 2006). Bonferroni correction should be implemented before "mining" for trait correlations that were not hypothesized, given the danger of an inflated false discovery rate (Garcia, 2003; Moran, 2003). In this work, trait correlations were considered a priori, thus data mining was not a consideration.

I conducted a principal component analysis (PCA; e.g., Sokal and Rohlf, 1995) for a set of 15 leaf traits. Data analysis and was conducted and graphic for Figure 3.1 was created using R computer programming language (Crawley, 2007), identify the key axes of covariation among traits and the traits that align with these key axes. Significantly correlated traits were not included in the PCA.

RESULTS

Analysis of variance for leaf composition traits across taxa

The 26 native Hawaiian *Euphorbia* taxa studied varied dramatically in climate variables, plant size, and leaf composition. Population elevation varied from 10-1695m across taxa and was lowest for taxa of coastal habitats and highest for wet forest taxa. The percent open sky varied 9-fold across taxa and was lowest for mesic forest taxa and highest for *E. sparsiflora* of bog habitat followed by taxa of coastal habitats. Three out of five climate traits (*MAP*, *VPD* and *MFVPD*) varied by more than twofold across taxa. The *MAP* varied 23-fold across taxa and was lowest for dry forest taxa and highest for wet forest taxa. The *MAT* varied 1.8-fold and was lowest for wet forest taxa and highest for coastal taxa, whereas the *MARH* varied 1.2-fold, and was lowest for coastal taxa and highest for wet forest taxa. The *VPD* and *MFVPD* varied by 2.4- to 2.8-fold and were lowest for wet forest taxa and highest for coastal taxa.

Plant size varied greatly across taxa. Plant diameter near the base varied 78-fold across taxa and was lowest for *E. sparsiflora* of bog habitat, followed by mesic forest taxa, and was highest on average for wet forest taxa. Plant height varied 135-fold across taxa, and was lowest for *E. sparsiflora* of bog habitat followed by taxa of coastal habitats, and was highest on average for wet forest taxa, with 4.7 m (*E. olowaluana*) of dry forest habitat having the tallest observed individuals in the field.

For 14 of 15 the leaf composition traits, there was two-fold or greater variation among taxa. The following relationships are in the order listed in Table 3.2. The *LMA* varied 8.2-fold across taxa and was lowest in mesic forest taxa and highest in dry forest taxa. Leaf *D* varied 5.3-fold across taxa and was lowest for wet forest taxa and highest for

dry forest taxa. Leaf Chl_{area} varied 2.6-fold across taxa and was lowest for dry forest taxa and highest for *E. sparsiflora* of bog habitat. The Chl_{mass} varied 5.2-fold across taxa, and was lowest for dry forest taxa and highest for *E. sparsiflora* of bog habitat followed by the mesic forest taxa. Leaf *Chl:N* varied 6.4-fold across taxa and was lowest for dry forest taxa and highest for mesic forest taxa. Leaf $\delta^{15}N$ varied 2.6-fold across taxa and was lowest in *E. sparsiflora* of bog habitat followed by taxa of wet forest taxa habitats, and highest for coastal taxa. The leaf $\delta^{13}C$ composition varied from -12.0 to -14.6 ‰ across taxa and was lowest for wet forest taxa and highest for coastal taxa. The P_{area} varied 7-fold across taxa and was lowest for *E. sparsiflora* of bog habitat followed by wet forest taxa and highest for dry forest taxa. Leaf P_{mass} varied 7-fold across taxa, lowest for wet forest taxa and highest for dry forest taxa. The leaf C_{mass} varied 5.2-fold across taxa and was lowest for mesic forest taxa and highest for dry forest taxa. The *N:P* varied 4.1-fold across taxa and was lowest for dry forest taxa and highest for *E. sparsiflora* of bog habitat followed by wet forest taxa. The *C:N* varied 3.9-fold across taxa and was lowest *E. sparsiflora* of bog habitat followed by coastal taxa and highest for dry forest taxa. The *C:P* varied 6.6-fold across taxa and was lowest for coastal taxa and highest for wet forest taxa.

Correlation of leaf composition traits and plant height with environment

Across taxa, several leaf composition traits were related to plant size and environmental traits. Composition traits N_{area}, $\delta^{15}N$, $\delta^{13}C$, and P_{area} were positively correlated with % open sky ($|r_s|$ and $|r_p|$ =0.33 - 0.67; $P < 0.05$) whereas *Chl:N* was negatively correlated ($|r_s|$ and $|r_p|$ = 0.43 - 0.65; $P < 0.05$). Plant height was positively correlated with elevation and *MARH* and negatively with *MAT*, *VPD* and *MFVPD* ($|r_s|$ and $|r_p|$ = 0.41 - 0.60; $P <$

0.05), whereas leaf $\delta^{15}N$ concentration was negatively correlated with elevation and *MARH* and positively with *MAT, VPD* and *MFVPD* ($|r_s|$ and $|r_p|$ = 0.39 - 0.65; $P < 0.05$). Leaf *D* and $\delta^{15}N$ were negatively correlated with *MAP* ($|r_s|$ and $|r_p|$ = 0.44 - 0.64; $P < 0.05$).

Variation among taxa establishing in open versus shade

All but four traits varied significantly between open- and shade- establishing taxa. Thus, elevation varied from 408 to 784 m between open- and shade establishing taxa respectively, and exposure varied 2.1-fold, and $\delta^{15}N$ varied 8.7-fold. For ten climate variables and plant traits open-establishing taxa had 1.1-1.7-fold higher values: *MAT*, *VPD*, *MFVPD*, stem diameter, *LMA*, *D*, N_{area}, N_{mass}, P_{mass} , and P_{area}. For seven climate variables and plant traits, shade-establishing taxa had 1.1 to 2.4-fold greater values: *MAP*, *MARH*, plant height, Chl_{mass}, *Chl:N*, *N:P*, and *C:P*. Open- and shade-establishing taxa did not differ significantly in C_{mass}, Chl_{area}, *C:N*, and $\delta^{13}C$.

Principal components analysis

The first two axes of the PCA explained 24.8% and 20.2%, respectively, of the variation in 15 key traits (Table 3.4). Axis one was aligned with traits associated with irradiance (correlations with PC axes run with log-transformed data, correlation of axis 1 with % open sky; $r = 0.49$; $P = 0.01$), while axis two aligned with traits associated with leaf mass per area ($r = -0.90$; $P < 0.001$; Figure 3.1). Additionally, taxon scores for PC1 differed between open- and shade-establishing taxa (*t*-test; $P < 0.001$); scores for PC2 did not differ between open- and shade-establishing taxa ($P = 0.14$). PC1 also correlated negatively with *MAP* ($r = -0.58$; $P = 0.002$) but not with other environmental variables,

i.e., elevation, *MAT*, *MARH VPD*, or *MFVPD* ($|r| = 0.22$ - 0.30; $P = 0.11 – 0.26$). The PC2 did not correlate with any environmental traits.

DISCUSSION

The 26 *Euphorbia* taxa varied significantly in plant size and leaf composition. Thus, 14 of 15 composition traits had two-fold or greater variation across taxa. Differences among species likely represent the combination of adaptation and plasticity. A common garden study was previously done on a subset of these taxa in which genetic variation was confirmed (Robichaux and Pearcy, 1980, 1984).

Because the Hawaiian *Euphorbia* include diverse life-forms subject to a broad range of habitats (Wagner et al., 1999), I expected that (i) I would find taxa across a wide range of elevation and levels of irradiance (ii), and that (iii) taxa would show significant size differences. Indeed, taxa ranged across 10-1695m in elevation, and irradiance varied 9-fold from mesic forest to bog followed by coastal taxa, and plant basal diameter and height varied nearly 80-fold and 135-fold respectively, with the smallest plants in bog and coastal habitats, and the largest on average in west forests. The increase in plant size from habitats of low to high *MAP* is consistent with a trend toward taller woody forms as competition for light and space increases under the closed canopy (Givnish, 1999).

I hypothesized (iv) that the five climate variables measured for these 26 taxa would vary significantly. This hypothesis was partially supported. Mean annual precipitation varied by nearly 23-fold variation across habitats, from the population of *E. skottsbergii* var. *audens* at the driest site, coastal west Molokai to *E. remyi* var. *kauaiensis* at the wettest site, “Blue Hole” in central Kauai. Additionally the *VPD* and *MFVPD*

varied 2.4-2.8-fold across taxa from wet forest taxa to taxa of coastal habitats. The *MAT* varied 1.8-fold across taxa, from wet forest to coastal taxa; notably, most of the wet forest sites occurred at higher elevation. The *MARH* varied 1.2-fold across taxa, from coastal habitats to wet forest.

I tested for differences in open- versus shade- establishing taxa for the composition and environment traits. I hypothesized (v) that open- versus shade-establishing taxa would vary in mean climate. There was a 376 m difference in average elevation between open- versus shade-establishing taxa, with open- establishing taxa occurring at higher elevations on average. This result was apparently driven by the two highest-elevation taxa in the study, *E. olowaluana* and *E. multiformis* var. *microphylla*, which inhabited an open canopy dry-forest in the saddle between Mauna Loa and Mauna Kea on Hawaii Island. The *MAT*, *VPD*, and *MFVPD* were higher for the sites of open-establishing taxa, as expected for drier exposed habitats. The *MAP* and *MARH* were higher for the sites of shade- establishing taxa, as expected given the association of denser forest canopy with moister climates.

I hypothesized (vi) that leaf composition traits would be significantly correlated with environmental factors. Indeed, N_{area}, $\delta^{15}N$, $\delta^{13}C$, and P_{area} were positively correlated with % open sky while *Chl:N* was negatively correlated. The positive relationships of N_{area} and P_{area} to % open sky were consistent with an increased biochemical allocation to photosynthesis and metabolism under greater irradiance (Givnish, 1988; Niinemets, 2001). The relationships of $\delta^{15}N$ and $\delta^{13}C$ to % open sky were also consistent with previous studies in the literature. Variation in $\delta^{13}C$ values across taxa were highest for drier, open habitat types, possibly due to bundle sheath leakage or due to (Farquhar et al.,

1989). A negative relationship of *Chl:N* with % open sky was expected, reflecting greater allocation to chloroplast light reactions relative to carbon fixation reactions for plants under greater shade. The $\delta^{15}N$ values were negatively correlated with elevation, *MAP* and *MARH*, and positively correlated with *MAT*, *VPA* and *MFVPD*. These relationships were also expected since the low elevation habitats considered in this study are usually the hottest, driest, and least productive, with highest *MAT* and soils richest in chemical nutrients (Dawson et al., 2002). Leaf density had a negative relationship with *MAP* which was expected, as in many vegetation systems plants adapted to reduced water availability have thicker and denser leaves (Lamont et al., 2002).

I hypothesized (vii) that one of the 15 composition traits, $\delta^{13}C$, would show narrow variation across the taxa. Indeed, taxa varied from only -14.5, to -12.0 ‰, consistent with all being C_4 photosynthetic. The ubiquity of C_4 photosynthesis is exceptional because typically C_4 plants are grasses or shrubs adapted to open, dry areas, whereas several of the Hawaiian *Euphorbia* subgenus *Chamaesyce* several have tree form, and the lineage includes and shade- and wet forest-adapted taxa (Pearcy and Troughton, 1975). The $\delta^{13}C$ range for all terrestrial plants is approximately -10 to -35‰, however, C_4 plants are narrowly represented at the high end of that range -10 to -14‰ (Cerling, 1999). The taxon with the highest $\delta^{13}C$ value was *E. deppeana* of the mesic habitat, at -12.0‰ and the lowest was *E. skottsbergii* var. *skottsbergii* of dry forest, at -14.6‰. I hypothesized that (viii) if there was variation in $\delta^{13}C$ values across the Hawaiian taxa the values would be highest for drier, open habitat types due to bundle sheath leakage or the "source air effect" in deeper shade (see Chapter 0 Introduction). This hypothesis was supported, as, on average, the habitat type with the lowest (most

negative) $\delta^{13}C$ values was the wet forest and the highest (least negative) values was the coastal habitat (Farquhar et al., 1989).

I hypothesized (ix and x) that *LMA, D*, and *T* would be higher in dry habitat types. Indeed, the *LMA* varied eight-fold across taxa and was lowest for taxa of mesic habitats and highest for taxa of dry forest habitats. This great variation in *LMA* was consistent with the taxa being adapted to a wide range of habitat types and irradiance (Cordell et al., 1998). I expected lower *LMA* for taxa of high rainfall, corresponding to shorter-lived leaves with less water storage tissue. Taxa that occur in habitats of low rainfall would tend to have higher *LMA* because of the need for longer-lived leaves with more water storage tissue, better adapted to xeric environments (Poorter et al., 2009). Leaf density and thickness are determinants of *LMA* (Castro-Diez et al., 2000). On average *D* varied more than 5-fold across taxa and like *LMA* was lowest for wet forest taxa and highest for dry forest taxa and *T* varied 1.7-fold from dry to wet forest taxa.

The leaf $\delta^{15}N$ varied significantly across the *Euphorbia* taxa and across habitat types. I hypothesized that (xi) foliar $\delta^{15}N$ values would be negatively correlated with *MAP* and positively correlated with *MAT* due to the probably lower N availability at high precipitation sties (Craine et al., 2009). This prediction was supported, as the foliar $\delta^{15}N$ values were lowest in bog followed by wet forest habitats and highest for taxa of coastal habitats.

Notably, N_{area} is equal to N_{mass} divided by *LMA*, and I hypothesized that (xii) N_{area} would be positively correlated with *LMA* while (xiii) N_{mass} would be negatively correlated with *LMA*, Additionally, I expected that (xiv) P_{area} and (xv) P_{mass} would change in the same direction as *N* concentrations given they both are associated with higher

photosynthetic rates that tend to occur in habitats of higher irradiance (Givnish, 1988; Wright et al., 2005; Wright et al., 2001; Petritan et al., 2010). These hypotheses were supported, as *N* and *P* concentrations were inter-correlated and varied 4- to 13-fold across taxa, from mesic and wet forest to dry forest and coastal taxa. I hypothesized (xvi) that C_{mass}, *C:N*, and *C:P* would be negatively related to N_{mass} and P_{mass}. My findings did not support expectation, as C_{mass} was not significantly correlated with *LMA*, and *C:N* and *C:P* were positively correlated with *LMA*. The reason for this lack of positive correlation is unknown, however, it is possible that the wide variety of plant tissue types present across this lineage (thick water storage tissue for some taxa, and large areas of airspace for others) could cause extremely variable carbon composition and influence relationships with *LMA* and other traits (Sack L, Pasquet-Kok J, Sporck, MJ, pers. ob.)

Across taxa Chl_{area} and Chl_{mass} were highly variable ranging from 2.6- to 5.2-fold. I hypothesized that (xvii) Chl_{area} would correlate positively and (xviii) Chl_{mass} would correlate negatively with irradiance. Only one of these hypotheses were supported as both traits exhibited lowest values in the exposed conditions of dry forest and highest values in the bog, with the shaded habitats of wet and mesic forests habitat as second highest. This pattern is consistent with the taxa in low light better harvesting available irradiance by allocating to greater chlorophyll per leaf area and per mass (Givnish, 1988). Foliar *Chl:N* was hypothesized to be (xix) higher in habitats of low irradiance (Givnish, 1988), since *Chl:N* is a shade-adaptation trait, reflecting investment in light capture (Chl) versus CO_2 capture, as most N in the leaves is found in the enzyme Rubisco. Thus, taxa with highest *Chl:N* occurred in mesic forest taxa, whereas taxa with lowest values occurred in the dry forest. I hypothesized (xx) that *N:P* would correlate negatively with rainfall and

irradiance because in such productive environments, plants would produce more RNA, rich in *P* relative to *N*, to achieve greater growth rates (Elser et al., 2000; Pasquet-Kok et al., 2010). This hypothesis was supported as the *N:P* in the leaf varied 4.1-fold across taxa and was lowest for dry forest taxa and highest for taxa of bog habitats followed by taxa from wet forests. Notably, this pattern may better reflect the relative poverty of P in the bog and wet, shaded forest habitat than biochemical allocation to higher growth rates for taxa in these habitats.

The results of previous work have shown many plant composition traits to be widely variable from open to shade establishing, including *N*, *P*, and chlorophyll concentration (Givnish, 1988). I hypothesized (xxi) that many of the leaf composition traits in this study would differ significantly between open and shade-establishing taxa. My results supported this expectation. The leaf composition trait with highest variation was $\delta^{15}N$, which varied more than 8-fold and was highest for open-establishing taxa and lowest for shade establishing taxa. This pattern may reflect the variation in nutrient cycling across a variety of habitat types (Vitousek and Sanford, 1986). Open establishing taxa also exhibited higher values for *LMA*, stem diameter, *D*, N_{area}, N_{mass}, P_{mass}, and P_{area}. These findings were consistent with results of previous studies of plants in other systems that showed that leaves of plants that establish in sun tend to be thicker and denser with greater accumulation of photosynthetic compounds than leaves of plants that establish in shade (Givnish, 1988; Ashton and Berlyn, 1994; Niinemets et al., 1998; Niinemets, 2001). By contrast, shade-establishing taxa had higher values for Chl_{mass}, *Chl:N*, *N:P*, *C:P*, and height while Chl_{area} was not significantly correlated with open or shade-establishing habitats. These findings are consistent with shade-establishing species

allocating more resources to light capture, and also to occupying habitats relatively poor in P. The PCA analysis showed that the first axis, which is most important in explaining the variation (Manly, 1994) reflected traits that are aligned with irradiance, and the second axis reflected traits aligned with *LMA*. The clustering of *LMA*-related traits indicates that many leaf traits are closely coupled and may change in correlation during evolution (Dunbar-Co et al., 2009; Table 3.4, Figure 3.1 - Figure 3.3).

I hypothesized (xxii) that plant height would be correlated with environmental factors. In the Hawaiian *Euphorbia*, plant height was indeed positively correlated with elevation and negatively with *MAT*. The highest elevation sites in which tree-form *Euphorbia* occurred in Hawaii tend to be rainforest sites (with the exception of *E. olowaluana* and *E. multiformis* var. *microphylla*). The negative correlation of height with *MAT* would thus be expected (Oleksyn et al., 1998). Additionally, plant height correlated positively with *MARH* and negatively with *VPD* and *MFVPD*. Because I was looking at what are likely genetically determined differences between the taxa this relationship is probably due to the tendency for plants of continuous forest to be taller and woody as to compete for canopy light (Givnish, 1999).

Overall, this study supported the importance of climate and environmental factors, and in particular, adaptation to contrasting irradiance as a determinant of differences in plant size and leaf composition in the Hawaiian *Euphorbia*, subgenus *Chamaesyce*. This trait variation, aligned with climate factors, arose in tandem with speciation within a short evolutionary time.

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TABLES

Table 3.1. List of Hawaiian *Euphorbia* taxa included in study, island of sampled population, maximum height observed in the field, habitat type ("Wet", "Mesic" and "Dry" were determined according to moisture regimes and elevation bands for coastal taxa, following Wagner et al., 1999, with an additional "Bog" specification for SPAR, which occurs exclusively in Wahiawa bog on Kauai. Federal conservation status: ***Endangered, **Species of concern; *Recommended for candidacy as endangered. Island abbreviations: H, Hawaii, Kah, Kahoolawe, Kau, Kauai, L, Lanai, Ma, Maui, Mo, Molokai, O, Oahu, †Not included in study due to lack of current knowledge of accessible populations.

Taxon	Authors of authority	Code name	Location	Islands (sampled island pop. in bold)	Max ht. (m)	Habitat	Open or Shade
Hawaiian taxa							
E. arnottiana var. *arnottiana***	(Endl.) O. Deg. & I. Deg.	ARNO	Aina Haina,	**O**	0.91	Mesic	Shade
E. arnottiana var. *integrifolia*	Hillebrand	ARIN	Kapuuakea,	**Ma**	0.43	Mesic	Shade
E. atrococca	(A. Heller) Croizat & O. Deg.	ATRO	Makaha Ridge	**Kau**	3.17	Dry	Shade
E. celastroides var. *amplectens*	(Sherff) O. Deg. & I. Deg.	CEAM	Hawaii Loa Ridge	**O**, all main	1.62	Dry	Open
E. celastroides var. *celastroides*	(Boiss.) Croizat & O. Deg.	CECE	NTBG	**Kau**	1.95	Dry	Open
E. celastroides var. *hanapepensis*	(Sherff) O. Deg. & I. Deg.	CEHA	Halemanu Rd. Kokee	**Kau**	1.61	Wet	Shade
E. celastroides var. *kaenana****	(Sherff) O. Deg. & I. Deg.	CEKA	Makua Valley	**O**	1.70	Coast	Open
E. celastroides var. *laehiensis*	(O. Deg., I. Deg. & Sherff) Koutnik	CELA	Waiopai	**Ma,** L	0.10	Coast	Open
E. celastroides var. *lorifolia***	(A. Gray) O. Deg. & I. Deg.	CELO	NTBG	**Kau**, Ma, L	5.30	Dry	Open
E. celastroides var. *stokesii*	(C. N. Forbes) O. Deg. & I. Deg.	CEST	Kilauea Point	**Kau**, Mo	1.13	Coast	Open
E. celastroides var. *tomentella***	(Boiss.) Koutnik	CETO	Waianae Kai	**O**	0.99	Mesic	Shade
*E. clusiifolia***	(Hook. & Arn.) Arthur	CLUS	Poamoho trail	**O**	2.44	Wet	Shade
E. degeneri	(Sherff) Croizat & O. Deg.	DEGE	Secret Beach	H**, Kau,** Ma, Mo, O	0.19	Coast	Open
*E. deppeana****	(Boiss.) Millsp.	DEPP	Pali Lookout	**O**	0.24	Mesic	Open
*E. eleanoriae***†	Lorence & W. L. Wagner	ELEA	Napali	**Kau**			
*E. halemanui****	(Sherff) Croizat & O. Deg.	HALE	Halemanu Rd. Kokee	**Kau**	3.18	Wet	Shade
*E. herbstii****	W. L. Wagner	HERB	Makaha Valley	**O**	0.91	Wet	Shade
*E. kuwaleana****	(O. Deg. & Sherff) O. Deg. & I. Deg.	KUWA	Kauaopuu Ridge	**O**	0.50	Dry	Open
E. multiformis var. *microphylla*	(Boiss.) O. Deg. & I. Deg.	MUMI	PTA	**H,** Ma, Mo, O	2.01	Dry	Open
E. multiformis var. *multiformis*	(Hook. & Arn.) Croizat & O. Deg	MUMU	Pahole	Ma, **O**	1.14	Mesic	Shade
*E. olowaluana***	(Sherff) Croizat & O. Deg.	OLOW	PTA	**H**	6.15	Dry	Open
E. remyi var. *hanaleiensis*†	(Sherff) O. Deg. & I. Deg.	REHA	Hanalei	**Kau**		Wet	Shade
E. remyi var. *kuaiensis**	(O. Deg. & Sherff) O. Deg. & I. Deg.	REKU	Blue Hole	**Kau**	3.12	Wet	Shade
E. remyi var. *remyi**	(A. Gray ex Boiss.) Croizat & O. Deg.	RERE	Kokee	**Kau**		Wet	Shade
*E. rockii****	(C. N. Forbes) Croizat & O. Deg.	ROCK	Koolau Summit Trail	**O**	2.36	Wet	Shade

Taxon	Authors of authority	Code name	Location	Islands (sampled island pop. in bold)	Max ht. (m)	Habitat	Open or Shade
E. skottsbergii var. *audens*	(Sherff) O. Deg. & I. Deg.	SKAU	W.Molokai	**Mo**	0.04	Coast	Open
E. skottsbergii var. *skottsbergii****	(Sherff) Croizat & O. Deg.	SKSK	Ewa Plain	Mo, **O**	1.04	Dry	Open
E. skottsbergii var. *vaccinioides***	(Sherff) Koutnik	SKVA	Central Molokai	Kah, Ma, **Mo**	1.28	Dry	Open
*E. sparsiflora***	(A. Heller) Koutnik	SPAR	Kanaele Bog	**Kau**	0.91	Bog	Open
Weed species							
E. hirta	(L.) Millsp.	HIRT	Manoa	**O**, all main	0.37	Weed	Open
E. hypericifolia	(L.) Millsp.	HYPE	Manoa	H, Kau, **O**, Ma	0.29	Weed	Open
E. prostrata	(Aiton)	PROS	Manoa	H, Kah, Kau, **O**, Ma, L	0.11	Weed	Open

Table 3.2. The minimum, mean, and maximum values for composition traits, field traits, and climate traits for native Euphorbia taxa and mean values +/- standard error for the weedy taxa. The vegetation type for the low and high value (B=bog, C=coastal, D=dry forest, M=mesic forest, W=wet forest), and for traits with a low or high value as bog, the habitat type representing the second highest or lowest value is included because there was only one bog taxa. Nested ANOVA significance results are presented, for taxon nested within habitat and taxon nested within open vs. shade-establishing and the mean +/- standard error for open, and shade taxa, all for native taxa; *P*-values for the taxon and open vs shade comparisons in columns 6-9 respectively. **P* < 0.05, ***P*=0.01 – 0.001, ****P* = <0.001.

Composition traits		Mean and range of trait values from taxa averages			Nested ANOVA with taxon nested within habitat			Nested ANOVA with taxon nested within open vs. shade establishing	
		Units	Min/mean/max (natives)	Weed mean +/- SE	Vegetation type low and high from avg. values, native taxa (low, high)	habitat	taxon	Mean +/- SE of open, mean +/- SE for shade taxa, Significance of open vs shade	taxon
LMA	leaf mass per area	$g \cdot m^{-2}$	15.5-**73.2**-127	44.4 ± 24.10	M, D	***	***	82.9 ± 7.30, 64.8 ± 6.4, ***	***
D	leaf density	$g \cdot cm^{-3}$	0.0732-**0.231**-0.387	0.25 ± 0.094	W, D	***	***	0.27 ± 0.017, 0.20 ± 0.02, *	***
Chl_{area}	chlorophyll per area		23.2-**46.0**-61.2	49.4 ± 3.57	D, C-**B**	***	***	46.5 ± 2.07, 46.4 ± 2.8, NS	***
Chl_{mass}	chlorophyll per mass		0.313-**0.745**-1.64	1.49 ± 1.03	D, C-**B**	***	***	0.67 ± 0.07, 0.82 ± 0.09, ***	***
N_{area}	nitrogen per area	$g \cdot m^{-2}$	0.167-**1.22**-2.18	1.1 ± 0.66	M, C	***	***	1.34 ± 0.09, 1.14 ± 0.14, ***	***
N_{mass}	nitrogen per mass	%	0.738-**1.76**-2.98	2.38 ± 0.44	W, C	***	***	1.9 ± 0.18, 1.72 ± 0.11, *	***
Chl:N	chlorophyll : nitrogen		25.6-**45.1**-162.4	74.6 ± 68.77	D, M	***	***	36.0 ± 2.07, 53.4 ± 9.8, ***	***
$\delta^{15}N$	delta ^{15}N	‰	-4.9- **2.4**- 12.9	5.17 ± 1.027	**B**-W, C	***	***	3.78 ± 1.25, 0.43 ± 0.66, ***	***
$\delta^{13}C$	delta ^{13}C	‰	-14.6- **-13.3**- -12.0	-13.0 ± 0.35	W, C	*	***	-13.08 ± 0.20, -13.41 ± 0.14, ***	***
P_{area}	phosphorus per area	$g \cdot m^{-2}$	0.463-**0.117**-0.323	0.23 ± 0.18	**B**-W, D	***	***	0.14 ± 0.019, 0.11 ± 0.02, ***	***
P_{mass}	phosphorus per mass	%	0.0633-**0.177**-0.439	0.48 ± 0.26	W, D	*	***	0.20 ± 0.031, 0.17 ± 0.03, ***	***
C_{mass}	carbon per mass	%	39.7-**42.3**-44.8	42.3 ± 0.35	M, D	NS	NS	42.6 ± .031, 42.3 ± 0.39, *	***
N:P	nitrogen : phosphorus		5.55-**12.2**-22.9	6.11 ± 1.50	D, W-**B**	NS	NS	11.10 ± 0.89, 12.84 ± 1.32, NS	NS
C:N	carbon : nitrogen		10.9-**26.0**-65.3	19.13 ± 4.22	**B**-C, D	NS	NS	28.4 ± 11.5, 27.1 ± 6.2 , NS	NS
C:P	carbon : phosphorus		32.3-**298.9**-834.1	112.6 ± 37.44	C, W	NS	NS	320.09 ± 156.9, 347.3 ± 82.6, NS	NS

Composition traits		Units	Mean and range of trait values from taxa averages: Min/mean/max (natives)	Weed mean+/- SE	Nested ANOVA with taxon nested within habitat: Vegetation type low and high from avg values, native taxa (low, high)	habitat	taxon	Nested ANOVA with taxon nested within open vs. shade establishing: Mean +/- SE of open, mean +/- SE for shade taxa, Significance of open vs shade	taxon
Field traits									
Diameter	diameter of stem/trunk	mm	1.7-**32.5**-133	2.6 ± 1.54	**B**-M, W	***	***	37.3 ± 10.4, 24.9 ± 6.0, ***	***
Height	plant height	m	0.035-**1.37**-4.73	0.22 ± 0.11	**B**-C, W	***	***	1.2 ± 0.33, 1.5 ± 0.24,***	***
Exposure	% open	%	11.0-**67.0**-100	86.7 ± 16.17	M, C-**B**	***	***	92.1 ± 3.2, 43.02 ± 8.3,***	***
Elevation		m	19.2-**582**-1695	71.2 ± 11.15	C, W	***	***	408.1 ± 140.2, 831.4 ±85.8,***	***
Climate traits									
MARH	mean annual relative humidity	%	69.3-**77.7**-82.9	70.70 ± 0.05	C, W	***	***	74.5 ± 1.2, 81.1 ± 0.34, ***	***
MAT	mean annual temperature	°C	13.3-**19.9**-23.8	23.2 ± 0.03	W, C	***	***	21.2 ± 0.88, 18.08 ± 0.50, ***	***
MAP	mean annual precipitation	mm	425-**2115**-9704	1746.3 ± 75.73	D, W	***	***	1236.4 ± 234.7, 2990.1 ± 779.4, ***	***
VPD	vapor pressure deficit	Kilo-pascals	0.324-**0.539**-0.899	0.84 ± 0.004	W, C	***	***	0.67 ± 0.055, 0.39 ± 0.018, ***	***
MFVPD	mole fraction VPD		0.0037-**0.0060**-0.0090	0.0083 ± 0.000055	W, C	***	***	0.0068 ± 0.00049, 0.0041 ± 0.00016,***	***

Table 3.3. Correlation results for 15 leaf composition traits as well as plant diameter and height with seven climate traits. Raw, log, and rank *r* values . Bold text means significant relationship between composition or morphology trait and climate trait, and P value significance indicated as follows: **P* < 0.05, ***P*=0.01 – 0.001, ****P* = <0.001. Symbols as in table 3.2.

Leaf Traits	%Open Sky	Elevation	MAT	MAP
LMA	0.351, 0.362, 0.283	-0.060, -0.162, -0.101	0.076, -0.035, 0.019	-0.062, -0.079, -0.208
D	0.340, 0.316, 0.277	-0.154, -0.290, -0.322	0.231, 0.147, 0.307	**-0.441***, **-0.492****, **-0.638*****
Chl_{area}	0.059, 0.039, 0.109	0.059, -0.036, 0.105	-0.112, -0.127, -0.097	0.055, 0.012, 0.021
Chl_{mass}	-0.316, -0.369^{X}, -0.293	-0.044, 0.145, 0.118	0.043, 0.000, -0.035	-0.030, 0.081, 0.189
N_{area}	**0.530****, **0.434***, **0.580****	0.092, -0.103, -0.005	-0.107, -0.130, -0.044	0.006, -0.126, -0.171
N_{mass}	0.332, 0.201, 0.425*****	-0.014, -0.065, -0.010	0.010, -0.070, 0.050	-0.084, -0.135, -0.207
Chl:N	**-0.427***, **-0.555****,**-0.649*****	-0.053, 0.174, 0.157	0.052, -0.080, -0.088	0.016, 0.190, 0.335
$\delta^{15}N$	**0.400*, 0.332, 0.465***	**-0.388**^{X}, **-0.583****, **-0.474***	**0.450***, **0.403***, **0.521****	**-0.446**, **-0.533****, **-0.580****
$\delta^{13}C$	**0.402***, **0.426***, **0.449***	-0.082, 0.068, -0.058	0.077, 0.087, 0.030	0.019, -0.043, -0.123
P_{area}	**0.539****, **0.619****, **0.673*****	0.102, -0.125, -0.178	-0.065, -0.048, 0.157	0.223, -0.325, -0.407*
P_{mass}	0.334, 0.282, 0.349	-0.033, -0.079, -0.012	0.070, 0.042, 0.051	-0.247, -0.283, -0.220
C_{mass}	0.310,0.355, 0.204	0.251, 0.180, 0.299	-0.234, -0.237, -0.306	-0.050, -0.196, -0.193
N:P	-0.246,-0.218, -0.148	0.110, 0.037, 0.048	-0.144, -0.071, -0.052	0.216, 0.260, 0.262
C:N	-0.197,-0.176, -0.381^{X}	-0.086, 0.081, 0.015	0.123, 0.056, -0.073	-0.054, 0.115, 0.163
C:P	-0.316, -0.303, -0.403	0.001, 0.074, 0.020	0.003, -0.007, -0.052	0.120, 0.297, 0.233
Diam	0.349, 0.289, 0.241	0.256, 0.287, 0.292	-0.270, -0.361, -0.356	0.106, 0.084, -0.026
Height	0.075, -0.122, -0.037	**0.553**, **0.597****, **0.522****	**-0.576**, **-0.562****, **-0.556****	0.199, 0.276, 0.164

Leaf Traits	MARH	VPD	MFVPD
LMA	-0.301, -0.244, -0.186	0.236, 0.127, 0.043	0.248, 0.147, 0.069
D	-0.382*, -0.365, -0.331	0.347, 0.293, 0.311	0.362, 0.317, 0.340
Chl_{area}	0.033, 0.012, 0.198	-0.041, -0.092, -0.138	-0.045, -0.089, -0.160
Chl_{mass}	0.194, 0.263, 0.257	-0.114, -0.167, -0.076	-0.127, -0.189, -0.113
N_{area}	-0.198, -0.181, -0.070	0.112, 0.031, -0.065	0.122, 0.052, -0.042
N_{mass}	-0.088, -0.020, -0.002	0.082, -0.042, -0.000	0.081, -0.040, -0.006
Chl:N	0.205, 0.283, 0.316	-0.131, -0.124, -0.100	-0.142, -0.151, -0.144
$\delta^{15}N$	**-0.647*****, **-0.647*****, **-0.612****	**0.619****, **0.579****, **0.521****	**0.629****, **0.600****, **0.556****
$\delta^{13}C$	0.036, 0.043, -0.025	-0.003, 0.031, 0.023	-0.007, 0.022, 0.019
P_{area}	-0.242, -0.307, -0.389[X]	0.130, 0.172, 0.197	0.151, 0.205, 0.250
P_{mass}	-0.171, -0.137, -0.161	0.134, 0.114, 0.086	0.143, 0.125, 0.122
C_{mass}	0.131, 0.131, 0.273	-0.172, -0.192, -0.349	-0.164, -0.177, -0.333
N:P	0.235, 0.186, 0.238	-0.183, -0.177, -0.122	-0.195, -0.195, -0.177
C:N	-0.041, 0.032, -0.000	0.055, 0.028, -0.023	0.060, 0.027, 0.011
C:P	0.110, 0.162, 0.153	-0.063, -0.111, -0.091	-0.68, -0.127, -0.123
Diam	-0.131, 0.092, 0.159	-0.038, -0.211, -0.330	-0.013, -0.189, -0.293
Height	**0.188**, **0.418***, **0.409***	**-0.363**, **-0.514****, **-0.551****	**-0.339**, **-0.498***, **-0.517****

Table 3.4. Factor-loadings of first two principal components for 15 traits determined for 26 Hawaiian *Euphorbia* taxa. PC 1= traits aligned with irradiance, PC 2= traits aligned with *LMA*. Trait abbreviations as in Table 3.3.

Variable	**PC1**	**PC2**
Eigenvalue	3.7	3.0
% of total variance explained	24.8	20.2
$\boldsymbol{Chl_{area}}$	-0.0283	-0.2797
Leaf mass per area (*LMA*)	-0.0344	-0.4712
Leaf area (*LA*)	-0.3677	0.0363
Thickness (*T*)	-0.3608	-0.1185
Density (*D*)	0.2208	-0.4144
Chlorophyll: N (*Chl:N*)	0.0058	0.4539
Nitrogen per mass (N_{mass})	0.1823	0.0003
Carbon per mass (C_{mass})	0.3372	-0.2110
Phosphorus per mass (P_{mass})	0.3761	0.2502
Nitrogen/ Phosphorus (*N/P*)	-0.2727	-0.2149
Carbon isotope ratio ($\delta^{13}C$)	0.3276	-0.1869
Lamina length/ Lamina width (*LamL/LamW*)	-0.0785	0.2192
Leaf perimeter squared/ leaf area (P^2/A)	-0.0258	0.2001
Vein orders (V_{orders})	-0.2178	-0.1990
Total vein density (Tot_{VD})	0.3993	-0.1597

FIGURES

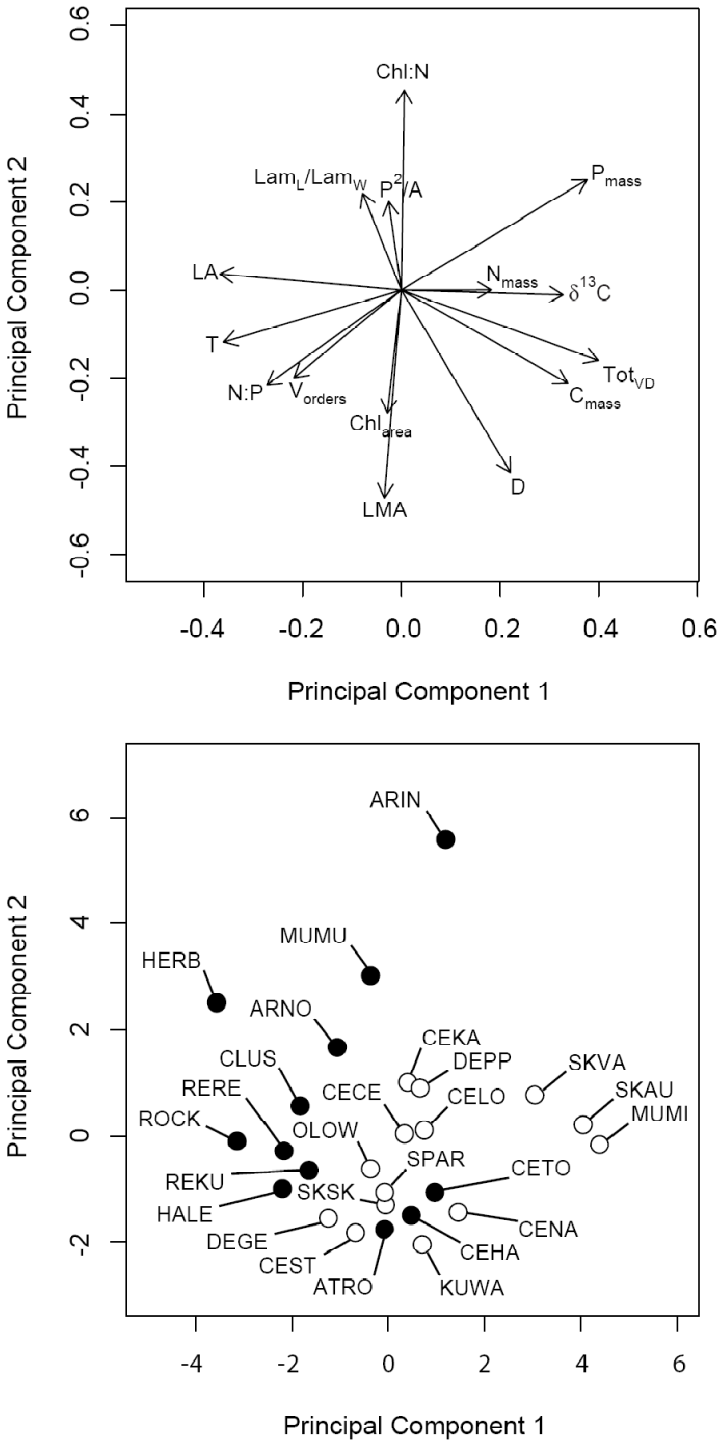

Figure 3.1. Top panel, principal components analysis of 15 leaf traits for 26 native Hawaiian *Euphorbia* taxa. PC1 = traits aligned with irradiance. PC2 = traits aligned with leaf mass per area (*LMA*). Bottom panel, plot of taxon loadings; open circles, open-establishing; closed circles, shade-establishing taxa. See table 3.1 species codes and table 3.2 for trait abbreviations.

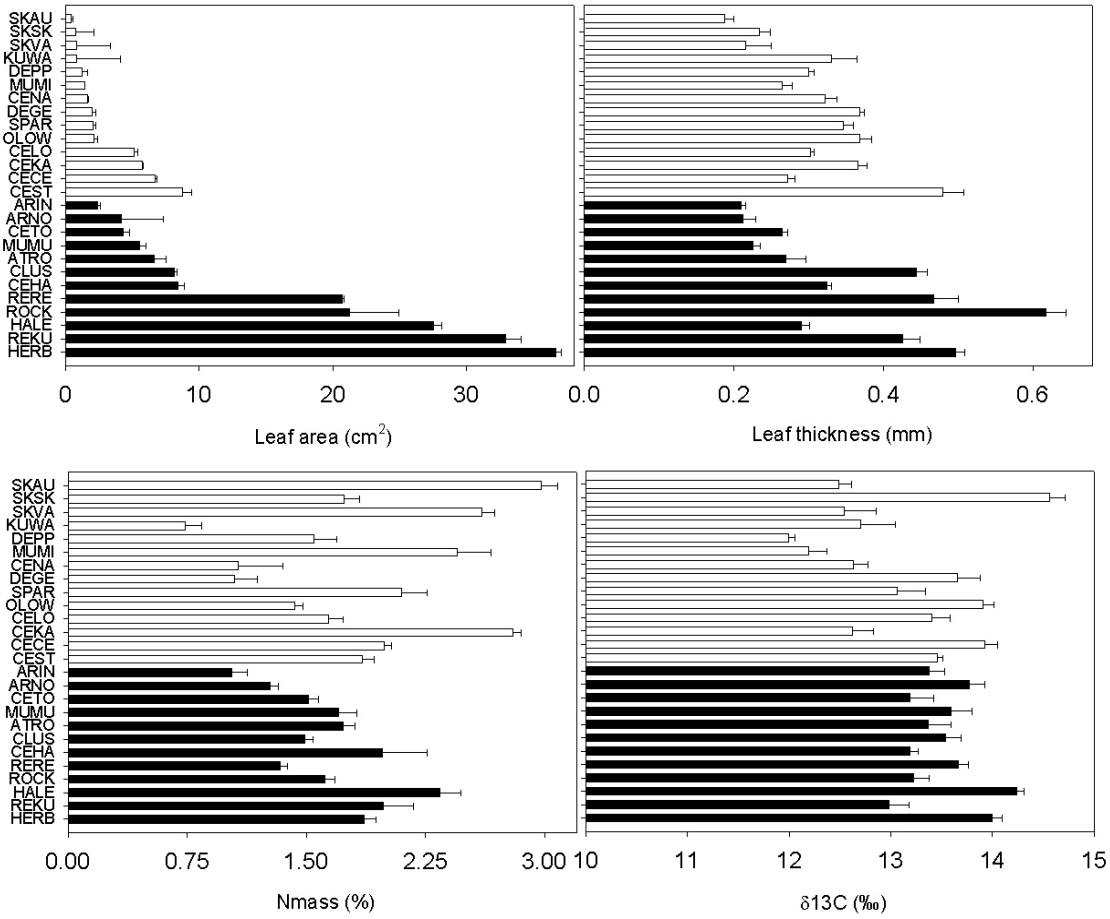

Figure 3.2. Taxon values for four traits aligned with irradiance for 26 native Hawaiian *Euphorbia* taxa (codes as in Table 3.1): leaf area (*LA*), leaf thickness, N_{mass}, and $\delta^{13}C$. Open bars indicate open-establishing taxa and closed bars indicate shade-establishing taxa. In each panel, taxa are arranged in order of smallest to largest *LA* within open- and shade-establishment categories.

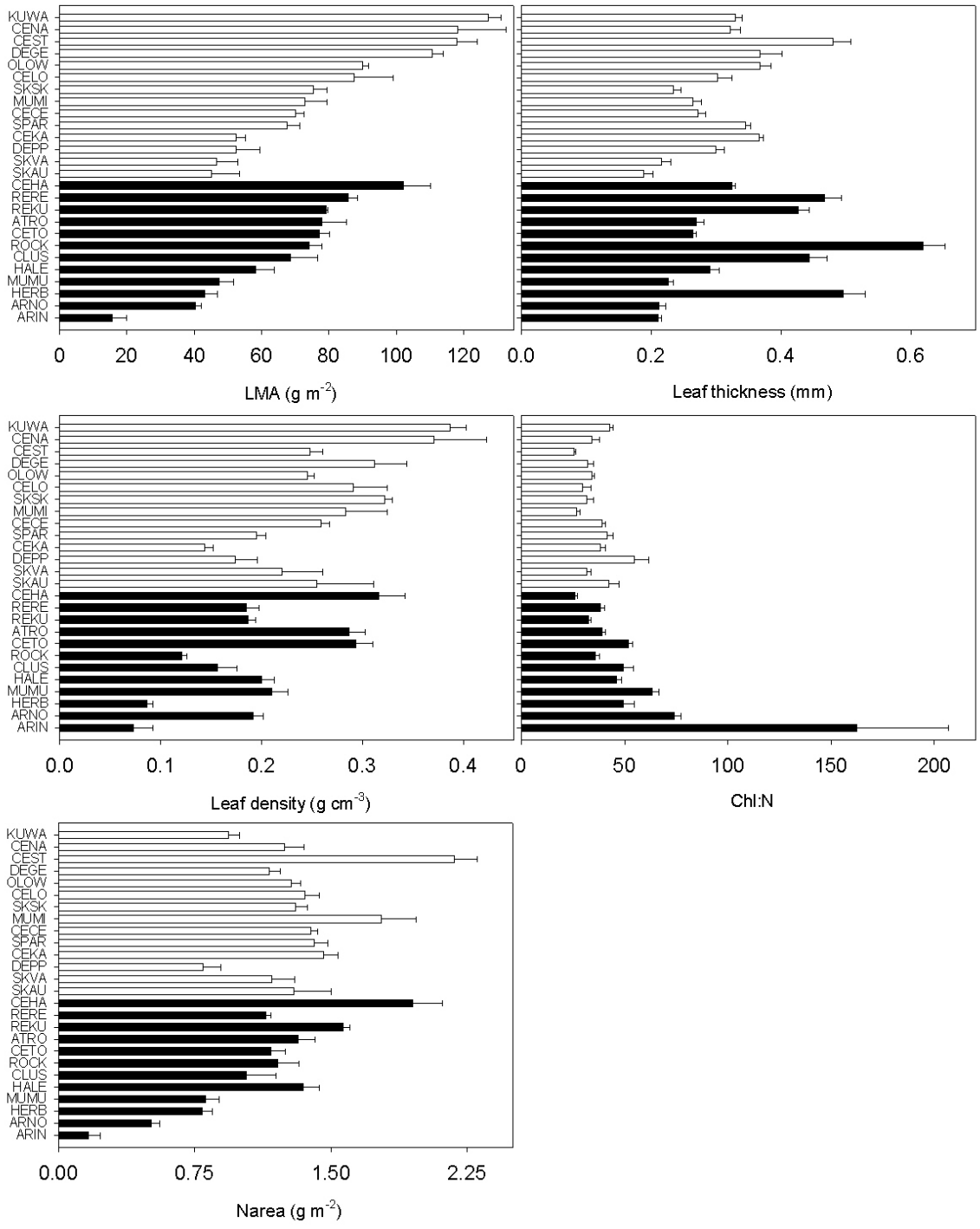

Figure 3.3. Taxon values for leaf mass per area (*LMA*) and four other traits aligned with *LMA*, for 26 native Hawaiian *Euphorbia* taxa (codes as in Table 3.1): leaf thickness, leaf density, *Chl:N*, and N_{area}. Open bars indicate open-establishing taxa and closed bars indicate shade-establishing taxa. In each panel, taxa are arranged in order of smallest to largest *LMA* within open- and shade-establishment categories.

APPENDICES

Appendix Table A3-1. Correlation matrix of select composition, stomatal, and venation traits for *Euphorbia* taxa. Three values presented for each correlation are Pearson correlation coefficients (r_p) first raw data, second log-transformed data, and third Spearman correlations (r_s) for all taxa. Values in bold face are significant at $P < 0.05$, level of significance is indicated by asterisks as follows: * < 0.05, ** < 0.01, *** < 0.001.

	Diam	**Height**
Height	**0.756***, 0.744***, 0.779*****	
Chl **area**	0.116, 0.211, 0.204	0.108, 0.070, 0.090
LMA	0.195, **0.523**, 0.495****	0.112, 0.284, 0.279
Chl **mass**	**-0.251, -0.479**, -0.454***	-0.234, -0.292, -0.298
LA	0.086, **0.421*, 0.453****	0.270, **0.599**, 0.617*****
T	0.349, **0.549**, 0.499****	0.185, **0.408*, 0.398***
D	0.024, 0.157, 0.075	-0.014, -0.029, -0.126
Chl:N	-0.293, -0.497**, -0.331	-0.291, -0.381*, -0.381*
N **area**	0.257, **0.466*, 0.380***	0.305, 0.351, 0.386*
N **mass**	0.077, -0.036, -0.025	0.052, -0.017, 0.006
C **mass**	0.055, 0.224, 0.260	0.139, 0.029, 0.107
P **area**	0.237, 0.166, 0.182	0.244, 0.075, 0.076
P **mass**	-0.013, -0.274, -0.172	-0.059, -0.265, -0.200
N:P	-0.122, 0.239, 0.181	0.079, 0.246, 0.282
C:N	-0.047, 0.038, 0.037	-0.180, 0.005, -0.019
C:P	-0.134, 0.212, 0.126	-0.103, 0.189, 0.135
δ^{15}N	-0.000, -0.246, -0.197	-0.050, -0.215, -0.144
δ^{13}C	0.011, -0.041, 0.003	-0.261, -0.357, -0.270
%*SD* **ad**	0.205, 0.033, 0.147	-0.001, -0.199, -0.085
SD **t**	0.437*, 0.091, 0.138	0.582, 0.139, 0.249
PL **tot**	-0.025, 0.124, 0.215	-0.222, -0.080, -0.041
%*GCA* **ad**	0.373, 0.138, 0.063	0.135, -0.035, -0.136
%*GCA* **t**	0.264, 0.205, 0.109	0.367, 0.268, 0.317
P **D**	0.103, 0.617, 0.032	0.292, 0.457, 0.137
SPI **t**	0.451*, 0.357, 0.357	0.350, 0.117, 0.179
%*SPI* **ad**	0.430X, 0.117, 0.233	0.149, -0.179, -0.061
P **dia**	-0.014, -0.342, -0.360	-0.051, -0.384, -0.553X
ECS **ad**	**-0.512*, -0.674***, -0.713*****	-0.586**, -0.695***, -0.617**
ECS **ab**	**-0.421, -0.692***, -0.620****	**-0.583**, -0.820***, -0.669****
VINA	-0.030, 0.039, -0.039	0.080, 0.244, 0.113
LamL/LamW	0.236, **0.445*, 0.573****	0.299, **0.694***, 0.794*****
P^2/A	-0.122, 0.077, 0.381*	-0.036, 0.413*, 0.620***
Vorders	0.175, 0.278, 0.301	0.370, 0.265, 0.270
MajVD	-0.022, -0.075, -0.036	-0.126, -0.309, -0.262
TotVD	-0.068, -0.215, -0.237	-0.110, -0.295, -0.345
1^{o}W	0.353, **0.635***, 0.588****	**0.497*, 0.684***, 0.675*****
minW	0.336, **0.604**, 0.642*****	0.196, 0.279, 0.323
Taxa missing this trait	RERE	RERE

	Chl **area**	***LMA***
LMA	0.230, 0.385*, 0.142	
Chl **mass**	-0.078, -0.008, 0.159	**-0.896*****, 0.924*****, -0.936*****
LA	0.136, 0.192, 0.264	-0.125, 0.179, 0.173
T	0.115, 0.109, 0.078	**0.368X, 0.597****, 0.543****
D	0.177, 0.381*, 0.091	**0.743*****, 0.701*****, 0.631*****
Chl:N	-0.477*, -0.391*, -0.027	**-0.611****, -0.842*****, -0.619*****
N **area**	**0.623****, 0.678*****, 0.595****	**0.563****, 0.794*****, 0.403***
N **mass**	**0.499****, 0.553****, 0.527****	**-0.379X, -0.212, -0.366X**
C **mass**	0.351, 0.352, 0.478**	0.088, 0.163, 0.154
P **area**	0.071, 0.201, 0.141	0.269, 0.324, 0.384*
P **mass**	-0.192, -0.039, 0.036	**-0.433*, -0.490****, -0.415***
N:P	0.473*, 0.294, 0.272	0.057, 0.314, 0.192
C:N	**-0.524****, -0.550****, -0.515****	**0.420***, 0.212, **0.389***
C:P	-0.073, -0.126, -0.104	**0.437*, 0.370*, 0.383***
δ^{15}N	0.036, 0.060, 0.010	0.042, -0.089, -0.105
δ^{13}C	-0.023, -0.003, 0.054	0.017, -0.096, -0.078
%*SD* **ad**	0.255, 0.257, 0.168	**0.524****, 0.325, **0.414***
SD **t**	0.092, 0.316, 0.175	0.279, 0.219, 0.211
PL **tot**	-0.047, -0.073, -0.053	0.312, 0.317, 0.325
%*GCA* **ad**	0.042, 0.054, 0.048	0.153, 0.072, 0.108
%*GCA* **t**	-0.020, -0.010, 0.027	0.009, 0.098, 0.005
P **D**	0.198, 0.339, 0.248	0.228, 0.249, 0.353
SPI **t**	0.176, 0.220, 0.299	0.331, 0.357, 0.365
%*SPI* **ad**	0.251, 0.251, 0.203	0.447*, 0.262, 0.314
P **dia**	-0.040, -0.081, -0.148	0.129, 0.150, 0.219
ECS **ad**	-0.228, -0.226, -0.292	**-0.499*, -0.613****, -0.678*****
ECS **ab**	-0.145, -0.132, -0.294	-0.181, -0.410X, -0.373
VINA	-0.219, -0.282, -0.337	-0.294, -0.266, -0.392*
LamL/LamW	0.119, 0.122, 0.105	-0.272, -0.055, -0.080
P^2/A	0.081, 0.017, 0.014	-0.190, -0.052, 0.175
Vorders	0.178, 0.133, 0.097	0.177, 0.314, 0.330
MajVD	-0.179, -0.170, -0.196	-0.035, -0.113, 0.013
TotVD	0.116, 0.132, 0.107	0.163, -0.025, 0.009
1^{o}W	0.208, 0.219, 0.234	0.164, **0.355X, 0.348X**
minW	0.238, 0.265, 0.245	**0.701****, 0.722*****, 0.739*****
Taxa missing this trait	NONE	NONE

	Chl **mass**	***LA***
LA	0.045, -0.125, -0.126	
T	**-0.429*, -0.609***, -0.566****	**0.583**, 0.680***, 0.670****
D	**-0.651***, -0.595**, -0.564****	-0.492**, -0.393*, -0.391*
Chl:N	**0.732***, 0.736***, 0.662*****	-0.093, -0.127, -0.014
N **area**	-0.497*, -0.570**, -0.274	0.058, 0.163, 0.135
N **mass**	0.362, **0.460*, 0.450***	0.104, -0.030, -0.111
C **mass**	-0.016, -0.009, -0.014	-0.250, -0.272, -0.131
P **area**	-0.267, -0.251, -0.364X	-0.231,-0.251, -0.226
P **mass**	**0.468*, 0.522**, 0.418X**	-0.245, -0.394*, -0.342
N:P	-0.025, -0.233, -0.140	0.358, **0.466*, 0.475****
C:N	-0.314, -0.456*, -0.463*	-0.246, -0.008, 0.034
C:P	-0.358, -0.455*, -0.380*	0.038, 0.353X, 0.290
δ^{15}N	-0.003, 0.210, 0.066	-0.052, -0.210, -0.266
δ^{13}C	0.038, 0.124, 0.118	-0.336, -0.429*, -0.448*
%*SD* **ad**	-0.405*, -0.233, -0.364X	**-0.530**, -0.592**, -0.366X**
SD **t**	-0.398, -0.110, -0.220	0.342, 0.221, 0.189
PL **tot**	-0.163, -0.344, -0.275	-0.615**, 0.332, -0.276
%*GCA* **ad**	-0.069, -0.035, -0.090	-0.479*, -0.396X, -0.474*
%*GCA* **t**	-0.112, -0.113, -0.033	0.434*, 0.444*, 0.372
P **D**	-0.197, -0.050, -0.290	0.422, **0.708*, 0.573***
SPI **t**	-0.233, -0.267, -0.239	-0.227, -0.135, 0.006
%*SPI* **ad**	-0.334, -0.166, -0.247	-0.486*, -0.617**, 0.421*
P **dia**	-0.144, -0.209, -0.229	-0.540X, -0.491, -0.561*
ECS **ad**	0.410**, 0.593**, 0.599****	-0.041, -0.280, -0.256
ECS **ab**	0.273, 0.397, 0.282	-0.099, -0.486*, -0.472*
VINA	0.151, 0.159, 0.298	0.573**, 0.624***, 0.320
LamL/LamW	0.228, 0.107, 0.085	0.113, **0.485**, 0.562****
P^2/A	0.209, 0.058, -0.204	-0.043, 0.224, 0.439*
Vorders	-0.277, -0.298, -0.291	0.192, 0.318, 0.265
MajVD	0.027, 0.063, -0.032	**-0.574**, -0.739***, -0.721****
TotVD	-0.039, 0.086, 0.058	**-0.636***, -0.741***, -0.770****
1^{o}W	-0.181, -0.295, -0.281	**0.665***, 0.862***, 0.856****
minW	**-0.597**, -0.657***, -0.673****	**-0.315, -0.032, 0.058**
Taxa missing this trait	NONE	NONE

	T	***D***
D	-0.304, -0.153, -0.194	
Chl:N	-0.316, -0.464*, -0.267	-0.504**, -0.615***, -0.512**
N_{area}	0.246, 0.389*, 0.107	**0.413*, 0.621***, 0.407***
N_{mass}	-0.165, -0.265, -0.295	-0.183, -0.024, -0.019
C_{mass}	-0.306, -0.222, -0.235	0.334, 0.402*, 0.318
P_{area}	-0.119, -0.095, -0.073	-.379X, 0.482**, 0.529**
P_{mass}	-0.422*, -0.571**, -0.517**	-0.123, -0.081, -0.060
N:P	0.243, **0.502**, 0.525****	-0.094, -0.068, -0.137
C:N	0.022, 0.215, 0.205	0.323, 0.060, 0.090
C:P	0.232, **0.521**, 0.452***	0.244, -0.019, 0.054
$\delta^{15}N$	-0.108, -0.252, -0.262	0.117, 0.125, 0.164
$\delta^{13}C$	-0.123, -0.186, -0.224	0.098, 0.059, 0.038
$\%SD_{ad}$	-0.204, -0.300, -0.100	**0.659***, 0.659***, 0.575****
SD_{t}	0.588**, 0.183, 0.203	-0.080, 0.102, 0.015
PL_{tot}	-0.469*, -0.134, -0.067	0.526**, 0.490*, 0.355
$\%GCA_{ad}$	-0.410X, -0.371, -0.455*	**0.423X, 0.399*, 0.492****
$\%GCA_{t}$	0.678**, 0.514**, 0.343	-0.409X, -0.318, -0.337
P_{D}	0.460, 0.422, 0.570*	-0.113, 0.035, -0.116
SPI_{t}	-0.188, -0.116, -0.062	0.403, 0.511**, 0.342
$\%SPI_{ad}$	-0332, -0.426,* -0.312	**0.670**, 0.665***, 0.613****
P_{dia}	0.341, 0.347, 0.390	-0.119, -0.085, 0.055
ECS_{ad}	-0.104, -0.275, -0.226	-0.383, -0.465*, -0.520*
ECS_{ab}	-0.046, -0.363, -0.220	-0.100, -0.222, -0.187
VINA	0.587**, 0.414*, 0.221	**-0.646***, -0.698***, -0.656****
LamL/LamW	-0.044, 0.169, 0.221	-0.232, -0.235, -0.255
P^2/A	-0.199, -0.081, 0.176	-0.056, -0.012, 0.048
Vorders	0.166, 0.299, 0.280	0.083, 0.120, 0.051
MajVD	-0.458*, -0.447*, -0.386*	0.378X, 0.271, 0.309
TotVD	-0.594**, -0.637***, -0.603***	**0.601**, 0.540**, 0.524****
$1^{o}W$	**0.445*, 0.634***, 0.588****	-0.183, -0.136, -0.148
minW	0.116**, 0.358X, 0.347X**	**0.618**, 0.569**, 0.498****
Taxa missing this trait	NONE	NONE

	Chl:N	***N*** **area**
N **area**	-0.764***, -0.941***, -0.752***	
N **mass**	-0.329, -0.261, -0.267	**0.475*, 0.425*, 0.616*****
C **mass**	-0.270, -0.300, -0.189	**0.429*, 0.389*, 0.445***
P **area**	-0.365, -0.570**, -0.696***	**0.539**, 0.541**, 0.657*****
P **mass**	0.243, 0.128, -0.051	**0.012, -0.098, 0.182**
N:P	-0.217, -0.199, -0.035	0.201, 0.257, 0.107
C:N	0.327, 0.262, 0.246	**-0.465*, -0.423*, -0.584****
C:P	0.014, 0.011, 0.132	-0.186, -0.069, -0.267
δ^{15}N	-0.253, -0.205, -0.404*	0.413*, 0.193, 0.300
δ^{13}C	-0.081, 0.024, -0.073	0.109, 0.001, 0.146
%*SD* **ad**	-0.458, -0.391*, -0.481*	**0.581**, 0.417*, 0.448***
SD **t**	-0.383, -0.301, -0.406*	0.343, 0.358, 0.351
PL **tot**	-0.055, -0.187, -0.081	0.031, 0.123, -0.002
%*GCA* **ad**	-0.179, -0.235, -0.379	0.350, 0.219, 0.384X
%*GCA* **t**	-0.105, -0.144, -0.140	0.058, 0.113, 0.038
P **D**	-0.288, -0.050, -0.140	0.362, 0.311, 0.328
SPI **t**	-0.274, -0.327, -0.341	0.363, 0.350, 0.418*
%*SPI* **ad**	-0.415X, -0.351, -0.392X	**0.559**, 0.383X, 0.441***
P **dia**	0.194, 0.170, 0.200	-0.202, -0.211, -0.409
ECS **ad**	0.279, **0.606**, 0.649****	**-0.511*, -0.558**, -0.683X**
ECS **ab**	0.053, 0.365, 0.294	-0.193, -0.326, -0.411X
VINA	0.225, 0.298, 0.503**	**-0.318, -0.343, 0.596****
LamL/LamW	0.040, -0.084, -0.105	-0.075, 0.124, 0.212
P^2/A	0.124, -0.002, -0.206	-0.184, 0.046, 0.222
Vorders	-0.161, -0.160, -0.109	0.019, 0.139, 0.025
MajVD	-0.107, -0.024, -0.151	0.086, -0.037, -0.028
TotVD	-0.144, -0.074, -0.259	0.306, 0.113, 0.268
1^{o}W	-0.165, -0.236, -0.135	0.210, 0.256, 0.210
minW	**-0.468*, -0.608***, -0.530****	0.531**, 0.573**, 0.497**
Taxa missing this trait	CELA	CELA

	N_{mass}	C_{mass}
C_{mass}	**0.458*, 0.384*, 0.441***	
P_{area}	0.305, **0.388*, 0.354X**	**0.461*, 0.416*, 0.371***
P_{mass}	**0.482*, 0.575**, 0.498****	0.403*, 0.269, 0.196
N:P	0.152, -0.060, -0.047	-0.008, -0.068, -0.094
C:N	-0.897***, -0.995***, -0.985***	-0.257, -0.308, -0.345
C:P	-0.610**, -0.666***, -0.592**	-0.180, -0.239, -0.189
$\delta^{15}N$	**0.451*, 0.446*, 0.522****	0.026, 0.014, 0.005
$\delta^{13}C$	0.245, 0.154, 0.298	**0.542**, 0.515**, 0.476****
$\%SD_{ad}$	0.181, 0.201, 0.226	0.310, 0.391*, 0.250
SD_{t}	0.012, 0.268, 0.275	-0.020, 0.029, 0.043
PL_{tot}	-0.152, -0.257, -0.230	0.148, 0.227, 0.250
$\%GCA_{ad}$	0.288, 0.270, 0.336	0.290, 0.276, 0.291
$\%GCA_{t}$	-0.007, 0.033, 0.065	-0.103, -0.093, -0.094
P_{D}	-0.126, -0.039, -0.161	-0.372, 0.028, -0.240
SPI_{t}	0.094, 0.063, 0.152	0.234, **0.443*, 0.386X**
$\%SPI_{ad}$	0.240, 0.256, 0.311	0.394, 0.472*, 0.342
P_{dia}	-0.250, -0.272, -0.408	-0.296, -0.277, -0.323
ECS_{ad}	-0.012, -0.018, -0.177	-0.190, -0.277, -0.405X
ECS_{ab}	0.196, 0.083, 0.020	0.017, -0.068, -0.159
VINA	-0.161, -0.193, -0.322	**-0.333, -0.402*, -0.392***
LamL/LamW	0.142, 0.215, 0.179	-0.262, -0.185, -0.079
P^2/A	-0.022, -0.011, -0.049	-0.228, -0.228, -0.139
Vorders	-0.201, -0.199, -0.276	-0.133, -0.123, -0.134
MajVD	0.302, 0.143, 0.111	0.421*, 0.345, 0.176
TotVD	0.274, 0.248, 0.370*	0.462*, 0.411*, 0.324
$1^{o}W$	-0.053, -0.116, -0.160	-0.067, -0.122, -0.029
minW	-0.097, -0.128, -0.061	0.294, 0.360X, 0.338
Taxa missing this trait	CELA	CELA

	P_{area}	P_{mass}
P_{mass}	**0.675***, 0.660***, 0.586****	
N:P	-0.618**, -0.621***, -0.527**	-0.657***, -0.808***, -0.817***
C:N	-0.228, -0.369*, -0.304	-0.332, -0.555**, -0.444*
C:P	-0.624**, -0.691***, -0.589**	-0.779***, -0.951***, -0.961***
$\delta^{15}N$	0.330, 0.337, 0.407*	0.337, **0.390*, 0.428***
$\delta^{13}C$	0.379X, 0.249, 0.244	**0.413***, 0.323, **0.364X**
%*SD* $_{ad}$	**0.396X, 0.486**, 0.406***	0.068, 0.210, 0.033
SD $_{t}$	0.193, 0.260, 0.317	-0.082, 0.115, 0.152
PL $_{tot}$	0.178, 0.118, 0.155	0.046, -0.150, -0.082
%*GCA* $_{ad}$	**0.450*, 0.479*, 0.515****	0.401, **0.404*, 0.443***
%*GCA* $_{t}$	-0.019, 0.029, 0.168	-0.054, -0.054, 0.120
P $_{D}$	0.517, 0.498, 0.382	0.180, 0.239, 0.082
SPI $_{t}$	0.526*, 0.430*, 0.541**	0.271, 0.142, 0.298
%*SPI* $_{ad}$	0.400, **0.516**, 0.405***	0.139, 0.313, 0.200
P $_{dia}$	-0.444, -0.440, -0.253	-0.449, -0.454, -0.455
ECS $_{ad}$	-0.352, -0.473*, -0.517*	-0.000, 0.068, 0.041
ECS $_{ab}$	-0.188, -0.247, -0.215	0.174, 0.140, 0.054
VINA	-0.366, -0.459*, -0.700***	-0.187, -0.245, -0.353
LamL/LamW	-0.061, 0.015, 0.000	-0.016, 0.021, 0.067
P^2/A	-0.145, -0.029, 0.180	-0.067, -0.069, -0.036
Vorders	-0.179, -0.233, -0.209	-0.377X, -0.440*, -0.477**
MajVD	0.344, 0.210, 0.183	0.492*, 0.310, 0.215
TotVD	**0.443*, 0.400*, 0.417***	**0.402*, 0.413*, 0.422***
1^oW	0.025, -0.027, 0.033	-0.206, -0.317, -0.172
minW	**0.483*, 0.403*, 0.458***	-0.044, -0.194, -0.145
Taxa missing this trait	CELA	CELA

	N:P	C:N
C:N	-0.212, 0.036, -0.017	
C:P	**0.577**, 0.781***, 0.770*****	**0.655***, 0.651***, 0.549****
δ^{15}N	-0.112, -0.212, -0.276	-0.368, -0.450*, -0.520**
δ^{13}C	-0.351, -0.329, -0.274	-0.004, -0.097, -0.223
%*SD*$_{ad}$	-0.034, -0.222, -0.021	-0.034, -0.157, -0.159
***SD*$_{t}$**	-0.166, -0.094, -0.048	-0.166, -0.253, -0.247
***PL*$_{tot}$**	-0.206, 0.017, -0.101	0.295, 0.278, 0.272
%*GCA*$_{ad}$	-0.336, -0.347, -0.368	-0.144, -0.227, -0.246
%*GCA*$_{t}$	0.041, 0.097, 0.007	-0.134, -0.049, -0.103
***P*$_{D}$**	-0.292, -0.424, -0.197	-0.074, 0.074, 0.145
***SPI*$_{t}$**	-0.405, -0.172, -0.237	-0.074, -0.017, -0.075
%*SPI*$_{ad}$	-0.097, -0.320, -0.173	-0.062, -0.199, -0.216
***P*$_{dia}$**	0.254, 0.365, 0.242	0.197, 0.231, 0.342
***ECS*$_{ad}$**	-0.008, -0.045, -0.016	-0.045, -0.003, 0.134
***ECS*$_{ab}$**	0.095, -0.046, -0.039	-0.051, -0.080, -0.050
VINA	0.169, 0.260, 0.372*	0.009, 0.151, 0.250
LamL/LamW	0.002, 0.088, 0.131	-0.259, -0.244, -0.216
P^2/A	-0.015, 0.033, 0.001	-0.064, -0.017, 0.016
Vorders	0.341, **0.377*, 0.368***	0.021, 0.187, 0.276
MajVD	-0.234, -0.261, -0.228	-0.099, -0.105, -0.032
TotVD	-0.181, -0.349, -0.372*	-0.091, -0.204, -0.281
1^{o}W	0.069, 0.289, 0.226	-0.082, 0.095, 0.114
minW	-0.181, 0.041, 0.053	0.169, 0.161, 0.109
Taxa missing this trait	CELA	CELA

	C:P	$\delta^{15}N$
$\delta^{15}N$	-0.347, -0.443*, -0.513**	
$\delta^{13}C$	-0.235, -0.316, -0.352	-0.123, -0.081, -0.066
%*SD*$_{ad}$	0.004, -0.268, -0.088	**0.447*, 0.439*, 0.438***
***SD*$_{t}$**	-0.094, -0.233, -0.257	-0.009, 0.169, 0.266
***PL*$_{tot}$**	0.099, 0.193, 0.126	0.168, 0.055, -0.046
%*GCA*$_{ad}$	-0.364, -0.400*, -0.436*	**0.605**, 0.596**, 0.566****
%*GCA*$_{t}$	-0.069, 0.048, -0.136	-0.151, -0.155, 0.084
***P*$_{D}$**	-0.226, -0.234, -0.164	0.281, -0.011, 0.202
***SPI*$_{t}$**	-0.390, -0.129, -0.269	0.291, 0.153, 0.230
%*SPI*$_{ad}$	-0.073, -0.369, -0.244	**0.445*, 0.469*, 0.480***
***P*$_{dia}$**	0.309, 0.443, 0.494	-0.315, -0.301, -0.379
***ECS*$_{ad}$**	-0.050, -0.043, 0.010	-0.144, -0.047, -0.095
***ECS*$_{ab}$**	0.057, -0.083, 0.005	0.393, 0.389, 0.349
VINA	0.141, 0.296, 0.398*	-0.394*, -0.429*, -0.391*
LamL/LamW	-0.223, -0.085, -0.128	-0.067, -0.170, -0.139
P^2/A	-0.060, 0.014, -0.039	-0.040, 0.022, 0.079
Vorders	0.272, **0.406*, 0.452***	-0.186, -0.234, -0.234
MajVD	-0.203, -0.257, -0.149	0.270, 0.238, 0.243
TotVD	-0.205, -0.392*, -0.404*	0.289, 0.351, 0.348
1^oW	-0.058, 0.285, 0.143	-0.106, -0.260, -0.244
minW	0.016, 0.135, 0.102	0.157, 0.049, -0.026
Taxa missing this trait	CELA	CELA

	$\delta^{13}C$	$\%SD_{ad}$
$\%SD_{ad}$	0.162, 0.321, 0.263	
SD_{t}	-0.176, -0.137, -0.079	0.004, 0.079, 0.112
PL_{tot}	0.333, 0.219, 0.330	**0.550**, 0.462*, 0.522****
$\%GCA_{ad}$	0.093, 0.112, 0.315	**0.858***, 0.783***, 0.875*****
$\%GCA_{t}$	-0.141, -0.136, -0.188	-0.368, -0.421*, -0.357
P_{D}	-0.326, 0..063, -0.401	**0.669*, 0.761*, 0.698***
SPI_{t}	0.083, 0.206, 0.211	**0.537*, 0.489*, 0.444***
$\%SPI_{ad}$	0.153, 0.357, 0.308	**0.988***, 0.998***, 0.979*****
P_{dia}	-0.058, -0.013, 0.035	0.095, 0.000, -0.152
ECS_{ad}	0.231, 0.188, -0.022	**-0.549*, -0.398X, -0.600****
ECS_{ab}	0.069, 0.124, 0.003	0.055, 0.165, 0.079
VINA	-0.184, -0.274, -0.190	**-0.695***, -0.910***, -0.743*****
LamL/LamW	-0.147, -0.231, -0.211	-0.195, -0.293, -0.164
P^2/A	-0.124, -0.267, -0.364X	-0.218, -0.194, 0.029
Vorders	-0.456*, -0.465*, -0.427*	-0.072, -0.175, -0.092
MajVD	0.295, 0.336, 0.382*	**0.408*, 0.566**, 0.480***
TotVD	0.271, 0.302, 0.396*	**0.558**, 0.688***, 0.471***
$1^{o}W$	-0.233, -0.312, -0.335	-0.342, -0.454*, -0.304
minW	0.245, 0.219, 0.233	**0.700***, 0.623***, 0.671*****
Taxa missing this trait	CELA	CELA, OLOW

	SD_t	PL_{tot}
PL_{tot}	-0.552*, -0.572**, -0.568**	
$\%GCA_{ad}$	0.168, 0.276, 0.181	0.394, 0.312, 0.382X
$\%GCA_t$	**0.838***, 0.699***, 0.667*****	-0.637**, -0.557**, -0.477*
P_D	**0.942***, 0.675*, 0.702***	0.091, -0.018, 0.060
SPI_t	0.423X, 0.244, 0.200	**0.431*, 0.623**, 0.521****
$\%SPI_{ad}$	0.026, 0.092, 0.163	**0.454*, 0.421*, 0.412***
P_{dia}	-0.872**, -0.885**, -0.852**	0.401, 0.373, 0.327
ECS_{ad}	-0.363, -0.263, -0.261	0.120, -0.160, -0.225
ECS_{ab}	-0.204, 0.072, 0.029	0.217, -0.143, -0.035
VINA	0.468*, 0.111, -0.217	-0.760***, -0.624**, -0.294
LamL/LamW	0.056, 0.098, 0.197	-0.225, -0.198, -0.179
P^2/A	-0.092, 0.069, 0.284	-0.062, -0.026, -0.086
Vorders	0.365, 0.192, 0.176	-0.026, 0.086, 0.019
MajVD	-0.157, -0.115, -0.091	0.217, 0.205, 0.276
TotVD	-0.379, -0.154, -0.050	0.437*, 0.361, 0.132
1^oW	0.432*, 0.218, 0.171	-0.340, -0.114, -0.135
minW	0.288, 0.181, 0.147	0.324, **0.429*, 0.526****
Taxa missing this trait	CELA, CEST, DEGE, OLOW, SPAR	CELA, CEST, SPAR

	%*GCA*$_{ad}$	**%*GCA*$_{t}$**
%*GCA*$_{t}$	-0.148, -0.092, -0.127	
***P*$_{D}$**	0.792*, 0.658X, 0.583	0.637, 0.394, 0.444
***SPI*$_{t}$**	**0.715***, 0.639**, 0.554****	0.100, 0.066, 0.208
%*SPI*$_{ad}$	**0.853***, 0.794***, 0.893*****	-.367, -0.417*, -0.344
***P*$_{dia}$**	-0.835**, -0.847**, -0.865**	-0.598, -0.622, -0.501
***ECS*$_{ad}$**	-0.473*, -0.480*, -0.435*	-0.207, -0.107, 0.012
***ECS*$_{ab}$**	0.017, 0.090, 0.215	-0.099, -0.083, 0.056
VINA	-0.576**, -0.686***, -0.743***	0.729***, 0.591**, 0.222
LamL/LamW	-0.067, -0.043, -0.085	0.138, 0.232, 0.277
P^2/A	-0.179, -0.009, 0.092	0.034, 0.118, 0.251
Vorders	-0.199, -0.236, -0.274	0.272, 0.261, 0.127
MajVD	**0.414X, 0.405*, 0.493***	-0.183, -0.239, -0.246
TotVD	**0.420X, 0.459*, 0.512****	-0.456*, -0.446*, -0.318
1^{o}W	-0.256, -0.232, -0.320	0.412X, 0.393X, 0.374
minW	**0.628**, 0.529**, 0.502***	-0.061, -0.003, -0.112
Taxa missing this trait	CELA, CEST, DEGE, OLOW, SPAR	CELA, CEST, DEGE, OLOW, SPAR

	P_D	SPI_t
SPI_t	0.828**, 0.677*, 0.576	
$\%SPI_{ad}$	0.504, 0.627, 0.557	**0.538*, 0.494*, 0.459***
P_{dia}	-0.008, -0.791*, -0.143	-0.795*, -0.792*, -0.608
ECS_{ad}	-0.696*, -0.855**, -0.756*	-0.330, -0.425*, -0.386
ECS_{ab}	-0.037, -0.606, -0.189	-0.096, -0.231, -0.159
VINA	-0.508, -0.641, -0.511	-.496*, -0.564**, -0.511**
LamL/LamW	0.065, 0.400, 0.051	-0.033, -0.037, 0.047
P^2/A	0.388, 0.744*, 0.332	-0.081, 0.075, 0.241
Vorders	0.239, -0.110, 0.165	0.064, 0.103, 0.058
MajVD	**-0.538X, -0.779*, -0.571***	0.020, 0.066, -0.042
TotVD	-0.513, -0.769*, -0.630*	0.153, 0.304, 0.086
1^oW	0.364, 0.721*, 0.369	0.264, 0.222, 0.323
minW	0.262, 0.624, 0.311	**0.698***, 0.659***, 0.580****
Taxa missing this trait	ARIN, ARNO, CELA, CETO, CLUS, DEPP , HALE,HERB, MUMI, MUMU,RERE, REKU, ROCK, SKAU	CELA, CEST, DEGE, OLOW, SPAR

	$\%SPI_{ad}$	P_{dia}
P_{dia}	-0.618, -0.583, -0.615	
ECS_{ad}	-0.536*, -0.393, -0.548**	0.642, 0.600, 0.649X
ECS_{ab}	-0.056, 0.138, 0.067	0.776**, 0.721*, 0.575
VINA	-0.660**, -0.906***, -0.742***	0.143, 0.199, 0.295
LamL/LamW	-0.083, -0.236, -0.074	-0.285, -0.363, -0.521
P^2/A	-0.192, -0.165, 0.141	-0.719**, -0.780**, -0.710**
Vorders	-0.160, -0.264, -0.199	0.402, 0.332, 0.247
MajVD	**0.415X, 0.592**, 0.475***	0.313, 0.364, 0.474
TotVD	**0.505*, 0.688***, 0.499***	0.416, 0.491, 0.483
1^oW	-0.261, -0.444*, -0.283	-0.495, -0.512, -0.523
minW	**0.731***, 0.625**, 0.641****	-0.486, -0.507, -0.345
Taxa missing this trait	CELA, CEST, DEGE, OLOW, SPAR	ARIN, ARNO, CELA, CETO, CLUS, DEPP, HALE, HERB, MUMI, MUMU, RERE, REKU, ROCK, SKAU

	ECS_{ad}	ECS_{ab}
ECS_{ab}	**0.527*, 0.638**, 0.575****	
VINA	0.083, 0.230, 0.558**	-0.057, -0.174, 0.076
LamL/LamW	-0.221, -0.410X, -0.464*	-0.349, -0.544**, -0.576**
P^2/A	-0.025, -0.246, -0.526*	-0.181, -0.315, -0.361
Vorders	-0.155, -0.152, -0.114	-0.230, -0.341, -0.331
MajVD	-0.053, 0.006, -0.034	0.403, 0.310, 0.269
TotVD	-0.192, -0.101, -0.036	0.059, 0.126, 0.080
1^oW	-0.372, -0.478*, -0.382	-0.514*, -0.671**, -0.609**
minW	-0.510*, -0.647**, -0.742***	-0.311, -0.378, -0.363
Taxa missing this trait	CELA, CEST, DEGE, HALE, OLOW, RERE, SPAR	CELA, CEST, CLUS, HALE, OLOW, RERE, ROCK, SPAR

	VINA	LamL/LamW
LamL/LamW	0.181, 0.317, 0.127	
P^2/A	0.076, 0.196, -0.119	**0.853***, 0.718***, 0.636*****
Vorders	0.055, 0.107, 0.197	-0.168, -0.038, 0.015
MajVD	-0.324, -0.445*, -0.219	-0.168, -0.220, -0.241
TotVD	-0.666***, -0.750***, -0.532**	-0.098, -0.238, -0.283
1^oW	0.210, 0.397*, 0.122	0.124, **0.502**, 0.558****
minW	-0.450*, -0.514**, -0.606***	-0.175, -0.053, 0.083
Taxa missing this trait	CECE, CEST	NONE

	P^2/A	Vorders	MajVD
Vorders	-0.203, -0.242, -0.180		
MajVD	-0.078, -0.192, -0.417*	-0.077, -0.091, 0.004	
TotVD	0.033, -0.112, -0.338	-0.010, -0.077, -0.128	**0.807***, 0.740***, 0.757*****
1^oW	0.011, 0.241, 0.523**	0.232, 0.256, 0.179	-0.663***, -0.715***, -0.706***
minW	-0.227, -0.119, 0.258	-0.024, 0.104, 0.128	0.089, 0.139, 0.158
Taxa missing this trait	NONE	NONE	NONE

	TotVD	1^oW
1^oW	-0.490**, -0.580**, -0.594**	
minW	0.269, 0.186, 0.116	0.011, 0.228, 0.245
Taxa missing this trait	NONE	NONE

GENERAL CONCLUSIONS

SUMMARY OF FINDINGS

The C_4 Hawaiian *Euphorbia* species have diversified broadly across habitat types in their overall growth form and show exceptional variation in their foliar characteristics. Thus, very large variation across this adaptively radiated lineage was found in leaf morphology and nutrient composition; in stomatal distribution, size and densities; the presence of papillae; and venation density including abundance of "vein islands." This work captures in detail a greater variation for leaf traits across taxa within a genus than has been reported for any other lineage to my knowledge.

The examination of the leaf micro-surface features for 26 of the native Hawaiian *Euphorbia* taxa revealed exciting results. I found dramatic variation in epidermal and stomatal traits, more that what was previously known to exist within closely related taxa. Stomatal size traits (PL_{ad}, PL_{ab}, GL_{ad}, GL_{ab}) varied strongly across vegetation types and correlated negatively with *MAT* and *VPD* and positively with *MAP*. Stomata were smaller in open- than shade-establishing taxa likely because smaller stomata may be able to close faster and/or more completely, or because selection for larger pore area is optimally resolved with more numerous small stomata than few large ones because this increases the pore per guard cell investment (Franks et al., 2009), or simply because achieving high stomatal pore area can be more easily realized by increasing the initiation of stomata, and thus greater stomatal number, rather than increasing the size of cells, including those of stomata, which may be more developmentally constrained. Stomatal distribution within this group was highly diverse. Species within given genera typically tend to be all hypostomatous, or to range from hypo- to amphistomaty, but tend not to include

hyperstomaty. Hyperstomaty is generally thought to be uncommon and extremely rare in plagiotropic leaves of terrestrial dicotyledons, and in fact such an occurrence may be unique in *Euphorbia*. The Hawaiian *Euphorbia* species include taxa representing all three stomatal distributions types with 12 amphistomatous, nine hypostomatous, and five hyperstomatous taxa. These distribution types are at least partially influenced by habitat type. The hypostomatous taxa tend to be associated with shade and higher rainfall, the amphistomatous taxa with high light and drier environments, and hyperstomatous taxa occur across a range of habitat types, but these taxa tend also to have a thick and continuous layer of water storage tissue occupying the lower portion of the internal leaf space which would hinder gas exchange on the abaxial surface.

Venation traits also showed exceptional variation. For 27 Hawaiian *Euphorbia* taxa, significant relationships existed between vein traits and climate, and other leaf traits. For example, major vein densities were significantly negatively correlated with leaf area, which was apparently linked with climate and habitat. These findings support the view that the major vein system is tightly developmentally linked to leaf size, and thus shows parallel evolution with leaf size across environments (Dunbar-Co et al., 2009; McKown et al., 2010; Sack et al., in prep), and small leaves are linked with high irradiance environments (Givnish 1987). The minor vein system was also associated with habitat. Minor vein densities were greater in open-establishing species when compared to shade-establishing species. Greater minor vein density is adaptive under high irradiance environments (Sack and Frole, 2006). Vein islands, an exceptional feature, were associated with wet and shaded environments. I considered that a possible advantage of vein islands in shaded habitats may be a reduction of total vein density and its associated

costs in construction and also in its shading of mesophyll. I also suggested that vein islands might not provide an adaptive advantage, and could be a case of a "neutral" retained mutation that could pose no disadvantage, especially in light of the fact that these are C_4 species. The apparent repeated evolution of high numbers of vein islands would suggest an adaptive significance, or else a repeatable mechanism for the emergence of a neutral mutation. Indeed, a neutral adaptation may have be related in evolution to increasing leaf size, for instance if vein xylem differentiation from the procambium were unable to keep pace with an accelerated or prolonged period of leaf expansion. Considering the relatively recent arrival of *Euphorbia* to Hawaii, my findings support the hypothesis that venation architecture can evolve rapidly following isolation and shows adaptation in response to a wide climatic gradient.

Consistent with their diversification across environments and into a wide range of woody growth forms, the 26 *Euphorbia* taxa varied significantly in plant size and leaf composition. Of the 15 traits that were the focus of my study of leaf morphology and composition, 14 varied more than two fold across habitats. Differences among species likely represent the combination of adaptation and plasticity. Plant stem diameter and height varied nearly 80-fold and 135-fold respectively from coastal to wet forest species. The increase in plant size from habitats of low to high *MAP* is consistent with a trend toward taller woody forms as competition for light and space increases under the closed canopy (Givnish, 1999). The N_{area} and P_{area} had a positive relationship with % open sky, as expected from greater biochemical allocation to photosynthesis and metabolism under higher irradiance (Givnish, 1988; Niinemets, 2001). The variation across habitats of $\delta^{15}N$ and $\delta^{13}C$ and their positive correlations with % open sky were also consistent with, and

importantly extended previous studies in the literature. Variation in $\delta^{13}C$ values across taxa were highest for drier, open habitat types, possibly due to bundle sheath leakage or due to a signal of source air (Farquhar et al., 1989). The leaf $\delta^{15}N$ varied strongly across the *Euphorbia* taxa and habitats. Open establishing taxa also exhibited higher values for *LMA*, stem diameter, *D*, N_{area}, N_{mass}, P_{mass}, and P_{area}. These findings are consistent with the fact that leaves of plants in other systems that establish in sun tend to be thicker and denser with greater accumulation of photosynthetic compounds than leaves of plants that establish in shade (Givnish, 1988; Ashton and Berlyn, 1994; Niinemets et al., 1998; Niinemets, 2001). Shade-establishing taxa, however, had higher values for Chl_{mass}, *Chl:N*, *N:P*, *C:P*, and height. These findings are consistent with shade-establishing species allocating more resources to light capture, and also to occupying habitats relatively poor in P. These findings for leaf nutrient composition points to a very strong functional importance given the emergence of pronounced differences within a rapid adaptive radiation from a single colonist across habitats.

A NOTE ON STUDIES OF RARE AND ENDANGERED PLANTS

During the course of this work, issues surrounding rare and endangered plants often resulted in logistical difficulties and important discussions. Some of the Hawaiian *Euphorbia* taxa (known as `akoko in the Hawaiian language) currently occur in specific single or few locations, with apparently narrow habitat niches, and thus "natural rarity" likely contributed to the current rare status of some of the taxa (Rabinowitz, 1981; Sakai et al., 2002). Endemism and rarity can often go hand in hand, as the conditions that culminate to allow a species to evolve in a unique way may very well be rare conditions

(Kruckeberg and Rabinowitz, 1985). However, it is hard to know for certain if some of these species have been naturally relatively rare throughout their evolution or if their being rare is only a recent phenomenon (Burney et al., 2001). Regardless of the "naturalness" of their rarity, there are many challenges currently facing the >300 federally listed endangered plant species in Hawaii as well as at least the same number of technically rare species that are not federally listed (Sakai et al., 2002). The rare Hawaiian *Euphorbia* and all other plant species "on the brink" in Hawaii are faced with habitat destruction by human land uses (primarily development, military training operations and unregulated recreational activities), the spread of invasive plant and animal species, and well as the potential for catastrophic events such as hurricanes or tsunamis that could annihilate entire populations if no refugia or satellite populations are established. Of the 29 currently recognized C_4 *Euphorbia* taxa in Hawaii, nine are federally listed endangered species (*E. celastroides* var. *kaenana, E. deppeana*, *E. eleanoriae, E. halemanui, E. herbstii, E. kuwaleana, E. remyi* var. *kauaiensis, E. remyi* var. *remyi, E. rockii, E. skottsbergii* var. *skottsbergii* ; http://www.fws.gov/endangered/), and others within the lineage age should be regarded as uncommon or rare taxa.

At times during my doctoral work, the question of its importance has been raised—and that of pure research on endangered species in general—and special justification requested for this research given a view that my work was not directly applied to solving the problem of these species' conservation and restoration. A thoughtful answer to this question could help to motivate and facilitate further important discovery. A complete answer requires first remembering, why we are conserving rare plants, or any part of our natural world, in the first place. There are at least five main

logical reasons for the conservation of biodiversity that are typically listed (e.g.,Clewell and Aronson, 2006). Depending on context, some of these reasons can be more compelling than others, but this can become a purely philosophical debate when the question is urgent, so I simply note that these are listed in no particular order:

Usefulness. The potential for medical breakthroughs and/or other applied uses that a plant species could provide that may never be discovered if the plant is not conserved.

Aesthetic, spiritual, or cultural value. In many cases even rare plants, or their existence, can carry human value.

Keystone species that provide ecosystem services. Some species may be significantly influential in determining the community structure of a given habitat, and their loss would be extremely detrimental to the community and potentially lead to the loss of services that maintain ecosystems or natural resources (Paine, 1969).

Right to exist. Many humans recognize that all species have an inherent right to life and out of respect to them as fellow biological species we are tasked with their conservation.

Science. Conserving species for the purpose of scientific understanding and realizing their potentially dramatic information content, both as a species, as well as in their interactions with the rest of the biota and environment. One major trait of the human species is our curiosity and desire to understand the intricacies of the world around us. This reason tends to grow increasingly central as more information about rare species and the sheer amount of information and understanding we can gain from them comes to light. We hope that new scientific discoveries will indeed feed into the previously named

reasons for conservation—because our interest, knowledge of uses, and even our respect for given species and their right to exist grows with new knowledge, and this leads to greater motivation and sometimes justification to protect them, especially urgent as extinction would lead to permanent loss of this information, knowledge, and understanding. Thus, science in itself is often particularly emphasized as a major, central reason for conservation of rare species (e.g., Hawaii Revised Statutes, 2010).

Of these reasons, all five may apply to the Hawaiian euphorbias, given their interactions with other members of the biota such as the endemic genus of leaf hoppers that lives solely on the native euphorbias or the mycorrhizal species that are reportedly associated with several of the *Euphorbia* taxa (e.g., Medeiros et al.; Zimmerman, 1970; Koske et al., 1992), role in Hawaiian lore and ethnobotany (Nagata, 1971), and unique value to science given their diversity of anatomy, development, physiology and ecology.

Based on this discussion, the importance and benefit of pure science is clear, as it justifies the importance of maintaining and conserving species. Indeed this work increases their "value," for example, by highlighting their features unique in the world's flora. This work could help us to consider which resources can and should be allocated to conservation. Further, this work potentially indicates new knowledge, and information that could be used for conservation. For example, the correlation of leaf traits with habitat can better characterize the locations to which species are adapted (Dunbar-Co et al., 2009), and thus, data from this project has been incorporated into the five year conservation reviews for the Hawaii Fish and Wildlife Service (Clark, 2009). This dissertation has increased the knowledge of these species' biology including their

relationships with their respective habitats, and also draws much positive attention to them in the eyes of science. I hope that this in some way contributes to the conservation of these plants. Thus, pure science is not only a fundamental reason for conserving the species in the first place (reason 5)—with its own necessary value and critical importance. This pure science can result in direct as well as indirect benefits to conservation.

FUTURE DIRECTIONS

This work emphasizes the need for greater conservation of all taxa within the lineage. Additionally, the development, physiology and tissue anatomy could benefit from additional work, especially with a focus on genetic mechanisms and phylogeny. Indeed, without proper genetic understanding of the relationships between these taxa, it is impossible to answer very specific historical questions about how, where, and when adaptive traits evolved. With a phylogenetic understanding, a clearer picture of the number of times traits evolved, for example, would be within our grasp. It would be exciting to know, for example, how many times vein islands evolved in this lineage, and whether it correlated with ancestral habitats, to gain a clearer answer to the question of whether or not that trait is adaptive in some way, or simply persisted due to low stabilizing selection. Work is ongoing on the phylogeny of all of the *Chamaesyce* clade of *Euphorbia* (Yang and Berry, in review), however, the directed attention that has been paid to the Hawaiian *Euphorbia* lineage is revealing new challenges given rapid evolution, apparent hybridization, lineage sorting.

I note that new systematic or taxonomic treatments of the lineage can benefit from the results of this study. The taxa of Hawaiian *Euphorbia* are currently determined by growth form, habitat and morphological observations and this work has greatly increased the quantitative detail of habitat and leaf morphology as well as composition. The findings in the present study show some striking differences (and similarities) between the species and varieties that could support the need for taxonomic rearrangement and motivate a full resolution of their evolutionary history and systematics. If adequate understanding and genetic resources can be compiled, this genus could serve as a world-class model for diversification of many traits central in plant biology, from biochemistry, development, and anatomy, to physiological and ecological function.

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