Demography and Genetic Diversity of the Endemic Tree Fern *Cibotium chamissoi* on O‘ahu Island, Hawai‘i: A Multi-Method Analysis of Population Dynamics, With Implications for Conservation

by

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Thesis directed by Susan W. Beatty

ABSTRACT

Despite their ecological importance and endemic status, little is known regarding the natural population dynamics of Hawaiian *Cibotium*. The primary goal of this research project was to determine the current population dynamics of the endemic tree fern, *Cibotium chamissoi* Kaulf. on O‘ahu Island. Demographic and molecular data were collected in order to determine recruitment, growth, mortality, and genetic diversity across existing size classes and natural habitats. Results indicate *C. chamissoi* demonstrates increasing growth with increasing size, and indeed can be considered slow growing species at an average of 3.8 cm/yr. Frond length and trunk top circumference were found to be better predictors of growth rate than total trunk length, although all morphological variables were significantly related to growth rate. Although recruitment was observed in half of our study sites, the rate of recruitment did not result in an increase in population size within any of the study plots at the conclusion of the study, due to greater observed rates of mortality. The oldest *C. chamissoi* that was measured had a trunk length of 428 cm at the conclusion of the study, and was calculated to be approximately 100 years old. In fact, it is likely that these tree ferns may be even older. Microsatellite results indicate that *C. chamissoi* is primarily outcrossing, with a breeding system that favors inter-gametophytic mating, as high levels of heterozygosity and genetic diversity were found within populations. Despite their high dispersal ability, however, several populations were found to have private alleles, indicating that gene flow among certain populations may be limited.

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CHAPTER ONE

INTRODUCTION

PURPOSE

Tree ferns of the genus *Cibotium* (Cibotiaceae Korall; *hāpu'u*) have a large presence in the wet and mesic forests of the Hawaiian Islands, and are considered to be common, endemic species that play an important ecological role where they occur. They may achieve very large sizes, maintaining a central, trunk-like rhizome that can reach over six meters tall on the island of Hawai'i (Rock 1913; Nelson and Hornibrook 1962; Wick and Hashimoto 1971), and sustain a crown of several fronds, each of which may extend up to seven meters long (Palmer 2003) and cover up to four square meters in leaf area (Durand and Goldstein 2001a; Arcand *et al*. 2008). *Cibotium* tree ferns form a closed sub-canopy in montane rainforests on the island of Hawai'i, reaching extremely high densities (Mueller-Dombois *et al*. 1981; Drake and Mueller-Dombois 1993; Kitayama *et al*. 1995), and have been identified as species of greatest conservation need among the Hawaiian flora and fauna (Mitchell *et al*. 2005). Despite their ecological importance and endemic status, little is known regarding the natural population dynamics of Hawaiian *Cibotium*. There have been no previous studies to determine natural rates of recruitment and mortality of *Cibotium* in their native habitat, nor have growth rates been measured for individuals over long time periods. Previous research indicates that these tree ferns are relatively slow growing on the island of O'ahu (Durand and Goldstein 2001a), and recruitment may be limited in some places, as several sampled populations were found to be absent of small and/or

intermediate individuals (Arcand 2007). Therefore, the primary goal of this research project is to determine the current population dynamics of the endemic tree fern, *Cibotium chamissoi* Kaulf. on O‘ahu Island. Demographic and molecular data were collected in order to determine recruitment, growth, mortality, and genetic diversity across existing size classes and natural habitats. Research objectives are generally motivated by conservation concerns that forest degradation and species extinctions are occurring at an alarming rate across the tropics, especially on islands (Caujape-Castells *et al*. 2010). Therefore, demographic and molecular studies of key species such as *Cibotium* may be useful to forest conservation and management practicioners.

The unique evolutionary history of Hawai‘i as an isolated hot spot archipelago has made it an exemplary case for studies of evolution, soil development, ecosystem function, tectonic geology, and island biogeography (i.e., see Wilson 1963; MacArthur and Wilson 1967; Carlquist 1974; Funk and Wagner 1995; Gagne and Cuddihy 1999; Price 2004). Scale and dispersal are common themes that unite much ecological and evolutionary research under the central tenant of island biogeography (MacArthur and Wilson 1967), and islands are ideal systems for examining patterns of dispersal and migration through studies of population genetics (Franks 2010). Studies of Hawaiian and other global tree fern species have been several (for example, see Becker 1976; Conant 1978; Seiler 1981; Tanner 1983; Ash 1987; Walker and Aplet 1994; Bittner and Breckle 1995; Bernabe *et al*. 1999; Chiou *et al*. 2001; Durand and Goldstein 2001a, 2001b; Vulcz *et al*. 2002; Ough and Murphy 2004; Korall *et al*. 2006; Schmitt and Windisch 2006; Mehltreter and Garcia-Franco 2008). However, there have been no previous projects that have combined the demograhic and molecular methods that I employ here to assess the population dynamics of an ecologically important island species. Long-term field studies of fern ecology *in situ* are

imperative to inform conservation management, and are significantly lacking in the literature (Arcand and Ranker 2008; Farrar *et al*. 2008; Mehltreter 2008). Furthermore, studies to facilitate the conservation of "keystone" or "umbrella" species can supply baseline ecological data for the management of forest habitat that may also benefit more vulnerable species (Caujape-Castells *et al*. 2010). With globally changing climate conditions and an environmentally uncertain future, Kramer and Havens (2009) suggest that research to maintain and restore resilient plant populations in the face of climate change will be most beneficial when ecology, demography, genetics, and evolutionary history are considered together under one unified approach.

Overview of Chapters, Objectives, and Research Questions

In Chapter One, I provide a broad overview of the topics investigated in Chapters Two and Three, including a background of the study species, a review of conservation concerns for endemic Hawaiian species and their habitats, the significance of the research, and a short explanation of how this project was conceived. In Chapter Two, I present the methods and results of a six year study of the natural growth, recruitment, and mortality of *C. chamissoi* on O'ahu Island. Growth rates as calculated for individuals from 16 *in situ* research plots are compared with growth rates of small greenhouse individuals. Specifically, I address the following research questions in Chapter Two: (i) Are there differences in *C. chamissoi* growth rate by size? If so, how should age estimates be calculated? (ii) At what average size do *C. chamissoi* reach fertility? (iii) What is the natural rate of *C. chamissoi* recruitment and mortality *in situ* over six years, and how does mortality vary by size and location? Chapter Three describes the methods and results of genetic analyses of the same tree fern individuals and populations as

measured in Chapter Two. I analyzed six polymorphic microsatellite loci, and present estimates of genetic diversity and clonal establishment for each sampled population. I address the following research question in Chapter Three: (i) What are the patterns of genetic diversity within and among populations of *C. chamissoi* on O‘ahu? (ii) What is the predominant method of *C. chamissoi* recruitment in natural populations: sexual reproduction of gameotphytes, or as ramets, distinguishable as vegetative clones? Chapter Four concludes with a summary and synthesis of results, with conservation implications.

BACKGROUND

HAWAIIAN TREE FERNS

Tree ferns are a significant component of most south temperate and tropical rainforests, especially of the wet tropical montane zone (Conant *et al*. 1994; Walker and Aplet 1994). As a group, tree ferns are classified based on molecular evidence to be placed in the order Cyatheales, and have been divided into eight families, 15 genera, and 663+ species (Smith *et al*. 2006). Korall *et al*. (2006) recognize the single genus of *Cibotium*, with 11 tree fern species worldwide within the family Cibotiaceae. They are distributed from eastern Asia, Malesia, Hawai‘i, southern Mexico, and Central America (Smith *et al*. 2006). Due to the combined effects of their considerable dispersal ability, and the high rates of speciation that occur on geographically isolated oceanic islands, ferns in general often demonstrate greater species diversity, higher rates of endemism, and higher proportions on oceanic islands as compared to the proportion of ferns in continental floral assemblages (Tryon 1970; Kramer 1993; Kessler 2010). This is clearly demonstrated in the two most recent treatments of the Hawaiian fern flora,

as of 144 native fern species, 106 are endemic, representing 74% endemism in Hawaiian ferns (Palmer 2003; Vernon and Ranker 2013). In comparison, the endemism of ferns and lycophytes of Costa Rica and Panama is estimated at 15% (Moran and Riba 1995; Moran 2008).

Among the endemic ferns of Hawai‘i, four species and one described hybrid occur in the genus *Cibotium*, with all but one species (*C. nealiae* O. Deg.) naturally found on the island of O‘ahu (Palmer 1994; 2003). Known locally as *hāpu‘u*, they are considered keystone species in the mesic and wet forests where they occur (Durand and Goldstein 2001a; Mueller-Dombois 2005). Previous studies have documented ferns to be habitat enhancers for other plants and animals (Medeiros *et al*. 1993; Ramos 1995; James and Burney 1997; Fjeldsa 1999; Loyn *et al*. 2001; Dobbs *et al*. 2003; Moran *et al*. 2003; Mueller-Dombois 2005), and *Cibotium* spp. do this in several ways. They are among the few Hawaiian species—and the most abundant—to grow directly from the mineral soil, whereas most other Hawaiian species have been found to commonly start as seedlings on logs above the soil surface (Cooray 1974; Santiago 2000; Mueller-Dombois 2005). *Cibotium* achieve subcanopy stature by growing a thick, erect rhizome, known as a caudex in tree ferns (Palmer 2003), and this caudex is covered by a dense mat of adventitious roots, which provides a preferential substrate for epiphytic establishment above the forest floor for canopy tree species such as *‘Ōhi‘a* (*Metrosideros polymorpha* Gaud.; Myrtaceae), other terrestrial vascular plants, and epiphytes (MacCaughey 1916; Mueller-Dombois *et al*. 1981; Medeiros *et al*. 1993; Santiago, 2000). *Ōhia* is an ecologically important, slow-growing canopy species, and is thought to rely on *Cibotium* spp. for regeneration. According to Rock (1913),

> Both the fern and the tree are often found growing together to such an extent that it is difficult to distinguish the tree trunk from the trunk of the fern. The natives have an idea that the *Hapu i‘i‘i* is the mother of the *‘Ōhia lehua*. The seeds of the *‘Ōhia lehua* often germinate in the crowns of the tree ferns, sending down their

> roots along the very fibrous, often water-soaked trunk. In time the fern begins to die and the *ʻŌhia lehua* is left standing with stilt roots of often 15 feet or more in height, after which the real trunk of tree commences (93).

In addition to providing an ideal substrate for plant establishment along their trunks (caudices), the large fronds of tree ferns play an important role in regulating light and moisture availability at the forest floor. They intercept, absorb, and re-direct a substantial amount of rainfall and fog drip at the sub-canopy layer (Takahashi *et al*. 2011; Arcand and Giambelluca, unpublished data), and *Cibotium* leaf-level transpiration rates were found to be similar to those of sampled canopy tree species (Kagawa *et al*. 2009), which in combination with their influence on the understory light regime, plays a large role in regulating the temperature, relative humidity, and moisture availability in the areas where they occur (Burton and Mueller-Dombois 1984).

Cibotium chamissoi, the species of focus in this study, is common from 150 – 1,200 m elevation in mesic to wet forests on Oʻahu, but less common above 800 m, and occasionally can be found as low as 50 m (Palmer 1994, 2003). It is scattered and uncommon on Molokaʻi, Maui, Lānaʻi, and Hawaiʻi, and has not been collected from Kauaʻi, the oldest of the Hawaiian Islands. In 1913, botanist Joseph Rock noted that *C. chamissoi* on the island of Oʻahu is much smaller and less common than on the island of Hawaiʻi, not forming the pure stands and covering large areas as it does on Hawaiʻi. However, *C. chamissoi* is the most common lower elevation tree fern on Oʻahu (Palmer 1994), growing in patches across a mosaic of intact and disturbed mesic to wet forest, and across a diversity of land ownership, public access, and management responsibilities. It has been noted to be the first *Cibotium* spp. encountered as one ascends in elevation, and at intermediate elevations it co-occurs with *C. menziesii* Hook., with which it has been found to hybridize on Oʻahu (*Cibotium chamissoi* x *C. menziesii* = *Cibotium* x *heleniae* D. D. Palmer) (Palmer 2003). *Cibotium chamissoi* is most often confused with *C. glaucum* (Sm.) Hook. & Arn.

in the field, but can be easily distinguished by a retained "skirt" of dead fronds, and the undersurfaces of the living fronds are light green with tan cobwebby hairs, as opposed to the light blue-gray to white (glaucous) undersurfaces of the fronds of *C. glaucum* (Palmer 2003; 1994).

The Hawaiian *Cibotium* spp. are slow growing and long-lived, although the average time it takes an individual to reach maturity is unknown, and growth rate measurements are complicated by the lack of information regarding time to trunk establishment of small individuals *in situ* (see Chapter Two). Based on laboratory and greenhouse trials, both the gametophyte and sporophyte of the Hawaiian *C. glaucum* have been classified as shade-requiring plants, with gametophytes demonstrating mortality and young sporophytes demonstrating restricted growth in full sunlight (Friend 1974). The adult sporophytes commoly occur in the forest understory with both *ʻŌhia* and *koa* (*Acacia koa* A. Gray; Fabaceae), the two most dominant and ecologically important native canopy tree species in the mesic and wet forests of Hawaiʻi. The density of tree ferns has been found to vary by substrate age on the island of Hawaiʻi, where Drake and Mueller-Dombois (1993) found mature *Cibotium* spp. to first appear on a 300 year flow and increase in density with age of substrate, achieving maximum density (approximately 3,000 individuals per hectare) on a 3,000 year flow. In another chronosequence study across the islands of Hawaiʻi, Molokaʻi, and Kauaʻi from youngest to oldest, Crews *et al*. (1995) found *Cibotium* spp. percent cover to increase from approximately 62% at Thurston (300 yr old) to 86% at Olaa (2100 yr), and then decrease with substrate age, from 70% at Laupahoehoe (20,000 yr), to 25% at Kohala (150,000 yr) on the island of Hawaiʻi, to 15% at Kolekole (Molokai: 1,400,000 yr), and nearly 0% at Kokee (Kauai: 4,100,000 yr). These studies

indicate that the Hawaiian *Cibotium* spp. are sensitive to variations in habitat such as light regime, forest structure, and soil nutrients.

THE FERN LIFE CYCLE

Ferns reproduce sexually via spores, which are very small, produced in great numbers, and distributed by the wind. A single frond of *C. chamissoi* produces 64 spores per sporangium (Gastony 1982), for an average of 700,000,000 spores per fertile frond (Dyer 1979). Ferns therefore have significant potential for dispersing across long distances, including multiple colonization events to remote islands, and heightened potential for inter-population gene flow within and among islands. For example, in the case of the Hawaiian fern genus *Dryopteris* Adans. (represented in Hawai‘i by 18 endemic and one native species), Geiger and Ranker (2005) found molecular evidence for five separate colonization events to the Hawaiian Islands, with most of the groups sharing closest relation to species from South East Asia. However, despite the potential for long-distance dispersal, mature ferns often drop spores in the neighborhood of parent plants (Sheffield 1996), so close in fact that Conant (1978) documented *Cyathea arborea*, a tree fern of 8 m tall, to drop the majority of its spores within 7.5 m of the parent. These findings lead to the possibility of two contrasting hypotheses regarding *C. chamissoi* populations on O‘ahu Island: 1) Populations may demonstrate high rates of gene flow and therefore little genetic differentiation among populations, or 2) Populations may be fragmented and/or geographically isolated to the extent that rates of gene flow are low, and therefore may demonstrate genetic differentiation among populations.

The fern breeding system presents additional considerations for predicting patterns in distribution and genetic exchange within and among populations. Ferns undergo two stages of

their general sexual life cycle: 1) the inconspicuous gametophyte, which germinates from a spore to a haploid (1n), usually heart-shaped, small green plant; and 2) the prominent diploid sporophyte plant (2n), which when mature, develop haploid (1n) spores. The sexual fern life cycle requires that the mature sporophyte produces viable spores, and that the gametophyte produces antheridia (male), archegonia (female), or both types of gametangia which then fertilize in one of three ways: intragametophytic selfing (union of sperm and egg from the same bi-sexual gametophyte); intergametophytic selfing (union of sperm and egg from different gametophytes, but from the same parental sporophyte); or intergametophytic crossing (union of sperm and egg from different gametophytes, from different parents) (Klekowski 1969; Ranker and Geiger 2008). Successful establishment and development of the gametophyte is largely dependant on the suitability of microhabitat (Cousens *et al*. 1985; Peck *et al*. 1990; Watkins *et al*. 2007; Farrar *et al*. 2008), which varies across spatial and temporal patterns of succession and disturbance (e.g. Beatty 1984; Hobbs and Huenneke 1992). Next, successful fertilization of fern gametophytes and the development of viable sporophytes depend on adequate availability of moisture, and in some cases, requires intergametophytic crossing (Farrar *et al*. 2008).

After successful fertilization of the gametophyte occurs *in situ*, the new sporeling may or may not persist and survive to maturity. Causes of mortality might be due to mechanical events, such as erosion along steep embankments, tree falls, burial by leaf litter, or predation. Light, temperature, and moisture are also extremely important environmental factors that limit the survival of sporelings. However, as demonstrated by Lloyd (1974) in the study of *C. glaucum* gametophytes, causes of sporeling mortality could be genetic, due to recessive lethal genes (genetic load) that may appear with inbreeding depression. Sexual recruitment could be limited by high genetic load and lethal alleles that may be present in populations with low genetic

diversity, leading to lack of sporophyte germination or high rates of mortality in small individuals.

The Hawaiian *Cibotium* are able to reproduce in two ways: by sexual reproduction and by lateral shoot development (Ripperton 1924; Ogura 1930). The patterns in method of recruitment of naturally-occurring tree fern populations have not been previously documented, and as noted by Mehltreter and Garcia-Franco (2008), "The capacity of tree ferns to ramify belowground has not been considered with regard to its spatial distribution…Future experiments could address this question with molecular methods to distinguish between trunk growth and leaf phenology of clonal and single trunks…" (pp. 11-12). In previous research, *C. chamissoi* on O'ahu was found to frequently maintain adventitious buds along the caudex, and 68% of large individuals were observed to have at least one adventitious bud (Arcand, 2007). In addition, many of the large individuals of this study have multiple caudices, which can be traced to a single large individual when connected aboveground. However, additional connections may occur underground as well. This growth habit raises the possibility that *C. chamissoi* groves may contain several genetically identicial individuals, and these may be identified as clones with the use of microsatellite analysis (see Chapter Three).

Conservation Concerns

Tropical ferns throughout the world are most threatened in centers of high endemism by the destruction and alteration of natural habitat (Gomez 1985; Arcand and Ranker 2008), and several species are especially vulnerable to extinction due to habitat and niche restrictions (Given 2002; Mehltreter 2008). An assessment by Wardlaw (2002) of the 1997 *IUCN Red List of Threatened Plants* concluded that 206 tree fern species (or 31% of the total species analyzed)

were threatened at that time. More recently in 2013, the *IUCN Red List of Threatened Species* unfortunately only reviewed 3% of all existing fern and fern allied taxa (315 species reviewed out of an estimated 12,000 globally described species), and reported 53.7% (or 167 species) of those evaluated are listed as critically endangered, endangered, or vulnerable (IUCN 2013). As pointed out by Mehltreter (2010), the very small number of species evaluated, and the strong taxonomic and geographic bias of evaluated species, prevents estimation of the actual threat status for most of the world's fern and lycophyte taxa. Mehltreter writes, "It is our incomplete knowledge of the abundance and ecology of ferns that impedes risk assessment," (2010: 347).

In Hawai‘i today, *Cibotium* spp. are considered common tree ferns, although as early as 1916 it was noted by MacCaughey that they were largest in size and achieve the greatest densities on the island of Hawai‘i. There, *Cibotium* spp. dominate the forest understory, and can reach heights over 20 feet tall, whereas on the island of O‘ahu, *Cibotium* spp. appear to be greatly reduced in distribution, size, and abundance. Causes of these variations in Hawaiian *Cibotium* densities between islands may be due to differences in soil nutrients with age of substrate (Crews *et al*. 1995), and the younger soils, taller forest structure, and larger areas of a flat topography may favor *Cibotium* spp. on the island of Hawai‘i (Kapua Kawelo, personal comm.; Matthew Keir, personal comm.; Joel Lau, personal comm.). In addition, it is thought that *Cibotium* populations on the island of O‘ahu have been reduced to a greater extent by various disturbances, including browsing pressure from feral pigs, predation from an introduced two-spotted leafhopper (Jones et al. 2000; Palmer 2003), invasive alien plant species, harvesting and collection for horticulture, habitat alteration and destruction, and climate change. It is likely that all of these factors have contributed to the reduced abundance and size of *Cibotium* on O‘ahu as compared to those on Hawai‘i. Indeed, the naturalization of non-native species, urban and rural

development, military occupation, and other human activities have taken their toll on the biota and ecosystem functions of the Hawaiian Islands, their cumulative effects having left Hawai‘i as the state with the highest number of federally listed species of threatened and endangered status in the United States (USFWS Endangered Species Database 2013) (Table 1.1).

State	***Animal spp.***	***Plant spp.***	***Total spp.***
Hawai‘i	61	338	399
California	123	180	303
Alabama	99	18	117
Florida	62	54	116
Texas	56	28	84
Georgia	38	22	60
Arizona	37	17	54
Oregon	34	16	50
Washington	28	9	37
South Carolina	15	19	34
Colorado	16	16	32
New York	18	7	25
Louisiana	18	3	21
Alaska	16	1	17
Minnesota	10	4	14
Montana	9	3	12
Maine	9	3	12
South Dakota	9	1	10
North Dakota	6	1	7

Table 1.1. Federally listed threatened and endangered species by selected states.
(USFWS Endangered Species Program, Species Reports 2013).

The most salient current threats to *C. chamissoi* populations and their habitat on the island of O‘ahu include invasive alient plant species, introduced mammals, harvesting and collection by people, and climate change. A more detailed summary of each of these topics is provided below.

Invasive Alien Plant Species

According to Wagner *et al*. (1999), over 1,000 non-native plant species have become naturalized in Hawai‘i, and while relatively few have become invasive, the ecological and economic effects of these few species are quite significant (Daehler *et al.* 2004). Invasive plant species often out-compete and displace native species, especially on oceanic islands, and subsequently ecosystem functions can become degraded or altered (Loope and Mueller-Dombois 1989; Schofield 1989; Vitousek and Walker 1989; Allison and Vitousek 2004b; Asner and Vitousek 2005; Mascaro *et al*. 2008). A plant species that is not a native component of local flora is considered “invasive” if the species demonstrates an ability to become established, increase in density, and expand in range while also causing the displacement, suppression, or degradation of native vegetation. The competitive advantages of invasive species have been demonstrated by measured higher specific leaf area, lower leaf construction costs, and higher photosynthetic rates in a comparison with native species on Hawai‘i (Baruch and Goldstein 1999). The authors hypothesize that invasive species may have been adaptively selected for their ability to compete for limited resources, explaining the demonstrated higher efficiency at resource capture when compared to native species, which are presumed to have evolved without such competitive selection (Baruch and Goldstein 1999).

The case of the introduced Australian tree fern, *Sphaeropteris cooperi* (Hook. ex F. Muell.) R. M. Tryon (Cyatheaceae), provides a good example of the various ways which invasive species achieve competitive dominance over endemic species of similar growth form. The Australian tree fern was reported to have escaped cultivation in upper Mānoa Valley on O‘ahu in 1950, and since has become naturalized on Kaua‘i, O‘ahu, Maui, and Hawai‘i (Medeiros *et al*. 1992; Palmer 2003). The relatively fast dispersal of *S. cooperi* throughout the

most populated islands of Hawai‘i can be traced to original human-facilitated introductions, as they are often used in landscaping and sold in plant nurseries, and have naturalized through wind-born spore dispersal from these source locations (Medeiros *et al*. 1992). The invasive *S. cooperi* has been found to grow faster, maintain a larger number of fronds, and reach fertility sooner than the native *Cibotium* spp. (Durand and Goldstein 2001a), and to exhibit a higher photosynthetic capacity and less photoinhibition in higher light regimes as compared to the native *Cibotium* spp. (Durand and Goldstein 2001b). This suggests that *S. cooperi* is more efficient at taking advantage of high-light environments such as those associated with disturbance (Durand and Goldstein 2001b). The invasive tree fern has also been found to suppress understory diversity and biomass (Medeiros *et al.* 1992), and to support less abundance and diversity of native epiphytes as compared to the native *Cibotium* spp. (Medeiros *et al.* 1993). In addition, *S. cooperi* leaf litter was found to decompose five times faster, produce two times more nitrogen (N), and three times more phosphorous (P) than the native *Cibotium glaucum* (Allison and Vitousek 2004b), and the leaf litter of *S. cooperi* was found to initially inhibit growth, and then increase foliar N content and total plant dry mass in three species of native plants, providing evidence that *S. cooperi* alters the nutrient dynamics of Hawaiian soils (Chau *et al.* 2013). These studies demonstrate why the Australian tree fern has been determined as, "…an invasive, disruptive species capable of radically modifying its habitat," (Medeiros *et al*. 1992), and "…a serious threat to Hawaiian ecosystems," (Palmer 2003: 245), "…especially those dominated by the native tree fern…" (Chau *et al*. 2013: 356).

Additional woody invasive plant species are known to alter the forest understory by forming dense, monotypic stands, reducing the abundance and diversity of native species through competitive exclusion, which may be aided by allelopathic methods in some species. On O‘ahu,

several species have been identified as invasive due to these traits, such as *Psidium cattleianum* Sabine (Myrtaceae; strawberry guava) and *Schinus terebinthifolius* Raddi (Anacardiaceae; Christmas berry) (Daehler 1998). *Schinus terebinthifolius* is a many-branched tree that can form a closed canopy, and is considered, "…a serious weed in many places, often forming dense thickets on steep slopes," (Wagner *et al*. 1999: 198) where few other species can coexist under its closed canopy. *Psidium cattleianum* also forms dense stands, prevents other plants from becoming established with allelopathic methods, is spread by feral pigs and birds, and is considered one of the most concerning invasive plant species in Hawai‘i (Wagner *et al*. 1999). Regeneration of native species was found to be extremely limited in lowland, exotic-dominated forests on windward Hawai‘i Island, where the invasive *Psidium cattleianum* was found to dominate the understory in over half of the study sites (Mascaro *et al.* 2008). A separate assessment of plant invasions in Hawai‘i reports that even at high elevations, and in protected fenced areas, the sampled plant communities contain several alien species (Daehler 2005). Indeed, both *P. cattleianum* and *S. terebinthifolius* occur commonly on O‘ahu, and frequently occur across my study plots (see Appendix 1), dominating the canopy at several sites.

The full removal of dense non-native plant canopy in Hawaiian mesic forests creates large gaps in the canopy and understory, increasing light availability and soil temperatures, which have been observed to be initially re-colonized more successfully by seedlings and root sprouts of invasive plants species, especially in the case of *Psidium cattleianum*. Other successful invaders of these gaps, and common in my study sites, include woody shrub species that form dense, impenetrable thickets such as *Clidemia hirta* (L.) D. Don (Melastomataceae; Koster's curse), *Lantana camara* L. (Verbenaceae), and *Ardisia elliptica* Thunb. (Myrsinaceae). An additional common invasive in closed-canopy mesic forests is the fern *Blechnum*

appendiculatum Willd. (Blechnaceae), which forms dense clonal colonies on the forest floor, competes with many native fern species (Palmer 2003), and was found to frequently occur in several of my research plots (see Appendix 2). *Blechnum appendiculatum* has been found to be favored in understory regeneration with ungulate exclusion on Kaua‘i (Weller *et al*. 2011), which is likely also the case on O‘ahu. It is understood that these gap-colonizing species are favored by disturbance, and are able to out-compete and possibly replace slower growing native species such as *C. chamissoi* and *Metrosideros polymorpha*.

Introduced Mammals

Introduced ungulates can also have devastating impacts to native island ecosystems, as isolated islands are more vulnerable to the effects of biological invasions due to their high proportions of endemic species which evolved in the absence of humans and grazing mammals (Carlquist 1974; Mueller-Dombois 1981; Wagner *et al*. 1985; Vitousek 1988; D'Antonio and Dudley 1995; Sakai *et al*. 2002). Feral goats and pigs on islands over-browse native vegetation, suppress native vegetation regeneration, facilitate invasive plant dispersal, and exacerbate erosion (Spatz and Mueller-Dombois 1973; Smith 1985; Cuddihy and Stone 1990; Stone *et al.* 1992; Dunkell *et al*. 2011). Feral pigs dig up large areas for mud wallows and forage, significantly altering the structure of the native understory (Spatz and Mueller-Dombois 1973; Mueller-Dombois *et al.* 1981; Smith 1985; Cuddihy and Stone 1990; Stone *et al.* 1992). Feral pigs are known to target the starchy cores of *Cibotium* trunks as a major food source, and even as early as 1916, MacCaughey wrote, “The pig hunters well know that fern groves are the favorite haunts of their game,” (p.4). Tree fern starch was found to compose over 90 percent of pig stomach contents in Kīlauea forest on Hawai‘i (Mueller-Dombois *et al.* 1981), and found to

compose the bulk of their diet in Kīpahulu Valley on Maui (Diong 1982). Pig herbivory of the trunk starch can cause mortality of the tree fern, and always does so if the fern growth apex is within reach (Mueller-Dombois *et al.* 1981). In a recent study by Murphy *et al*. (2013), widespread feral pig damage was observed to occur at their study site in Laupāhoehoe Forest on Hawai‘i Island, and was associated with decreased growth and survival of tree ferns.

Feral pigs occur on all of the main islands in Hawai‘i, and are locally hunted as an important source of food. However, public hunting access is a culturally sensitive and political issue in Hawai‘i, which often involves conflict between pig hunters, land managers, and conservationists. Since the arrival of Polynesians with the Polynesian pig (*Sus scrofa vittatus*), and later European introductions (*Sus scrofa scrofa*) (Tomich 1986), pork has been an important part of the local diet. However, land managers with conservation mandates in Hawai‘i invest a considerable amount of time and money mitigating the impacts of feral ungulates. For example, in the three national parks of Hawai‘i, pig control costs approximately $100 per pig per year (Rodolfo Zuniga, unpublished data, as cited in Pimentel *et al*. 2000). Areas identified as important for conservation, often including critical habitat for endangered species, have been shown to benefit with the protection of ungulate exclosure fences (Loope and Scowcroft 1985; Cabin *et al.* 2000). However, without additional control of invasive vegetation and rodents, native canopy tree species regeneration was found to be limited (Cabin *et al*. 2000; Weller *et al*. 2011). In addition, rats (*Rattus rattus*, black rats; *R. norvegicus*, Norway rats; and *R. exulans*, Pacific rats) are invasive species that seriously impact insular floras (Cuddihy and Stone 1990), and have been shown to limit the regeneration of native species within fenced areas (Pender *et al.* 2013), while also facilitating the distribution of non-native species (Shiels and Drake 2011). Approaches to conservation of native species in Hawai‘i may therefore require the control of

invasive plant species, rodent populations, and feral ungulates simultaneously. Lowland areas in Hawai‘i have become dominated by exotic species in forests that generated on lands after being subjected to centuries of agriculture and development (Mueller-Dombois and Fosberg 1998), and as accompanied by changing climate conditions, these “novel ecosystems” (Hobbs *et al*. 2006; Seastedt *et al.* 2008) will require novel approaches to management.

Current and Historic Human Uses of Tree Ferns

Tree ferns are used by people locally and globally for several purposes, such as a growth substrate for orchids, as decorative artistic containers, in gardens and landscaping, and as poles for construction of buildings, fences, and roads. Some tree fern species are especially useful for local construction due to their superior resistance to rot and termites, such as *Cyathea manniana* in Tanzania (Zilihona *et al*. 1998). Historically, the international trade of certain tree fern species has led to conservation concern that harvesting may threaten populations where they naturally occur. For example, *Cibotium barometz* (L.) J. Sm. has been listed in Appendix II of the Convention of International Trade of Endangered Species (CITES) due to its harvest for the Chinese herb medicine trade and the ornamental plant trade (Zhang *et al.* 2002). The commercial collection of tree ferns for horticultural use in landscaping (Vulcz *et al*. 2002), and harvesting for use as a growth substrate by orchid growers (Conant *et al*. 1994), has led to the listing of *Cibotium barometz*, all species of the family *Cyathea*, and all American species of *Dicksonia* in Appendix II of CITES for trade monitoring (Zhang *et al*. 2002; CITES 2011). However, several additional tree fern species are still considered vulnerable to overharvesting, such as the Old World species of *Dicksonia* which are not protected by CITES; *Dicksonia antarctica* is exported from Tasmania to supply Australian and European markets, and is considered endangered by

commercial sale, yet is not protected or monitored (Unwin and Hunt 1996; Mehltreter 2010). Additional tree fern species are considered to be of conservation concern from local harvesting pressures, such as *Alsophila setosa* in southern Brazil, where harvesting for the orchid industry and landscaping was among the threats to local populations (Schmitt and Windisch 2006). In Mexico, adventitious tree fern roots, known locally as *maquique*, are harvested and sold worldwide as a substrate for growing orchids and other epiphytic plants, and due to the damages observed to natural tree fern populations, several species have been protected under Mexican law, such as *Cyathea divergens* var. *tuerckheimii* R.M. Tryon, *Cyathea fulva* M. Martens et Galeotti (Eleuterio and Perez-Salicrup 2006), and *Alsophila firma* (Baker) D.S. Conant (SEMARNAT 2002; Mehltreter and García-Franco 2008). Despite these protections, harvesting continues to occur, in large part to satisfy local and regional tourism demand (Eleuterio and Perez-Salicrup 2006, 2009).

In Hawai‘i, similar uses of *Cibotium* are known to occur, but current harvesting practices and quantities have not been studied. Historically, tree ferns in Hawai‘i have been harvested for several purposes: the trunks for use in landscaping (Joe 1964; Teho 1971) and for their starchy cores in times of famine in ancient Hawai‘i to feed people, and also as regular hog feed (MacCaughey 1916; Fosberg 1942); manufactured logs for plant supports industry (Nelson and Hornibrook 1962), and for a short-lived starch industry (Ripperton 1924); the *pulu* (the fine hairs) for a short-lived mattress and pillow stuffing industry; and the fiddleheads for feeding livestock, and people (Teho 1971). MacCaughey (1916) reported that the local use of *Cibotium* trunks for the construction of hanging baskets, fern-boxes, and orchid pots by gardeners and florists had depleted the local woodlands of the older tree ferns near Honolulu in 1916.

According to Joe (1964), bare-root tree ferns from Hawai‘i began arriving in the mainland United States around 1952. Reports indicated they were being commercially harvested from Kaua‘i, Maui, and in the Puna District of Hawai‘i, and as the Hawaiian ferns grew more popular on the mainland, shipments from Hawai‘i increased. Unfortunately, Joe does not report volumes or pricing, but at the time of her report in 1964, she found Hawaiian tree ferns to be "…commonly planted in public places, office buildings, and private homes, where they may be grown in pots, in raised planting beds, and in gardens, indoors or outdoors. These ferns are among the most common tree ferns in ornamental use and may now have surpassed the Australian tree fern and Tasmanian tree fern in numbers," (Joe 1964: 138). The shipments inspected from Hawai‘i to the mainland included two species of *Cibotium*: *C. glaucum* and *C. menziesii*. Apparently at this time, *C. chamissoi* was noted to be "not in the trade," and "rare in cultivation," (Joe 1964: 139).

Currently it is legal for a person to obtain a permit for harvesting Hawaiian *Cibotium* from certain state forest areas, and harvesting is also commonly practiced on private lands. Living and dead trunks of *Cibotium* are available to purchase from two large retail chains on Hawai‘i, Maui, O‘ahu, and Kaua‘i Islands, and also can be purchased regularly online via eBay with shipping to the mainland United States. Because they are being used locally or shipped from Hawai‘i to the mainland U.S.A. (within-country), and since they are not listed in the CITES appendices, quantities of *Cibotium* exports from Hawai‘i to the mainland United States or to other countries are not monitored by CITES, nor are they prohibited.

Climate Change

Air temperature in Hawai‘i is linked to sea surface temperature (SST), and until 1970, has been strongly linked to the Pacific Decadal Oscillation (PDO) (Diaz and Giambelluca 2012). Generally, warm SST anomalies in the tropical eastern North Pacific and along the west coast of North America are associated with the positive phase of the PDO (Mantua *et al.* 1997), which are linked with multi-decadal dry periods (Chu and Chen 2005). Timm *et al*. (2011) found that the frequency of heavy rainfall events in Hawai‘i during the wet season (October – April) from 1958 – 2005 can largely be predicted by the Pacific-North American index (PNAI) and the El Niño-Sothern Oscillation index (SOI). El Niño events in Hawai‘i are highly correlated with deficient winter rainfall (Chu and Chen 2005). However, a shift has been observed to more frequent light precipitation, and less frequent heavy and moderate precipitation since the 1980’s in Hawai‘i (Timm *et al*. 2011). Wet season precipitation across the Hawaiian Islands during the late 21st century was found to be 5 – 10 % reduced, and 5% increased during the dry season (Timm and Diaz 2009). Regional surface temperature trends in Hawai‘i have been rising relatively rapidly over the last 30 years, and despite recent cooling associated with the PDO, surface temperatures in Hawai‘i have remained elevated (Giambelluca *et al*. 2008).

Global circulation models based on projections under various emission scenarios provided by the Intergovernmental Panel on Climate Change (IPCC) predict an increase in atmospheric CO_2 and other greenhouse gasses, raising the global mean temperature and sea level, and influencing storm patterns and precipitation (IPCC 2007). In Hawai‘i, evidence of these changes have been recently documented: temperatures have significantly increased at higher elevations over the last 60 years as measured by multiple weather stations across the state (Giambelluca *et al.* 2008), and sea surface temperatures are also warming, exhibited by

widespread coral bleaching and measured warmer temperatures in the oceans surrounding the Hawaiian Islands (Jokiel and Brown 2004). Further ecosystem-wide effects of warming are anticipated, as an increase in mean annual temperature has been shown to have an increase in soil-surface respiration in Hawaiian tropical montane wet forests (Litton *et al*. 2011), and changes in temperature and precipitation are likely to affect rates of evapotranspiration and streamflow (Safeeq and Fares 2012). A warmer and drier climate may act as a chronic disturbance for moisture-dependent plant species, and for spore-bearing plants like ferns, a decrease in moisture may limit the fertilization necessary to produce new sporophytes from natural gametophyte populations. During my study period, annual rainfall on O‘ahu was significantly lower than the 60-year average, and average air temperature exhibited a significant warming trend (see Chapter Two). With climate change scenarios predicting a continued increase in air temperature, we might expect to see *C. chamissoi* populations exhibit a demographic response to these changing growing conditions in the future.

Research Significance

This research will be a rare undertaking to link molecular evidence and six years of *in situ* data collected on the population demography of *Cibotium chamissoi* in explaining biogeographic patterns of recruitment, persistence, and mortality across the heterogeneous landscape of O‘ahu. Currently no literature exists which clarifies the reproductive strategies of naturally-occurring *Cibotium* spp., to determine whether vegetative or sexual reproduction is the predominant strategy, and under which natural, *in situ* conditions one or the other is more prevalent. Nor are there any published studies on the time to establish a caudex, or growth rates

of small *Cibotium*, or any other tree ferns for that matter; all published studies to date have documented the growth rates of individuals with an established caudex. Because age is assumed to be relative to caudex length, and growth rates may vary by size and habitat, this study will provide a more accurate method of age estimation across habitat types for *C. chamissoi* on O‘ahu. Findings of this research will compare mature *C. chamissoi* growth rates to previous growth rate findings by Durand and Goldstein (2001a) in their study of mature *C. chamissoi* at various elevations on O‘ahu.

Conservation efforts recognize that maintaining ecologically healthy forests is a crucial component in protecting critical habitat for endangered species. Wardlaw (2002) writes, “Ideally, conservation should be *in situ* and involve maintaining the ecosystem that contains the species” (p. 396). In addition, tropical montane forests are critical zones for watershed conservation, and they are important in maintaining soil stability and water quality. In Hawai‘i, montane forests are critical for surface and ground water supply (Giambelluca 1983; Giambelluca *et al*. 1986), and as tree ferns often comprise a significant portion of the tropical montane forest vegetation, baseline data is needed to assess current population dynamics and projected response of *Cibotium* to future ecological and climatological conditions.

Land managers are interested in using native tree ferns in restoration projects. There is also considerable private and commercial demand for tree ferns in landscaping and orchid horticulture, though current harvesting activities of *Cibotium* have not been investigated. Research to determine the natural population dynamics of *C. chamissoi* as it varies across habitats is crucial for understanding the potential impacts of harvesting activities to local populations. Furthermore, the ecological role of *C. chamissoi* in the mid to low elevation forest

on O‘ahu is not well understood. It is difficult to distinguish whether its presence is facilitative of a more native plant community, or whether it is limited to certain habitat parameters.

A comparison of the levels of genetic exchange at multiple scales will inform land managers whether the genetic integrity of geographically separate *C. chamissoi* populations should be maintained or enhanced in restoration plantings. For example, can nursery-propagated *C. chamissoi* from spores collected in the Wai‘anae Mountains of O‘ahu be planted in the Ko‘olau Mountains of O‘ahu? Would supplemental plantings of genetically different individuals encourage natural recruitment in areas where recruitment has been limited in recent years? Sporeling recruitment appears to be very limited in preliminary results from permanent growth plots on O’ahu, but *C. chamissoi* may be able to maintain abundance through its growth of multiple trunks and, along these trunks, additional trunk sprouts that are thought to be vegetative clones of the parent tree fern. If high rates of clonal establishment are documented within populations, then vegetative propagules might be sustainably harvested by land managers from naturally occurring populations of *Cibotium* to be transplanted into appropriate habitat areas targeted for restoration, without causing mortality of the parent individual.

PROJECT ORIGINS

This project was undertaken in collaboration with several management agencies, including O‘ahu Army Natural Resources (OANR), which is composed of civilian field staff contracted through the Research Corporation of the University of Hawai‘i to manage the endangered and threatened species and ecosystems occurring in Army training areas, the Hawai‘i Department of Land and Natural Resources Forest Reserve System and Natural Area Reserve

System, Ahupua‘a O Kahana State Park, and University of Hawai‘i Lyon Arboretum. The research was conceived, in part, to fulfill a request by OANR staff for more information regarding natural *Cibotium* population dynamics. The first botanist for OANR, Kapua Kawelo, was hired in 1995 after the U.S. Fish and Wildlife listed many new Hawaiian plant species as federally endangered. Kawelo hired additional field staff and began the large task of surveying and planning to ensure the survival of the endangered plants and animals that occur within the vast and remote forests of Army military installations. Before I began the graduate program at University of Hawai‘i, I was hired to work for OANR in 2002 as a natural resource technician, newly out of college with a B.A. degree in conservation biology from the University of Wisconsin. At that time, the OANR staff had grown to approximately eight. My work involved collecting rare plant cuttings and seeds, conducting rat control with poisoned bait and snap-traps, controlling feral goats and pigs around sensitive areas and endangered plants, fence monitoring, remote helicopter operations, remote camping, surveying for rare and endangered plants, monitoring endangered tree snails and endangered forest birds, and outplanting of nursery-grown endangered plants species to appropriate habitat. The OANR staff spend many hours conducting weed control, and my daily tools might include a weed whacker, herbicide backpack sprayer, hatchet, machete, chainsaw, handsaw, clippers, and a sickle. Sometimes we were required to wear protective bomb gear for safety while working in areas of unexploded ordinance.

Attempting to keep up with the “original” OANR staff in the field was challenging, but it was also motivational, and we all sought to become stronger and faster because of their exemplary work ethic. For example, one of the summer interns took to carrying small boulders in his backpack to get into better shape when he did not think he had enough gear to carry for the day. The dedication of Kawelo and OANR staff has been an inspiration to many in Hawai‘i, and

it was this field experience that led me to instigate a research project that could be useful to land managers in an applied sense. I was interested in the study of *Cibotium chamissoi* because it is a common, native species that is considered ecologically important, yet is still poorly understood.

CHAPTER TWO

POPULATION DEMOGRAPHY OF A HAWAIIAN TREE FERN (*CIBOTIUM CHAMISSOI* KAULF.): GROWTH RATE, RECRUITMENT, AND MORTALITY IN RELATION TO SIZE AND HABITAT

INTRODUCTION

Despite the growing evidence of their ecological importance, little is known regarding the population dynamics of endemic Hawaiian tree ferns (*Cibotium* spp.; Cibotiaceae) in areas of their natural habitat. They are thought to be long-lived, slow growing species, as previous research has estimated *Cibotium glaucum* on the island of Hawai‘i at ages over 100 years (Walker and Aplet 1994). Demographic studies of such long-lived species require long-term datasets, because population dynamics of long-lived species can be highly variable on shorter time scales due to varying conditions associated with habitat disturbance, climate, and competition (Oostermeijer *et al*. 1996; Pfeifer *et al*. 2006; Jongejans and de Kroom 2005; Jongejans *et al*. 2010). No previous research has included recruitment, mortality, and growth data for *Cibotium* populations across a range of habitats in Hawai‘i. Demographic studies to measure leaf production and calculate growth rates of global tree fern species have been several, i.e. *Cyathea arborea* and *Alsophila senilis* in Puerto Rico (Conant 1976), *Alsophila salvinii* in El Salvador (Seiler 1981), *Cyathea pubescens* in Jamaica (Tanner 1983), *Sphaeropteris senilis* in Venezuela (Ortega 1984), *Leptopteris wilkesiana* and *Cyathea hornei* in Fiji (Ash 1986, 1987), *Alsophila erinaceae*, *Alsophila polystichoides*, *Cyathea delgadii*, *Cyathea nigripes*, *Cyathea pinnula*, and *Cyathea trichiata* in Costa Rica (Bittner and Breckle 1995), *Cyathea caracasana* in Colombia (Arens 2001), *Alsophila setosa* in southern Brazil (Schmitt and Windisch 2006), and

Alsophila firma in Mexico (Mehltreter and Garcia-Franco 2008). However, studies to document the rates of reproduction and mortality of naturally occurring tree fern populations in general are missing from the literature.

The objectives of this study were to determine the natural regeneration, mortality, and growth rate of *C. chamissoi* as monitored across a diversity of habitats *in situ* on the island of O‘ahu. Results from previous research (Arcand 2007) indicate that for areas outside of ungulate exclosure fences in the Wai‘anae Mountains, *C. chamissoi* was reduced in abundance and was in overall decline through lack of recruitment and absent intermediate individuals. However, in the higher rainfall Ko‘olau Mountains, *C. chamissoi* appeared to be maintaining a relatively healthy population structure in all of the four measured plots, despite them being unfenced and accessible to feral pigs. These plots were measured once for a snapshot view of *C. chamissoi* population structure, which limited our understanding of population dynamics of this species over time. Long-term ecological data comparing growth rates, recruitment, and mortality among size classes and habitats do not exist. Therefore, in this chapter I report the results of repeated growth measurements and observations of recruitment and mortality for sixteen permanent *C. chamissoi* plots over a maximum of six years (2005 – 2011).

Methods of measuring rate of growth, and subsequently estimating age, are complicated for tree ferns due to their lack of a true woody trunk, without growth rings to count. Instead, lignified sclerenchymatic plates provide the support that allows a tree fern trunk to grow vertically (Large and Braggins 2004). For the purpose of simplicity, I use the term “trunk” to refer to this structure, as this is commonly used interchangeably for caudex (Palmer 1994), rhizome, and stem in tree ferns (Sharpe and Mehltreter 2010). Two approaches to measure tree fern growth include: 1) assessment of leaf production rates, and 2) repeated measurement of

trunk height (Schmitt and Windisch 2006; Sharpe and Mehltreter 2010). Some tree fern genera, such as *Cyathea*, *Leptopteris*, and *Alsophila* exhibit leaf scars along their trunks, which when analyzed with rate of leaf production, can be counted to estimate growth rate and age (Seiler 1981; Tanner 1983; Ash 1986; Ash 1987; Schmitt and Windisch 2006). For example, Tanner (1983) found that trunk height poorly predicted time for trunk growth of tree fern *Cyathea pubescens* Mett. ex Kuhn in Jamaica over one year, and Tanner suggests that the better method of determining growth rate with *Cyathea* species involves measuring the rate of leaf production and counting the number of leaf scars along the trunk. However, the Hawaiian tree ferns of the genus *Cibotium* grow with a thick layer of adventitious roots and leaf bases covering the leaf scars along the trunk, disallowing use of the leaf-scar method to measure growth without causing mortality of the fern. Ripperton (1924) estimated growth rate and age of *Cibotium* spp. using the leaf production and scar-counting method by harvesting eight individuals, stripping their bark (adventitious roots), and measuring their attributes; results indicate a possible over-estimation in growth rate (see Table 2.1).

	Mean leaf number	Leaf production (lvs / yr)	Leaf life span (months)	Trunk growth (cm / yr)	Timing of leaf production	Site
Cibotium chamissoi [a]	3 - 6	2.4 – 4.8	10.6 - 12	3.0	April; Aug/Sept	O‘ahu Island
Cibotium spp. [b]	5	--	21	5.1	Jan - March	Hawai‘i Island
Cibotium glaucum [c]	9 – 11	3.6 – 4.3	18 - 39	4.4 – 6.5	Feb - April	Hawai‘i Island
Cibotium spp. [d]	--	5	18 – 24	11.01	Early spring	Hawai‘i Island
Cibotium taiwanense [e]	5	3	15 – 26	--	Feb - April; July/Aug	Northern Taiwan

Table 2.1. Studies of *Cibotium* spp. that include growth data. [a] Durand and Goldstein (2001a); [b] Wick and Hashimoto 1971; [c] Walker and Aplet 1994; [d] Ripperton 1924; [e] Chiou *et al*. 2001.

Previous research has documented growth rates for *C. chamissoi* over one year on the island of O‘ahu at an average of 3.0 cm/yr for individuals of 1.5 to 2.0 meters in height, and

individuals were found to produce 0.2 - 0.4 leaves per month (2.4 – 4.8 leaves/year), with a mean leaf-life of 10.6 - 12 months (Durand and Goldstein 2001a). This observed growth rate is a bit lower than two estimated for the closely related species *C. glaucum* on the island of Hawai‘i, at 4.4 – 6.5 cm/yr (measured over two years), on younger and older sites, respectively (Walker and Aplet 1994), and 5.08 cm/yr (Wick and Hashimoto 1971) (Table 2.1). These studies also selected for larger individuals, with trunk heights greater than 30 cm, and may limit the accuracy of age estimates for Hawaiian *Cibotium*. Both methods of measuring tree fern growth rate have been noted to lack the ability to determine growth rate before initial development of the trunk (Seiler 1981; Tanner 1983; Schmitt and Windisch 2006), and, “for all calculations of age, a critical problem is that the time required by young sporophytes to develop an erect trunk is not known,” (Bittner and Breckle 1995: 40). I address this problem by including in this chapter the growth rates of *C. chamissoi* started from spores and grown under nursery conditions by fern horticulturalist Kay Lynch on O‘ahu during the study period (Lynch, unpublished data). These data will be compared with observations of naturally occurring young *C. chamissoi* as measured in the field.

Some authors have estimated tree fern ages based upon the assumption of a constant growth rate over the life of the fern (Seiler 1981; Bittner and Breckle 1995), although other authors have acknowledged the limitations of this method (Arens 2001; Sharpe and Mehltreter 2010). Sharpe and Mehltreter (2010) suggest that age estimates for *in situ* tree ferns are, “…complicated by several problems such as gradually decomposing older tissue in rhizomes of understory ferns, and variable growth rates throughout the life of an individual tree fern,” (Sharpe and Mehltreter 2010: 61). Variations in growth rates throughout the life of a tree fern may be in part due to size, as previous studies suggest an increased rate of leaf production with

increased tree fern height (Tanner 1983), increased growth rate with increased stem height (Ash 1986), and overall increase in biomass production with increase tree fern height, until individuals fall over (Ash 1986). Variable growth rates among tree fern individuals of different habitats may also be related to different forest canopy structure and/or different successional stages after disturbance (Schmitt and Windisch 2006).

RESEARCH QUESTIONS

i) Are there differences in *C. chamissoi* growth rate by size? If so, how should age estimates be calculated?

H: Small C. chamissoi (15 cm trunk length or less) grow more slowly than intermediate or large individuals.

ii) At what average size do *C. chamissoi* reach fertility?

H: Fertile fronds of C. chamissoi will emerge when individuals are between 20—30 cm in total trunk length.

iii) What is the natural rate of *C. chamissoi* recruitment and mortality *in situ* over six years, and how does mortality vary by size and location?

H_1: Recruitment will be generally low, especially in the drier Wai'anae plots.

H_2: Mortality will be high for sporelings, and low for intermediate and mature individuals.

METHODS

STUDY AREA

The island of Oʻahu is of volcanic origin ca. 3.7 million years old, located 22 degrees north of the equator, and 1,574 km^2 in area (Wagner *et al.* 1999). The Koʻolau Mountains shape the windward, eastern side of the island, where the northeasterly trade winds cause higher levels of rainfall. Maximum annual rainfall at the summit of the Koʻolau range reaches 7,000 mm, and 1,500 mm along the windward coast (Giambelluca *et al.* 1986). The Waiʻanae Mountains form the leeward, western side of the island where rainfall is typically much lower, especially along the coast. Mount Kaʻala in the Waiʻanae range is the highest point on Oʻahu at 1,225 m, and it receives upwards of 2,000 mm annual rainfall (Giambelluca *et al.* 1986).

Nursery-grown *C. chamissoi* were started by Kay Lynch as spores collected from wild individuals, and sown in a lab at the University of Hawaiʻi, Mānoa between 2001 and 2007. Sporelings that germinated were eventually transferred to Lynch's fern nursery, Laʻau Hawaiʻi, located in Kahaluʻu on the northeast side of Oʻahu to be grown, transplanted, and measured (Lynch, unpublished data).

Sixteen permanent *in situ* plots, 10 x 20 meters in area, were first established and measured in 2005-2006 (Fig. 2.1) (Arcand 2007), and were measured approximately annually from 2005 to 2011. *In situ* plots were located in areas of accessible *C. chamissoi* groves in an attempt to include at least 15 individuals to capture current population structure at different sites.

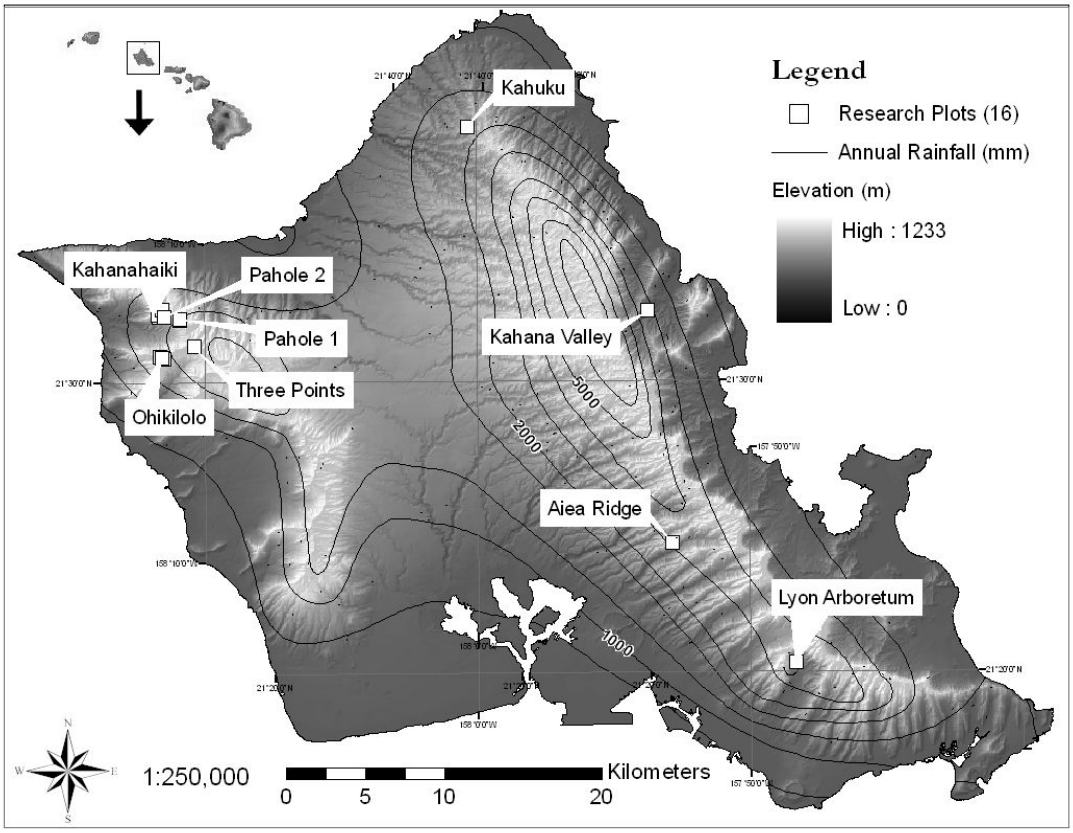


Figure 2.1. Location of Research Plots, O‘ahu Island, HI (Arcand 2007). The high elevations in the west form the Wai‘anae Mountains, and in the east the Ko‘olau Mountains.

Sites were selected in the western Wai‘anae range to be compared inside and outside of fenced ungulate exclosures, and paired at similar elevation, aspect, and slope. At some of these sites, however, *C. chamissoi* density was sparse, and so plots were located to include as many individuals as possible. Twelve plots located in the Wai‘anae Mountains were established at elevations from 585 –920 m, occur on slope gradients between 7 to 37 degrees, north-northwest to east-northeast in aspect, with 1383 – 1764 mm annual rainfall (Table 2.2). Four unfenced plots located in the eastern Ko‘olau Mountains occur at elevations from 135—440 m, occur on slope gradients between 27 to 37 degrees, north-northeast to south-southeast in aspect, with 2356 – 3836 mm annual rainfall (Table 2.2).

Plot Name	Plot Code	Mountain Range	Fenced	Non-Native Mgmt.	Elevation (m)	Aspect	Slope °	Annual Rainfall (mm)	Forest Type	Dominant Overstory Species	Native	Non-Native
Pahole In 1	W_Pah1-a	Waianae	yes	no	635	NE	34	1383	Mesic	*Schinus terebinthifolius*		X
Pahole Out 1	W_Pah1-b	Waianae	no	no	675	NE	24	1513	Mesic	*Psidium cattleianum*		X
Pahole In 2	W_Pah2-a	Waianae	yes	no	670	NE	34	1425	Mesic	*Schinus terebinthifolius*		X
Pahole Out 2	W_Pah2-b	Waianae	no	no	685	NE	37	1513	Mesic	*Psidium cattleianum*		X
Kahanahaiki In	W_Kiki-a	Waianae	yes	yes	590	NW	7	1406	Mesic	*Metrosideros polymorpha*	X	
Kahanahaik Out	W_Kiki-b	Waianae	no	no	600	NW	19	1384	Mesic	*Metrosideros polymorpha*	X	
Kahanahaiki Out Steep	W_Kiki-c	Waianae	no	no	585	NW	34	1384	Mesic	*Metrosideros polymorpha*	X	
Ohikilolo In	W_Ohik-a	Waianae	yes	yes	920	NE	20	1485	Mesic	*Metrosideros polymorpha*	X	
Ohikilolo Out	W_Ohik-b	Waianae	no	yes	883	NE	32	1476	Mesic	*Acacia koa*	X	
Ohikilolo Out Weedy	W_Ohik-c	Waianae	no	no	867	NW	31	1485	Mesic	*Schinus terebinthifolius*		X
3 Points In	W_3Pts-a	Waianae	yes	yes	841	NE	13	1764	Mesic	*Metrosideros polymorpha*	X	
3 Points Out	W_3Pts-b	Waianae	no	no	841	NE	22	1764	Mesic	*Metrosideros polymorpha*	X	
Kahuku MTA	K_Kahuku	Koolau	no	no	337	NE	37	2356	Mesic	*Acacia koa*	X	
Kahana State Park	K_Kahana	Koolau	no	no	135	SE	27	3812	Mesic	*Pandanus tectorius*	X	
Aiea	K_Aiea	Koolau	no	no	440	SE	28	3123	Wet	*M. polymorpha / A. koa*	X	
Lyon Arboretum	K_Lyon	Koolau	no	yes	230	NE	29	3836	Wet	*Simarouba glauca*		X

Table 2.2. Summary Description of Research Plots. Mean annual rainfall was interpolated using data provided by *The Rainfall Atlas of Hawai'i* (Giambelluca *et al.* 2011), based on data during the 30-year period 1978 – 2007. Native / non-native refers to the dominant overstory species listed for each site.

Plot measurements of percent cover by native and non-native species, *C. chamissoi* density, and canopy density are provided in Appendix 3. Mean annual rainfall was estimated by interpolation at each plot location using data provided by *The Rainfall Atlas of Hawai'i* (Giambelluca *et al.* 2011) (Fig. 2.2), which is based on data collected from multiple stations during the 30-year period 1978 – 2007. These rainfall estimates do not include fog drip, which is moisture from cloud droplets directly intercepted by vegetation. Plots that are located more distant from the nearest weather station reflect a greater reliance on interpolated rainfall values and a higher degree of uncertainty.

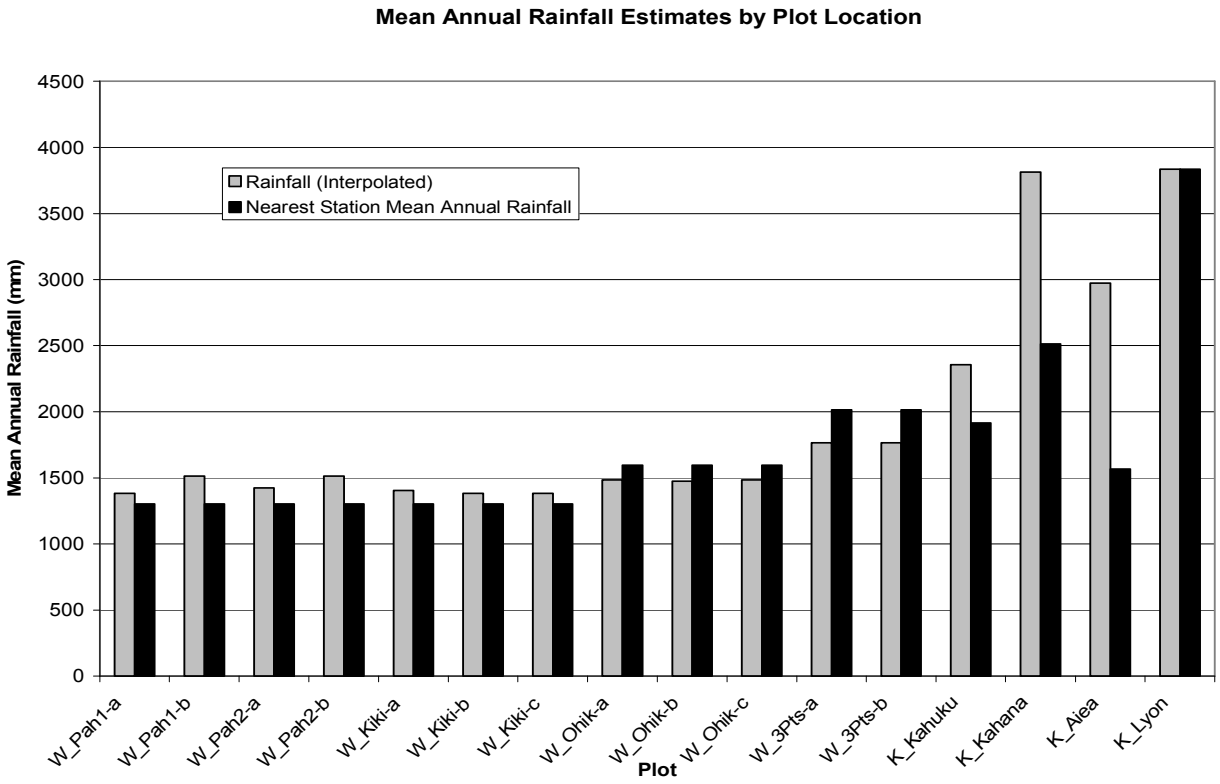


Figure 2.2. Interpolated mean annual rainfall (grey bars) and measured mean annual rainfall for the nearest weather station (black bars) for each plot location. Data provided by *The Rainfall Atlas of Hawai'i* (Giambelluca *et al*. 2011), for the 30-year period 1978 – 2007.

The following plot descriptions have been adapted from my M.A. thesis (Arcand 2007).

Pahole and Kahanahāiki

Four research plots were placed within the Pahole Natural Area Reserve (NAR), which is managed by the state of Hawai‘i as part of the Natural Area Reserve System, and is on the eastern boundary of Kahanahāiki in the Wai‘anae Mountains (see Fig. 2.3).

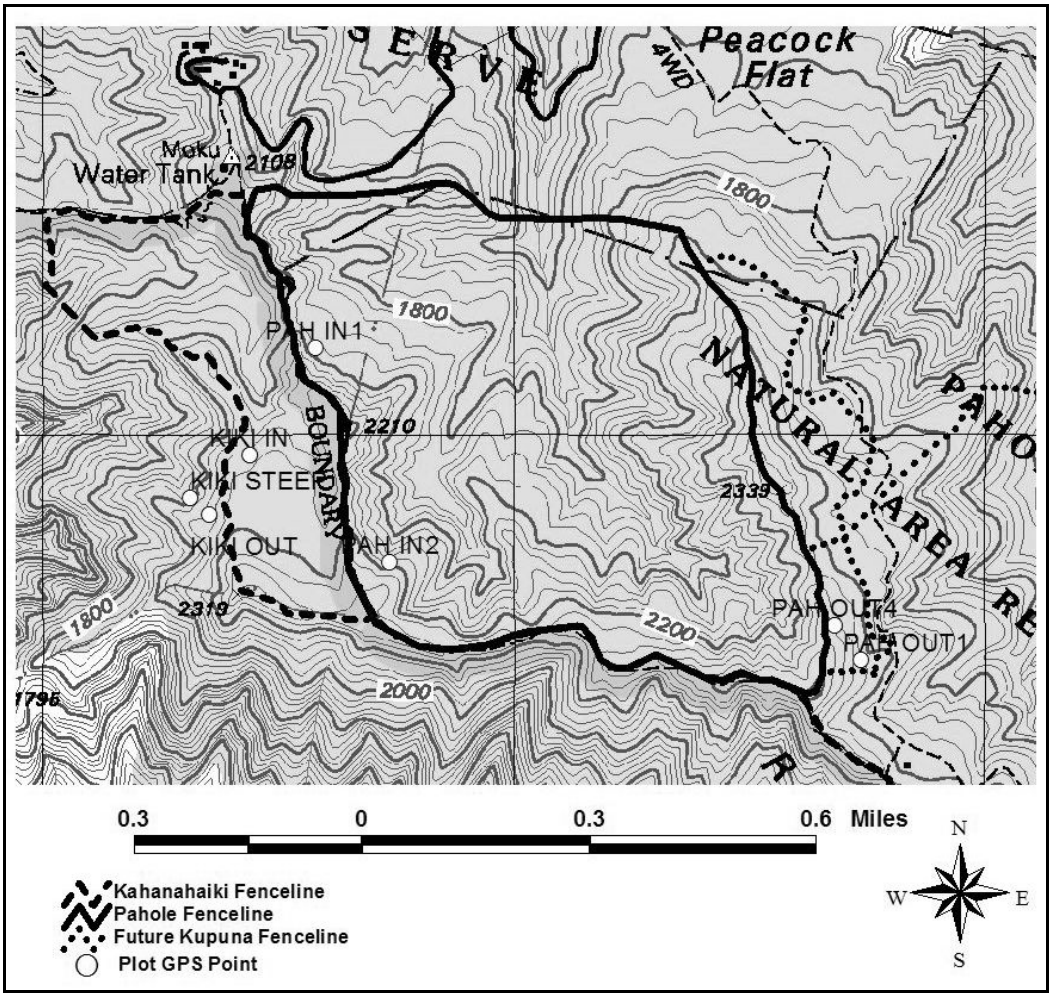


Figure 2.3. Kahanahāiki and Pahole Plot Locations, Wai‘anae Mountain Range. Kahanahāiki fence indicated by long dashes, Pahole fence by solid black, and the Kapuna fence by dots.

Two fenced Pahole plots were located on slopes near the ridge dividing the two valleys, where the canopy was dominated by the non-native *Schinus terebinthifolius*. The indigenous fern *Microlepia strigosa* was found to dominate the understory at the Pahole In 1 site, and the non-native fern *Blechnum appendiculatum* dominated the understory at the Pahole In 2 site. The two

unfenced Pahole plots were located in an area that was in the process of fence construction, in a new addition enclosed by the Kapuna fence (see Fig. 2.3), where the canopy was dominated by the non-native *Psidium cattleianum*. The understory of both the Pahole Out 1 and Out 2 sites were also found to be dominated by *P. cattleianum*. The Pahole fence was completed in December of 1996, and pig free 11 months later in 1997 (T. Takahama, pers. comm.). Pahole gulch fence encompasses 217 acres. The Kapuna fenceline was enclosed in 2006, and pig-free by 2007 (T. Takahama, pers. comm.).

Near Pahole, three additional research plots are located within the Kahanahāiki Management Unit in the Mākua Military Reservation along the northeastern rim of Mākua Valley (Fig. 2.3). The area is managed by Army Natural Resources (ANR), a contracted group of civilian employees within the Environmental Division of the Department of Public Works of the U.S. Army Garrison, Hawai‘i. The native canopy tree *Metrosideros polymorpha* dominated all three study sites in the Kahanahāiki area. The understory was dominated by the native fern *Microlepia strigosa* for the Kahanahāiki In site, and by the non-native grass *Paspalum conjugatum* for the Kahanahāiki Out site. A third plot, Kahanahāiki Out Steep, was measured along a steep slope flanking a seasonal waterfall outcropping, where the non-native fern *Blechnum appendiculatum* dominated the understory.

The Kahanahāiki fence was constructed and the enclosures made pig free by 1998 (Army Natural Resources Center 2004). Kahanahāiki fence encompasses 90 acres. Feral pig and goat control has been conducted by ANR staff outside the fence along the ridge adjacent to the two unfenced Kahanahāiki plots, in addition to the larger area around the Kahanahāiki fence beginning in 1998 (Army Natural Resource Center 2004), and also by NAR staff in the Pahole

NAR and vicinity. Occasional pig incursions occurred within both fences during the study period, but did not leave evidence of disturbing the research plots, and were eventually removed.

Vegetation throughout the Kahanahāiki and Pahole area is considered mesic forest. Non-native plant control efforts have continued within both fenced areas and in small areas outside of fences, with more intense management focused around endangered plant and animal populations, and areas with higher native species diversity. The landscape history of Pahole, and likely areas of Kahanahāiki, involves disturbance caused by logging and cattle. Little history of the area has been published, but according to a long-time Pahole NAR specialist on Oʻahu:

> "I know that the forests in Waialua were cut for fire wood and for the earliest boilers at the mill before they changed over to coal. When I started there still was a working ranch below the NAR and we had at least 2 incursions of cattle into the heart of the reserve. During one of those roundups the old cowboy told me about how they used to run cattle across the lower portion and high ridges of what became the NAR. Additionally, water works were built to capture and convey fresh water to the ranch down below. There's still evidence of that infrastructure around today," (Takahama, pers. comm).

ʻŌhikilolo

Three ʻŌhikilolo research plots were located within ʻŌhikilolo Management Unit along the southern ridgeline of Mākua Valley in Mākua Military Reservation, managed by ANR. The vegetation is considered mesic forest, and the canopy was dominated by the native tree *Metrosideros polymorpha* at the ʻŌhikilolo In site, the native *Acacia koa* at the ʻŌhikilolo Out site, and the non-native *Schinus terebinthifolius* at the ʻŌhikilolo Out Weedy site. The understory was dominated by the indigenous sedge *Carex meyenii* at the ʻŌhikilolo In site, the endemic fern *Deparia prolifera* at the ʻŌhikilolo Out site, and the non-native fern *Blechnum appendiculatum* at the ʻŌhikilolo Out Weedy site.

The fenced forest exclosure was built and free of goats by 1999 and encompasses approximately 2.5 acres. An additional fenceline runs the length of ʻŌhikilolo ridge in order to reduce the browsing pressure of feral goats within Mākua Valley from neighboring goat source populations, and goat populations were noted to be significantly reduced by 2000, although some goats still inhabited lower Mākua Valley in greatly reduced numbers until 2004 (Kapua Kawelo, pers. comm.). Feral pigs are thought to be absent or rare in the area because of the steep cliffs and slopes below and along the ridge, and ANR has never detected pigs in the area (Army Natural Resource Center 2004). The ANR staff and volunteers have spent a significant amount of time conducting weed control inside the W_Ohik-a plot and outside the fence in the W_Ohik-b plot (Jane Beachy, personal comm.).

Three Points

Two research plots at the Three Points site were located within Mokulēʻia Forest Reserve, bordering the southeast rim of Mākua Valley and managed by the state of Hawaiʻi. The approximately six acre fenced exclosure surrounds mesic forest, and was constructed and declared pig free in 2001. *Metrosideros polymorpha* was the dominant canopy tree species at both sites, and inside the fence the understory was dominated by the non-native shrub *Rubus argutus*. The understory at the Three Points Out site was dominated by the non-native *Psidium cattleianum*.

Pig browsing pressure in this area was high, and occasionally small pigs have been able to breach the fence. In 2006, at least two small pigs were inside the fence and had been digging in an area adjacent to the research plot. The plot was intentionally delineated to avoid the area disturbed by pig digging, and hunters removed the pigs soon thereafter. Prior to fence

construction, 44 pigs were removed from the area by the Department of Land and Natural Resources in cooperation and with the support of ANR between January 2000 and October 2000 (Army Natural Resource Center 2004), which is quite numerous for the short time span of 10 months. Since Mokulēʻia Forest Reserve has been opened to hunters, an additional 15 pig catches from the area had been reported by September 2003 (Army Natural Resource Center 2004). Weed control has been, and continues to be conducted at Three Points inside the fence: ANR collaborates with the State for an offsite management partnership.

Kahuku

The single Kahuku plot was located within Kahuku Military Training Area, on the northern leeward side of the Koʻolau Mountain range, and is managed by ANR. The plot was located near a ridge 4WD road that is occasionally used by military vehicles and soldiers for training activities. During the study period, road construction caused a high amount of erosion to occur in parts of the plot, which likely influenced mortality and growth of small plants on the more exposed, steep areas at this site. Weed control has not been previously conducted within the area, which is considered to be mesic-wet forest vegetation. The dominant canopy species was the native tree *Acacia koa*, and the indigenous woody climber *Freycinetia arborea* was the dominant understory species. There is no public hunting in the vicinity of the research site.

Kahana

The research plot in Kahana Valley State Park was located on the windward side of the Koʻolau Mountains at approximately 135 m elevation, close to the valley bottom on the northern side of the valley. The vegetation community is considered to be coastal mesic forest (Wagner *et*

al. 1999) with the indigenous tree *hala* (*Pandanus tectorius* Parkinson ex Zucc.) dominant in both the overstory and understory. No weed control had been conducted within the research area. According to Hawai‘i State Division of Forestry and Wildlife records, public hunters reported a total of 20 feral pigs harvested in Kahana Valley over 10 months in 2005 – 2006. Public hunting continues to be allowed in Kahana.

‘Aiea Ridge

The ‘Aiea Ridge plot was located within the State of Hawai‘i managed Honolulu Watershed Forest Reserve in the Ko‘olau Mountains. Located near the main ridge hiking trail in a less steep area, non-native and invasive plant species appear to be dispersed along the trail and were observed to infiltrate the plot area. *Metrosideros polymorpha* and *Acacia koa* co-dominated the forest canopy at this site, and the naturalized shrub *Rubus rosifolius* was found to dominate the understory. There were recent signs of disturbance caused by feral pigs. Public hunting is allowed, and hunters with dogs were in the vicinity as I surveyed the research plot in 2006. Non-native plant management has not been conducted within the area. During the study period, research tags marking plot corners and several ferns were removed by someone unknown, but this did not interfere with subsequent data collection as plots had been previously mapped.

Lyon Arboretum

The Lyon Arboretum is located in the Ko‘olau Mountains and managed by the University of Hawai‘i in Mānoa Valley. Feral pig control has been administered in the Arboretum through hunting. The plot is located in “Valley 4C” within a dense *C. chamissoi* grove. Weed management was conducted over roughly three days in the area of the research plot in 1981 (Ray

Baker, pers. comm.). Canopy and understory species in the area are predominantly exotic species either intentionally planted or spread from collections introduced by Arboretum staff. The canopy was dominated by the non-native tree *Simarouba glauca*, and the understory was dominated by the indigenous fern *Sphenomeris chinensis*.

CLIMATE

Mean monthly temperature and rainfall data for O‘ahu Island were available for the years 1951 – 2011 from Honolulu International Airport Station (# COOP:511919), located at 2.1 m elevation, 21.32° N, 157.93° W, as reported by the National Climactic Data Center (NCDC) of the National Oceanic and Atmospheric Administration (NOAA), Annual Climate Summary, Quality Controlled Local Climatological Data (Fig. 2.4).

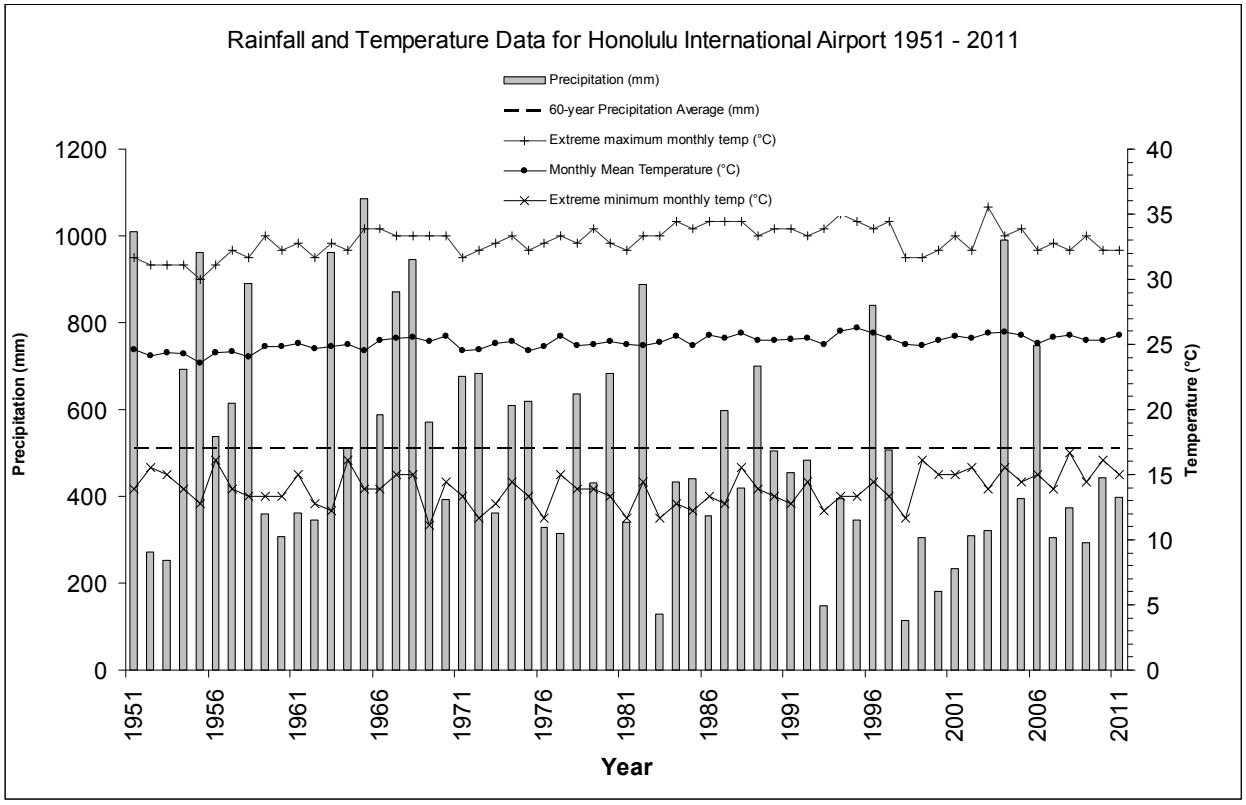


Figure 2.4. Mean annual temperature and rainfall as reported by the NCDC of NOAA, Annual Climate Summary, Quality Controlled Local Climatological Data for Honolulu. Gray bars indicate precipitation (mm), and the dotted line indicates the 60-year precipitation average (mm); both can be read on the left y-axis. The lines marked with crosses, dots, and x's indicate extreme maximum, mean, and extream minimum monthly temperature (°C) respectively, and can be read on the right y-axis.

Mean monthly temperature was averaged by year, and results of standard linear regression in SPSS demonstrate a statistically significant increase over time, of approximately 2.3 °C over the past 60 years ($y = 0.0214x - 17.289 \pm 0.387$ *SE*, $R^2 = 0.4959$, $P < 0.01$). Precipitation as reported by the NCDC of NOAA appears quite variable, but annual totals demonstrate a negative trend, with less precipitation in recent years ($y = -4.64x + 9704.5 \pm 231.2$ *SE*, $R^2 = 0.1143$, $P < 0.01$) (Fig. 2.5).

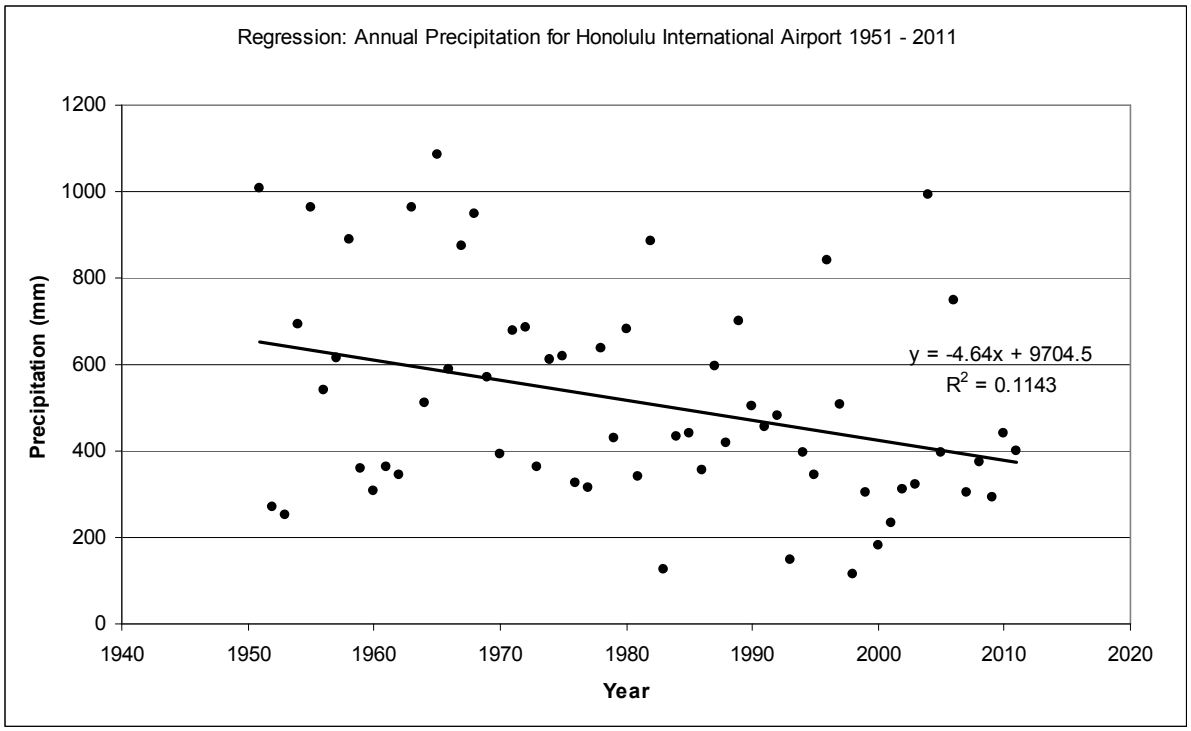


Figure 2.5. Rainfall as reported by the NCDC of NOAA, Annual Climate Summary, Quality Controlled Local Climatological Data for Honolulu. Line represents standard linear regression of annual precipitation ($y = -4.64x + 9704.5 \pm 231.2$ *SE*, $R^2 = 0.1143$, $P < 0.01$).

The effects of urban centers on local temperature measurements have been demonstrated to cause an overestimation in warming regional air temperature trends due to the urban heat island effect (Oke 1982; Li and Bou-Zeid 2013). However, when surface temperature data were analyzed both with and without the urban station data for Hawai‘i, Giambelluca *et al.* (2008) found no significant effect of the urban station data, and in fact mean temperatures were slightly warmer when the urban station data was removed (Giambelluca *et al*. 2008). Therefore, the data as reported for Honolulu by the NCDC of NOAA are likely representative of general atmospheric trends on O‘ahu Island over the past sixty years.

Generally Hawai‘i experiences warmer and drier weather in the summer, from about May to September, and wetter, cooler weather in the winter, when there are longer periods of widespread rainfall and cloud cover from October to April (Wagner *et al*. 1999). Prevailing

surface winds are from the northeasterly direction throughout the year, severe storms are infrequent, and temperatures are mild and equable year round (Wagner *et al*. 1999). This pattern is demonstrated in the monthly average temperature and cumulative rainfall data for Honolulu International Airport during the study period, 2005 – 2011 (Fig. 2.6).

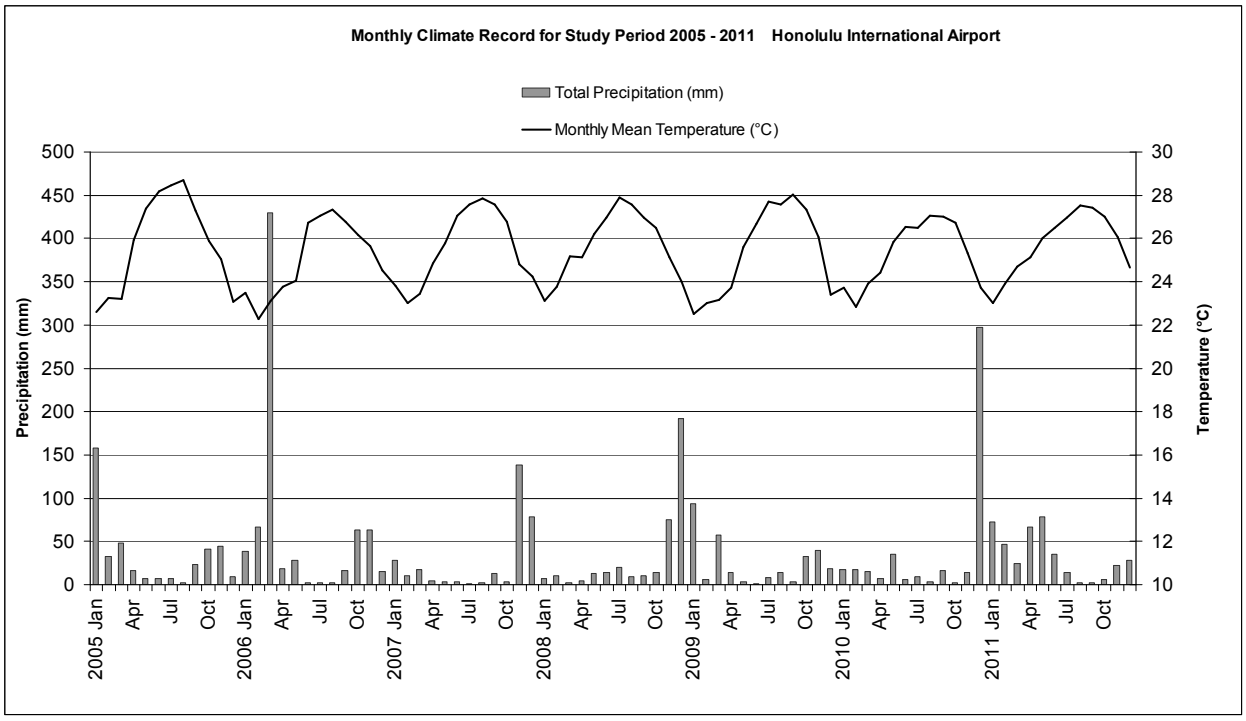


Figure 2.6. Monthly average temperature and cumulative rainfall data for Honolulu International Airport during the study period, 2005 to 2011, as reported by NCDC of NOAA, Annual Climate Summary, Quality Controlled Local Climatological Data. Precipitation (mm) indicated by gray bars can be read on the left y-axis, and temperature (°C) indicated by the solid line on the right y-axis.

Monthly mean temperature was observed to fluctuate between a low of 22.3 °C (72.1 °F) in the winter months, and a high of 28.7 °C (83.6 °F) in the summer, with maximum precipitation falling in the winter months. It is clear that during the study period, annual precipitation has generally remained well below the 60-year average (512.7 mm/year), with March of 2006 receiving the highest precipitation at 429.8 mm.

NURSERY GROWTH DATA

The following information has been shared by horticulturalist Kay Lynch based upon her own research for 46 *C. chamissoi* individuals that were started in the laboratory, and grown after germination at the plant nursery La‘au Hawai‘i (Lynch, unpublished data). Knop’s solution was used as a spore-germination medium to provide nutrients such as potassium nitrate, magnesium sulfate, calcium nitrate, and potassium phosphate; after sporophytes germinated they were transferred to half-strength Murashige and Skoog (1962) growth medium (Lynch 2011). Gametophyte cultures were maintained, and produced zero to many sporophytes, which were then transferred to tubes or tubs to grow until approximately three inches tall (Lynch 2011) (Fig. 2.7). Next, sporelings were transferred to a mixture of peat and pearlite (ratio 1:2) in covered tubes or tubs, and pre-hardened in the laboratory for approximately one to two months by gradually loosening and then removing the lid (Lynch, pers. comm.) (Fig. 2.7).

Figure 2.7. Images of a newly emerged sporeling of *C. chamissoi* from gametophyte culture (left), and sporelings being pre-hardened in tubes (right).

Sporophytes were then transferred to two inch pots with peat and pearlite (ratio 1:2), and Nutricoat 13-13-13 (Fig. 2.8). Newly potted individuals were covered with inverted clear 24-oz. cups with ventilation holes punched in the tops for 10 days in the outdoor nursery, after which

the cups were removed. The light regime is maintained with 66% shade cloth and individuals were irrigated by overhead spray once every other day (Lynch, pers. comm.).

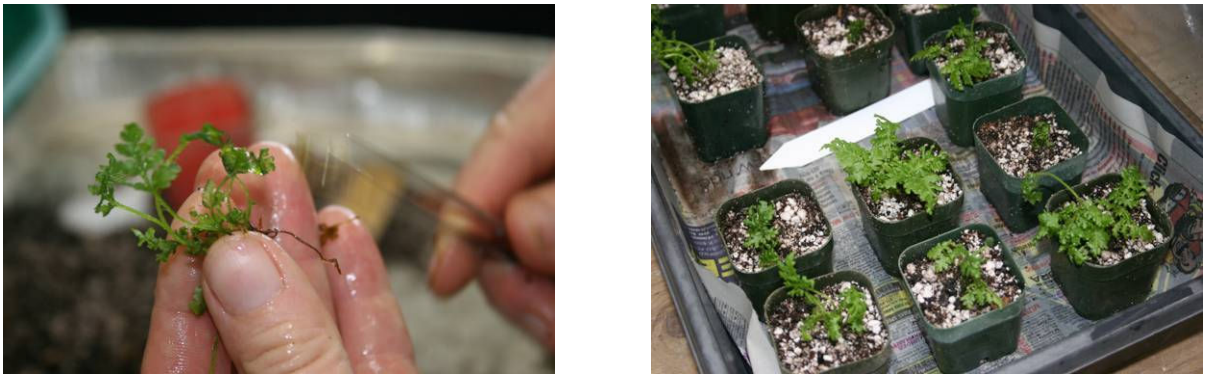


Figure 2.8. One *C. chamissoi* sporeling 6 – 8 months old (left), at time of first planting into 2 inch pots (right).

The date of first potting sporelings from germination tubes into two inch pots with soil medium was recorded as the starting date for calculation of growth rates, and total trunk height from soil medium to growth apex was measured in 2010-2011. However, it takes an additional 6 – 8 months from the time of sporophyte germination in the gametophyte tubs to planting into the two inch pots (Lynch, pers. comm.), and this time will be included to adjust age estimates of nursery-grown individuals.

IN SITU GROWTH DATA

Repeated measurements of *C. chamissoi* trunk length were recorded for each individual within each plot approximately annually from 2005 – 2011, depending on the study site and access (see Table 2.3).

Plot	Date1	Date2	Date3	Date4	Date5	Date6	Time1	Time2	Time3	Time4	Time5	Total Years
W_Pah1-a	16-May-06	28-Jun-08	9-Jan-09	4-Dec-09	14-Jul-10	21-Jul-11	2.12	0.53	0.90	0.61	1.02	5.18
W_Pah1-b	11-May-06	26-Jun-08	9-Jan-09	4-Dec-09	14-Jul-10	25-Jul-11	2.13	0.54	0.90	0.61	1.03	5.21
W_Pah2-a	16-May-06	28-Jun-08	9-Jan-09	4-Dec-09	14-Jul-10	21-Jul-11	2.12	0.53	0.90	0.61	1.02	5.18
W_Pah2-b	19-May-06	28-Jun-08	9-Jan-09	4-Dec-09	24-Jul-10	25-Jul-11	2.11	0.53	0.90	0.64	1.00	5.19
W_Kiki-a	25-Aug-05	16-Jun-08	18-Jan-09	29-Sep-09	24-Jun-10	11-Aug-11	2.81	0.59	0.70	0.73	1.13	5.96
W_Kiki-b	27-Sep-05	16-Jun-08	18-Jan-09	29-Sep-09	24-Jun-10	20-Jul-11	2.72	0.59	0.70	0.73	1.07	5.81
W_Kiki-c	27-Aug-05	16-Jun-08	18-Jan-09	29-Sep-09	24-Jun-10	20-Jul-11	2.81	0.59	0.70	0.73	1.07	5.90
W_Ohik-a	7-Nov-05	4-Jun-08	21-Jan-09	13-Oct-09	15-Jun-10	30-Nov-11	2.58	0.63	0.73	0.67	1.46	6.07
W_Ohik-b	17-Aug-05	5-Jun-08	21-Jan-09	14-Oct-09	14-Jun-10	29-Nov-11	2.80	0.63	0.73	0.67	1.46	6.29
W_Ohik-c	16-Aug-05	4-Jun-08	N/A	13-Oct-09	15-Jun-10	29-Nov-11	2.80	N/A	1.36	0.67	1.46	6.29
W_3Pts-a	14-Feb-06	31-May-08	14-Jan-09	1-Oct-09	19-Jul-10	28-Jul-11	2.29	0.62	0.71	0.80	1.02	5.45
W_3Pts-b	14-Feb-06	31-May-08	14-Jan-09	1-Oct-09	19-Jul-10	28-Jul-11	2.29	0.62	0.71	0.80	1.02	5.45
K_Kahuku	17-Apr-06	30-Jun-08	N/A	24-Sep-09	21-Jul-10	27-Jul-11	2.21	N/A	1.24	0.82	1.02	5.28
K_Kahana	12-Apr-06	14-Jun-08	19-Jan-09	2-Dec-09	7-Jul-10	16-Jul-11	2.18	0.60	0.87	0.59	1.02	5.26
K_Aiea	8-Apr-06	30-May-08	8-Jan-09	6-Nov-09	17-Jul-10	14-Jul-11	2.15	0.61	0.83	0.69	0.99	5.27
K_Lyon	6-Apr-06	18-Jun-08	17-Jan-09	N/A	16-Jul-10	1-Aug-11	2.20	0.58	N/A	1.49	1.04	5.32
Min	16-Aug-05	30-May-08	8-Jan-09	24-Sep-09	14-Jun-10	14-Jul-11	2.11	0.53	0.70	0.59	0.99	5.18
Max	19-May-06	30-Jun-08	21-Jan-09	4-Dec-09	24-Jul-10	30-Nov-11	2.81	0.63	1.36	1.49	1.46	6.29

Table 2.3. Dates of measurements (Date1 – Date5), and time between measurements, for all plots (Time1 – Time5).

Initial measurements at dates one and two included the entire trunk. It became apparent at date two that the older caudex tissue gradually decomposes, and so permanent markers were installed along the trunk with wire that was attached in a way to allow for some expansion in trunk circumference without harming the plants, while maintaining the same location. Trunk length measurements below and above the marker were recorded at time of installation, and from this point on, growth was measured from the marker to the trunk apex. At date five it was becoming difficult to see the wire on some of the individuals, due to the covering over by adventitious roots; therefore the wire was replaced with a galvanized nail. This pin marker method is similar to that used for *C. glaucum* on the island of Hawai‘i by Walker and Aplet (1994), and *C. chamissoi* on the island of O‘ahu by Durand and Goldstein (2001a). Small individuals were measured from soil level to growth apex and were not installed with permanent trunk markers if doing so would have harmed the individual, as would be the case for individuals with a small trunk circumference.

At each time of measurement, each fern was relocated and measured for total trunk length (TTL), ranked for overall health (dead, poor, moderate, healthy), and examined for fertility (presence/absence of spore-bearing fronds). Additional morphological data was collected for each fern during the first and last times of measurement, including trunk top circumference (TTC), canopy height from ground (CH), longest frond length (LFL), and number of healthy fronds (HF), which were classified as those greater than 50% green. Trunk circumference was measured just below where the fronds attach at the stem apex. In the case of extremely dense populations, density by size class (sporeling, immature, mature) was recorded for the entire 10 x 20 m area in 2006, but the morphological measurements of individual ferns were monitored across half the plot for an area of 10 x 10 m in subsequent plot visits.

Growth rates were calculated by subtracting new trunk length from previous trunk length, and then dividing by the time between measurements:

((TL2 – TL1) / (Date2 – Date1)) / 365 days yr $^{-1}$ = Growth Rate 1

Growth rates were calculated between each period of measurement (GR1 – GR5), and also averaged across the entire study period (AvGR). Ferns that tipped over, became inaccessible due to tree falls, were categorized in poor health, or died after the initial measurement were excluded from growth rate analyses. Additional outliers were identified and excluded in cases of trunk decomposition or measurement error. Size classes one through seven were assigned to individuals based on TTL, from smallest to largest as follows: size class 1 = 0.1 – 9.9 cm; size class 2 = 10.0 – 19.9 cm; size class 3 = 20.0 – 39.9 cm; size class 4 = 40.0 – 99.9 cm; size class 5 = 100.0 – 199.9 cm; size class 6 = 200.0 – 299.9 cm; and size class 7 = 300.0 – 428 cm.

Data Analysis

Statistical analyses were performed with SPSS. Average *C. chamissoi* growth rate (dependent variable) by size class at measurement date three (independent variable) was analyzed by one-way ANOVA. Effect-size was calculated to indicate the relative magnitude of the differences between the means with the eta-squared statistic:

Eta squared = Sum of squares / Total sum of squares

Normality was assessed for morphological and growth rate variables by inspecting histograms, normal probability plots, and generating skewness and kurtosis values. The Kolmogorov-Smirnov statistical test for normality was also examined, and all variables were found to suggest

violation of the assumption of normality. Therefore variables were log-10 transformed for correlation and regression analyses, and results were compared with those of untransformed variables to confirm that transformations improved normality and the results of the analyses.

The relationship between growth rate and morphological variables, including total trunk length, trunk top circumference, canopy height, longest frond length, and number of healthy fronds, was explored with Pearson product-moment correlation coefficient. Standard bivariate regressions were performed with average growth rate as the dependent variable, and total trunk length, trunk top circumference, canopy height, longest frond length, and number of healthy fronds as the independent variables.

Average growth rate by size class was used to estimate trunk age, similar to the methods of Mehltreter and Garcia-Franco (2008). The maximum trunk age of each size class was calculated as the sum of the age for all preceding size classes, plus the estimated time from sporeling germination to trunk establishment, with the following equation adapted from Mehltreter and Garcia Franco (2008):

$$\text{Trunk age max}_{\text{size class i}} = c_0 + \sum_{i=1}^{\text{size class}} \frac{TLI_i}{g_i}$$

where the constant c_0 represents time to trunk establishment, and was set to .7626 from the results of regression analyses performed on nursery grown *C. chamissoi* sporelings (see results below). Trunk length increment (TLI) indicates the range in trunk length corresponding to each size class, and g_i is the mean annual trunk growth per size class i.

Recruitment and mortality rates were calculated for each plot and reported as number of new or dead ferns, respectively, and then divided by the total initial ferns of that plot and reported as a percentage.

RESULTS

NURSERY GROWTH RATES

Linear regression analyses of nursery grown *C. chamissoi* (n = 46) indicate that trunk length is a moderate predictor of growth rate in a linear relationship ($R^2 = 0.28$, $p < 0.001$) (Fig. 2.9a), and this relationship is improved with the log-10 transformed variables ($R^2 = 0.598$, $p < 0.001$) (Fig. 2.9b). When the untransformed variables are fitted with a logarithmic curve, the predictive ability of trunk length is substantively the same ($R^2 = 0.505$, $p < 0.001$) (Fig. 2.10).

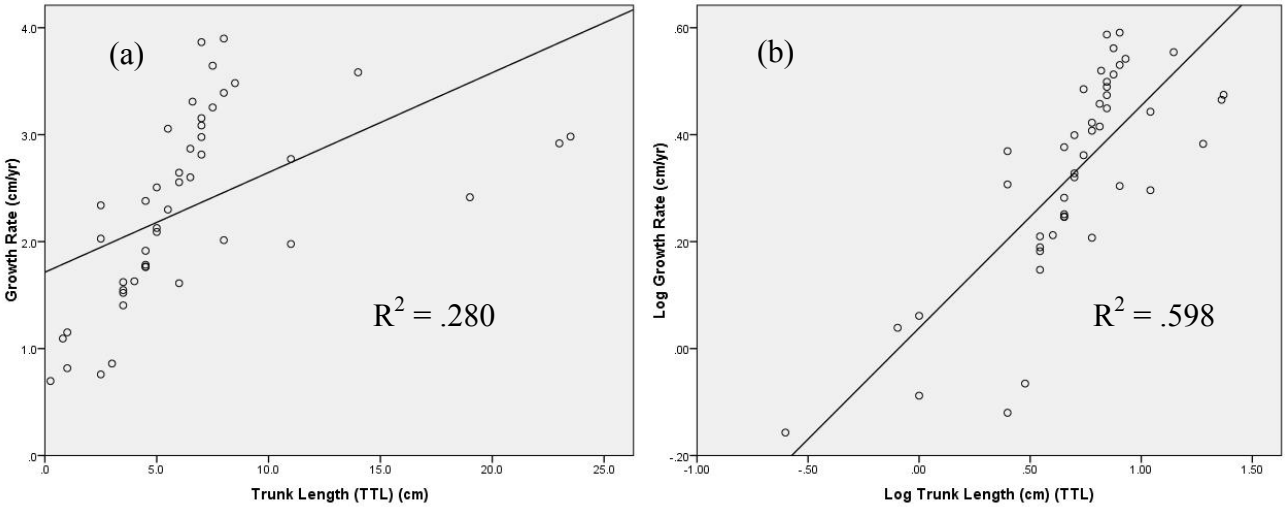


Figure 2.9. Linear regression of nursery *C. chamissoi* growth rate (cm/yr) (y-axis) by trunk length (cm) (TTL) (x-axis), for (a) untransformed variables ($R^2 = 0.280$, $p < 0.001$; $y = 0.093 * x + 1.714$), and (b) log-10 transformed variables ($R^2 = 0.598$, $p < 0.001$; $\log y = 0.416 * \log x + 0.038$).

Nursery Linear Regression Model Summary (a)

R	R Square	Adjusted R Square	Std. Error of the Estimate
.529	.280	.263	.746

The independent variable is TTL.

Coefficients

	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
	B	Std. Error	Beta		
TTL	.093	.023	.529	4.132	.000
(Constant)	1.714	.184		9.290	.000

Nursery Linear Regression Model Summary (b)

R	R Square	Adjusted R Square	Std. Error of the Estimate
.774	.598	.589	.124

The independent variable is LogTTL.

Coefficients

	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
	B	Std. Error	Beta		
LogTTL	.416	.051	.774	8.096	.000
(Constant)	.038	.040		.953	.346

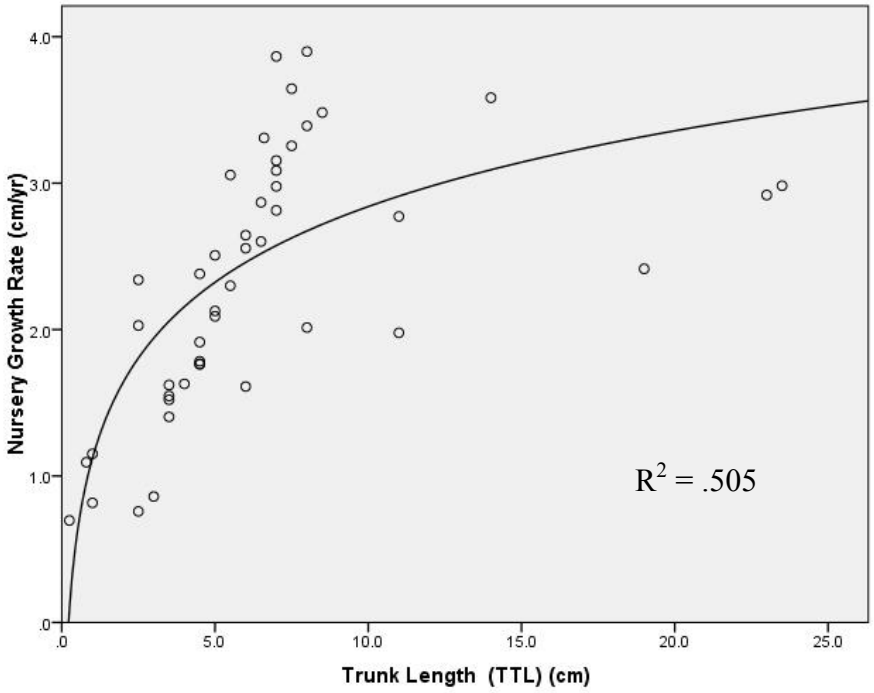


Figure 2.10. Logarithmic regression for nursery *C. chamissoi* growth rate (y-axis), as predicted by trunk length (x-axis), $R^2 = 0.505$, $p < 0.001$; $y = 1.122 + 0.746 * \log(x)$.

Model Summary

R	R Square	Adjusted R Square	Std. Error of the Estimate
.710	.505	.493	.619

The independent variable is TTL.

Coefficients

	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
	B	Std. Error	Beta		
ln(TTL)	.746	.111	.710	6.696	.000
(Constant)	1.122	.202		5.562	.000

Mean growth rates of nursery-grown *C. chamissoi* were found to increase with size class, from 2.26 cm/yr in size class one, to 2.69 cm/yr in size class two, and 2.95 cm/yr in size class three (Table 2.4).

Size Class	N	Mean Trunk Length (cm)	Mean Number of Fronds	Mean Growth Rate (cm/yr)
1	40	5.004	6.857	2.258
2	4	13.750	5.500	2.687
3	2	23.250	3.500	2.951

Table 2.4. Mean growth rates and morphological data for nursery-grown *C. chamissoi*.

Nursery-grown *C. chamissoi* demonstrated a negative relationship between trunk length and number of fronds ($R^2 = 0.232$; $y = -0.1601x + 7.5587$), with mean number of fronds ranging from 6.9 in size class one, to 5.5 and 3.5 fronds for size classes two and three, respectively.

AGE OF NURSERY-GROWN TREE FERNS

Actual age of *C. chamissoi* grown from spores in the nursery was plotted against trunk length ($R^2 = 0.801$, $p < 0.001$) (Fig. 2.11).

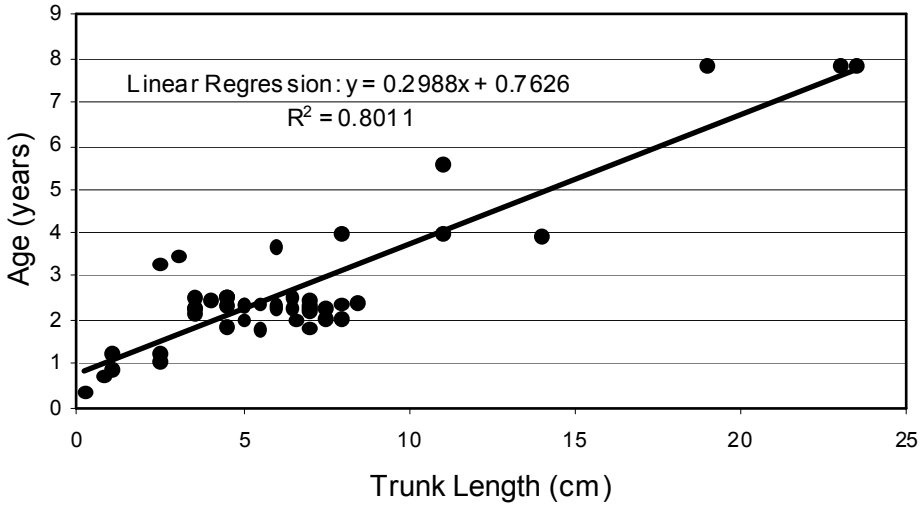


Figure 2.11. Linear regression for nursery-grown *C. chamissoi* age by trunk length ($R^2 = 0.801$, $p < 0.001$; $y = 0.2988x + 0.7626$).

Using the linear regression equation generated, it is possible to estimate the age of a nursery-grown tree fern by trunk length ($y = 0.2988x + 0.7626$), such that an individual with a trunk length of 10 cm is approximately 3.7 years old. Similarly, an individual with a trunk length of 20 cm would be approximately 6.7 years old when grown under nursery conditions.

In Situ Growth Rates by Size Class

The Results of a one-way between groups ANOVA to explore differences in growth rate by size class demonstrate a statistically significant, sequentially increasing rate of growth rate with size class, with the exception of ferns in the largest size class, which slightly decline in growth rate ($F(6, 222) = 8.5$, $p < 0.0005$) (Fig. 2.12).

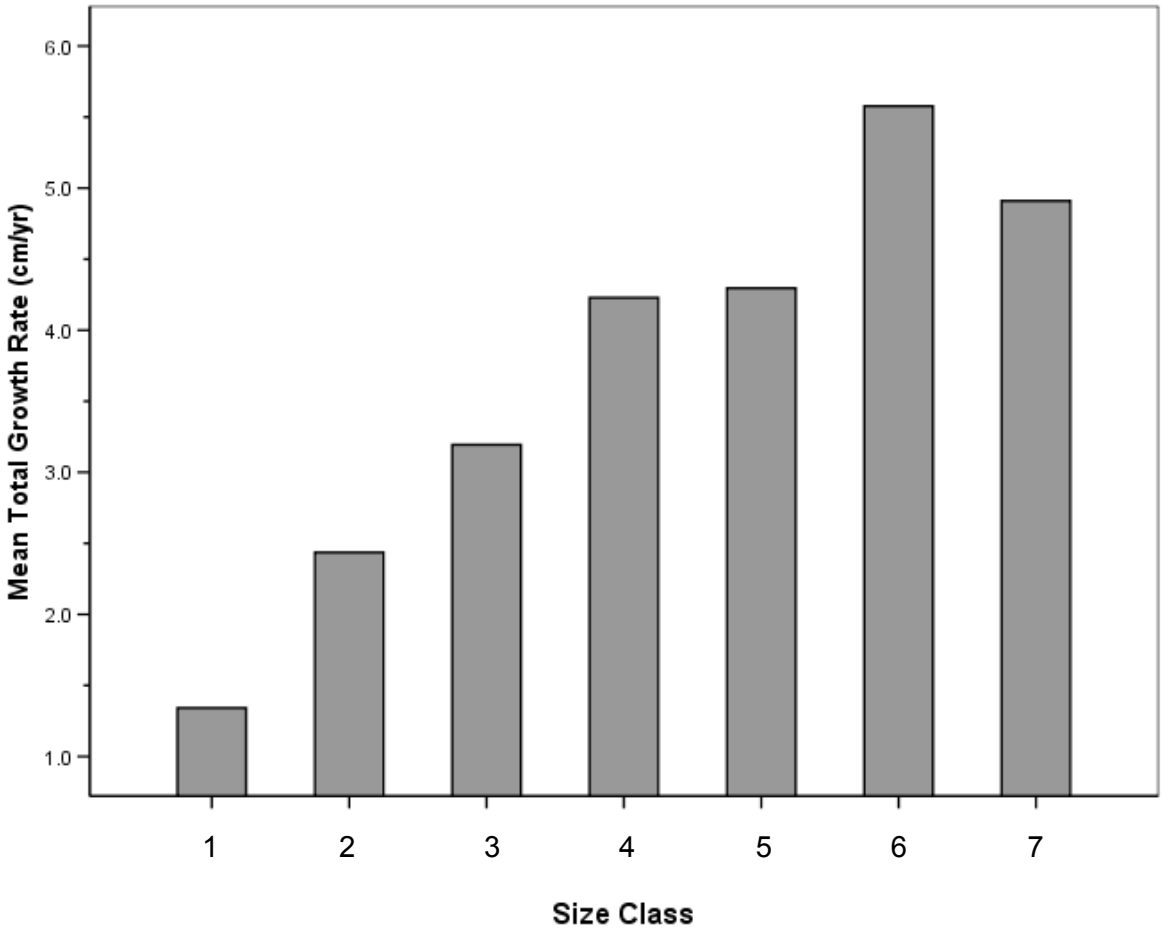


Figure 2.12. Mean growth rate (cm/yr) as averaged over the entire study period for all *C. chamissoi* by size class. Size classes assigned by measured trunk length as follows: (1) .1 – 9.9 cm; (2) 10 – 19.9 cm; (3) 20 – 39.9 cm; (4) 40 – 99.9 cm; (5) 100 – 199.9 cm; (6) 200 – 299.9 cm; (7) 300 – 428 cm.

Mean growth rate is lowest for size class one (1.3 cm/yr), and maximum for size class six (5.6 cm/yr), although growth rates for size classes four and five appear to be similar (4.2 and 4.3 cm/yr, respectively) (Table 2.5).

One-Way ANOVA Results: Mean *C. chamissoi* Growth Rate (cm/yr) By Size Class

SC	Size	N	Mean	SD	SE	95% Confidence Interval Lower	Upper	Min	Max
1	0.1 - 9.9 cm	14	1.3414	1.44337	0.38576	0.5080	2.1748	-0.17	5.20
2	10 - 19.9 cm	22	2.4352	1.90908	0.40702	1.5888	3.2816	0.17	7.11
3	20 - 39.9 cm	42	3.1940	1.69486	0.26152	2.6658	3.7221	0.33	7.59
4	40 - 99.9 cm	73	4.2280	2.23200	0.26124	3.7073	4.7488	0.94	11.96
5	100 - 199.9 cm	55	4.2960	2.01352	0.27150	3.7517	4.8404	1.24	10.26
6	200 - 299.9 cm	17	5.5773	3.40728	0.82639	3.8254	7.3292	1.65	13.88
7	>= 300 cm	6	4.9101	1.77600	0.72505	3.0463	6.7739	2.12	6.83
	Total	229	3.8240	2.32213	.15345	3.5217	4.1264	-0.17	13.88

Table 2.5. Results of one-way ANOVA mean growth rate (cm/yr) by size class (SC), assigned by trunk length in centimeters (Size) at measurement date three.

The actual difference in mean scores between size class groups was large, with an effect size of 0.19 calculated using eta squared. Minimum growth ranged from -0.17 cm/yr in size class one to 2.12 cm/yr in size class seven. Maximum growth rate ranged from 5.20 cm/yr in size class one to 13.88 cm/yr in size class six.

Average growth rates for size classes four and five, as calculated by plot, region, and mountain range, are presented in Table 2.6 below. Some of the plots were missing individuals in these size classes, or contained a sample size of $N = 1$. Therefore, growth rate comparisons between plots should be interpreted with caution.

Plot	*C. chamissoi* Average Growth Rate				Regional Mean Growth Rate for Size Classes 4 and 5	
	Size Class 4	(*N*)	Size Class 5	(*N*)		(*N*)
W_Pah1-a	5.112	*1*	4.244	*2*		
W_Pah1-b	–	–	–	–		
W_Pah2-a	2.894	*2*	4.849	*11*		
W_Pah2-b	–	–	9.159	*1*	5.252	*17*
W_Kiki-a	4.988	*8*	2.641	*2*		
W_Kiki-b	–	–	3.239	*3*		
W_Kiki-c	2.939	*3*	5.849	*2*	3.931	*18*
W_Ohik-a	3.174	*12*	3.675	*15*		
W_Ohik-b	3.976	*1*	4.639	*9*		
W_Ohik-c	4.451	*1*	4.708	*3*	4.104	*41*
W_3Pts-a	4.024	*8*	–	–		
W_3Pts-b	–	–	–	–	4.024	*8*
Waianae Total	**3.945**		**4.778**		**4.328**	***84***
K_Kahuku	3.988	*9*	7.482	*3*	5.735	*12*
K_Kahana	5.000	*8*	8.740	*2*	6.870	*10*
K_Aiea	7.170	*11*	11.958	*1*	9.564	*12*
K_Lyon	2.946	*25*	4.665	*3*	3.805	*28*
Koolau Total	**4.776**		**8.211**		**6.493**	***62***

Table 2.6. Mean growth rates by plot, region, and mountain range for *C. chamissoi* individuals of size class four (40 – 99.9 cm trunk length) and size class five (100 – 199.9 cm trunk length). (*N*) indicates sample size; growth rates are reported in cm/yr. Plot codes: *W_ = Waianae Mountain Range; K_ = Koolau Mountain Range; a = inside fence; b = outside fence; Kiki-c = Kahanahaiki outside fence steep site; Ohik-c = Ohikilolo outside fence non-native site.*

Tree fern growth rates appeared variable across habitats, despite restricting comparisons to individuals of similar size. The minimum and maximum growth rates in size class four were observed at 2.89 cm/yr and 7.17 cm/yr, respectively. Minimum and maximum growth rates in size class five were observed at 2.64 cm/yr and 11.98 cm/yr, respectively. Regional mean growth rates for *C. chamissoi* were found to be greatest at Aiea (9.56 cm/yr), and least at Lyon (3.81 cm/yr). Across both size classes combined, mean growth rates were lower for plots in the Wai‘anae Mountains (4.33 cm/yr), than for those in the Ko‘olau Mountains (6.49 cm/yr).

ESTIMATING AGE OF *IN SITU* TREE FERNS

Maximum trunk ages for naturally occurring *C. chamissoi* were estimated based upon total trunk length, and results of mean growth rate by size class as reported in Table 2.4. Estimated ages by size class are reported in Table 2.5. Individuals in size class one with a TTL of approximately 10 cm were estimated at 8.1 years old, and the largest individual in size class seven (428 cm trunk length) was estimated to be 99.8 years old (Table 2.7). In comparison, estimating age by applying the mean growth rate across all sizes of 3.82 cm/yr, the largest individual of 428 cm trunk length would be 112 years old.

SC	Size (TTL)	Estimated Age at Max TTL (years)
1	0.1 - 9.9 cm	8.1
2	10 - 19.9 cm	12.2
3	20 - 39.9 cm	18.4
4	40 - 99.9 cm	32.6
5	100 - 199.9 cm	55.8
6	200 - 299.9 cm	73.7
7	300 - 428 cm	99.8

Table 2.7. Estimated maximum age of *C. chamissoi* by size class, calculated from mean growth rates.

Growth rates for all *C. chammisoi* by size class as calculated between each time of measurement are presented in Figure 2.13 below. For a more detailed visual of growth rate variation during the study period for individuals of size class one in Kahanahaiki In, 3 Points In, Kahana, and Aiea plots, see Appendix 4.

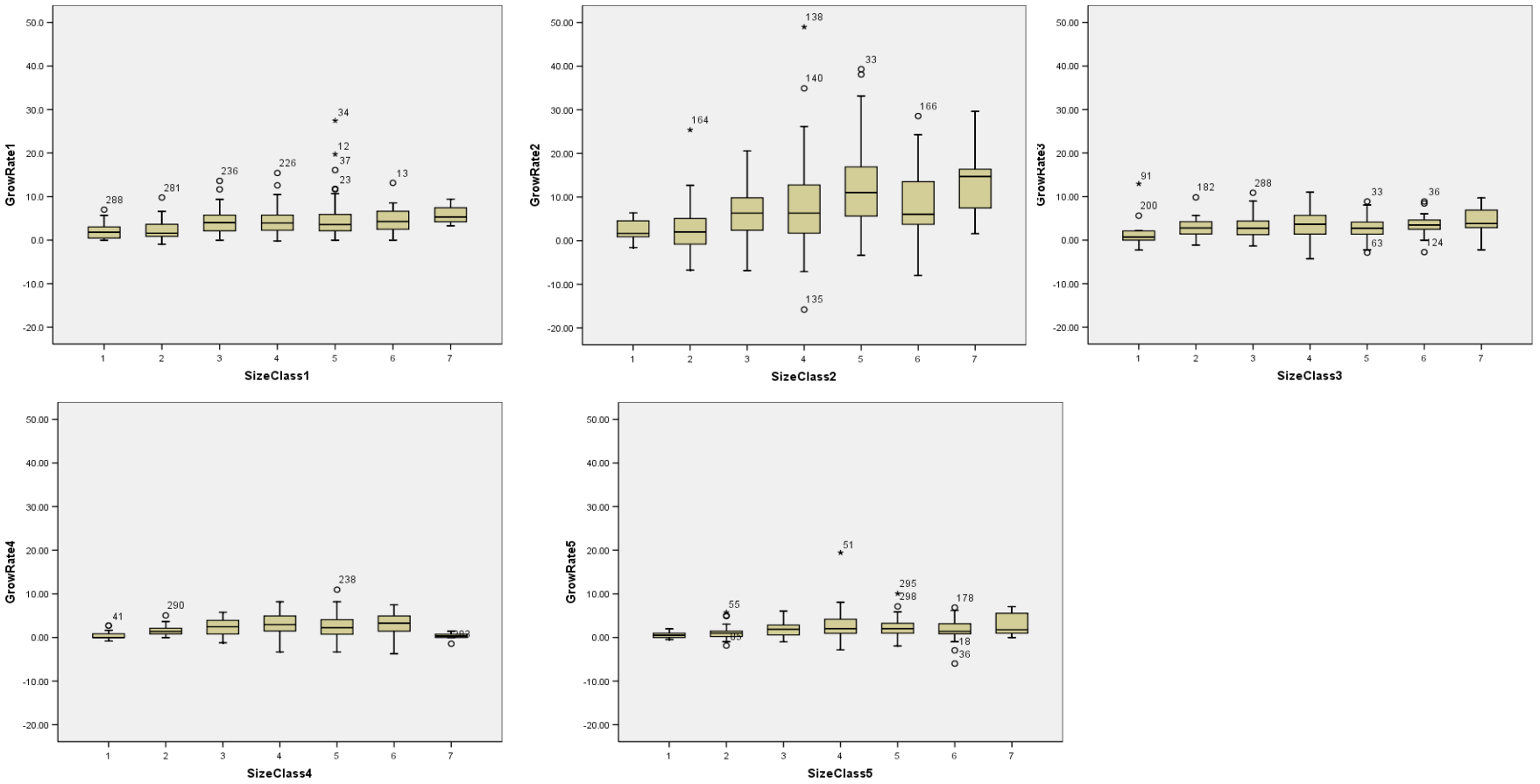


Figure 2.13. *Cibotium chamissoi* growth rates in cm/yr (y-axis), at measured times 1 – 5, by size class (x-axis). Size classes assigned by measured trunk length as follows: (1) .1 – 9.9 cm; (2) 10 – 19.9 cm; (3) 20 – 39.9 cm; (4) 40 – 99.9 cm; (5) 100 – 199.9 cm; (6) 200 – 299.9 cm; (7) 300 – 428 cm. Boxes indicate 50% of cases; central line represents median value, with whiskers indicating max and min values. Circles and stars identify outliers and extreme outliers, respectively.

NATURAL GROWTH RATES AND MORPHOLOGY

Mean morphological data for each size class are provided in Table 2.8. Mean trunk top circumference, longest frond length, and canopy height were found to increase with size, with the exception of size class seven.

Size Class	N	Mean Trunk Length (cm)	Mean Trunk Top Circumference (cm)	Mean Longest Frond Length (cm)	Mean Canopy Height (cm)	Mean Number of Fronds	Mean Growth Rate (cm/yr)
1	12	5.542	5.917	47.083	28.583	2.750	1.341
2	19	14.632	13.526	88.526	56.421	2.211	2.344
3	48	28.573	22.000	143.438	102.708	3.021	3.194
4	97	65.660	36.284	221.258	174.320	3.897	4.228
5	70	142.949	41.129	250.870	196.015	3.200	4.296
6	24	226.875	44.604	262.208	228.125	3.250	5.577
7	6	345.833	39.250	206.667	146.333	3.667	4.910

Table 2.8. Mean *in situ* morphological data for *C. chamissoi* by size class.

Mean trunk top circumference was greatest in size class six (44.6 cm) and smallest in size class one (5.9 cm), as was mean longest frond length (2.62 m in size class six; 0.47 m in size class one), and mean canopy height (2.28 m in size class six; 0.29 m in size class one). However, mean number of healthy fronds appeared to be more variable, with maximum number of fronds observed in size class seven (3.7 fronds), and minimum number of fronds observed in size class two (2.2 fronds).

Results of Pearson product-moment correlation between log-10 transformed morphological variables and growth rates indicate significant, positive correlations between all variables (Table 2.9).

Pearson Correlation: Log-10 Transformed Morphological Variables and Growth Rates

		LogTTL6	LogTTC6	LogCH6	LogLFL6	LogHF6	LogGR5	LogAvGR
LogTTL6	Pearson Correlation	1	.827**	.752**	.782**	.144*	.296**	.618**
	Sig. (2-tailed)		.000	.000	.000	.016	.000	.000
	N	281	276	275	277	278	231	254
LogTTC6	Pearson Correlation	.827**	1	.916**	.929**	.371**	.537**	.727**
	Sig. (2-tailed)	.000		.000	.000	.000	.000	.000
	N	276	278	275	277	278	230	249
LogCH6	Pearson Correlation	.752**	.916**	1	.933**	.436**	.511**	.709**
	Sig. (2-tailed)	.000	.000		.000	.000	.000	.000
	N	275	275	277	277	277	229	248
LogLFL6	Pearson Correlation	.782**	.929**	.933**	1	.354**	.493**	.714**
	Sig. (2-tailed)	.000	.000	.000		.000	.000	.000
	N	277	277	277	279	279	231	250
LogHF6	Pearson Correlation	.144*	.371**	.436**	.354**	1	.427**	.463**
	Sig. (2-tailed)	.016	.000	.000	.000		.000	.000
	N	278	278	277	279	280	231	251
LogGR5	Pearson Correlation	.296**	.537**	.511**	.493**	.427**	1	.540**
	Sig. (2-tailed)	.000	.000	.000	.000	.000		.000
	N	231	230	229	231	231	231	209
LogAvGR	Pearson Correlation	.618**	.727**	.709**	.714**	.463**	.540**	1
	Sig. (2-tailed)	.000	.000	.000	.000	.000	.000	
	N	254	249	248	250	251	209	265

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Table 2.9. Pearson correlation coefficients generated for log-10 transformed morphological variables at last time of measurement: total trunk length (TTL6), trunk top circumference (TTC6), canopy height (CH6), longest frond length (LFL6), and number of healthy fronds (HF6); growth rate as measured between dates 5 and 6 (GR5), and averaged across the six-year study period (AvGR).

Total trunk length demonstrates the strongest correlations with TTC ($r = 0.83$, $n = 276$, $p < 0.01$), and LFL ($r = 0.78$, $n = 277$, $p < 0.01$). Trunk top circumference was most strongly correlated with LFL ($r = 0.93$, $n = 277$, $p < 0.01$), as was CH ($r = 0.93$, $n = 277$, $p < 0.01$). Number of healthy fronds was most strongly correlated with average growth rate ($r = 0.46$, $n = 251$, $p < 0.01$), and with canopy height ($r = 0.44$, $n = 277$, $p < 0.01$). Growth rate as measured between dates 5 and 6 (GR5) was most highly correlated with TTC ($r = 0.54$, $n = 230$, $p < 0.01$), and so was growth rate as averaged across the six-year study period (AvGR) ($r = 0.73$, $n = 249$, $p < 0.01$). Average growth rate was more strongly correlated with TTL ($r = 0.62$, $n = 254$, $p < 0.01$), than GR5 and TTL ($r = 0.296$, $n = 231$, $p < 0.01$).

The correlations between repeated measures of trunk length (TTL1 – TTL6) and growth rates (GR1 – GR5; AvGR) are shown in Table 2.10 below. Trunk length generally demonstrates a stronger, positive relationship with average growth rate (r = 0.43 – 0.62), than it does with GR1 – GR5 (r = 0.26 – 0.51), although all correlations are significant at the 0.01 level. These positive correlation values demonstrate that as *C. chamissoi* trunk length increases, so does growth rate.

Pearson Correlations: Log-10 Transformed Total Trunk Length and Growth Rates

		LogTTL1	LogTTL2	LogTTL3	LogTTL4	LogTTL5	LogTTL6	LogGR1	LogGR2	LogGR3	LogGR4	LogGR5	LogAvGR
LogTTL1	Pearson Correlation	1	.982**	.971**	.969**	.952**	.952**	.396**	.386**	.200**	.106	.094	.425**
	Sig. (2-tailed)		.000	.000	.000	.000	.000	.000	.000	.005	.108	.162	.000
	N	335	305	267	261	305	270	263	202	197	232	223	253
LogTTL2	Pearson Correlation	.982**	1	.986**	.986**	.980**	.982**	.512**	.422**	.252**	.216**	.171*	.524**
	Sig. (2-tailed)	.000		.000	.000	.000	.000	.000	.000	.000	.001	.010	.000
	N	305	317	270	265	308	274	274	208	197	236	225	259
LogTTL3	Pearson Correlation	.971**	.986**	1	.993**	.988**	.988**	.492**	.486**	.174*	.211**	.228**	.509**
	Sig. (2-tailed)	.000	.000		.000	.000	.000	.000	.000	.021	.002	.001	.000
	N	267	270	277	233	275	246	234	210	174	212	202	228
LogTTL4	Pearson Correlation	.969**	.986**	.993**	1	.997**	.997**	.530**	.495**	.343**	.263**	.238**	.611**
	Sig. (2-tailed)	.000	.000	.000		.000	.000	.000	.000	.000	.000	.001	.000
	N	261	265	233	272	271	241	233	181	203	202	200	225
LogTTL5	Pearson Correlation	.952**	.980**	.988**	.997**	1	.998**	.523**	.501**	.345**	.263**	.260**	.593**
	Sig. (2-tailed)	.000	.000	.000	.000		.000	.000	.000	.000	.000	.000	.000
	N	305	308	275	271	317	281	268	210	203	240	231	264
LogTTL6	Pearson Correlation	.952**	.982**	.988**	.997**	.998**	1	.496**	.508**	.395**	.284**	.296**	.618**
	Sig. (2-tailed)	.000	.000	.000	.000	.000		.000	.000	.000	.000	.000	.000
	N	270	274	246	241	281	281	247	195	190	230	231	254
LogGR1	Pearson Correlation	.396**	.512**	.492**	.530**	.523**	.496**	1	.202**	.337**	.447**	.280**	.753**
	Sig. (2-tailed)	.000	.000	.000	.000	.000	.000		.006	.000	.000	.000	.000
	N	263	274	234	233	268	247	274	186	175	212	203	246
LogGR2	Pearson Correlation	.386**	.422**	.486**	.495**	.501**	.508**	.202**	1	.188*	.242**	.264**	.584**
	Sig. (2-tailed)	.000	.000	.000	.000	.000	.000	.006		.026	.001	.001	.000
	N	202	208	210	181	210	195	186	210	140	174	165	189
LogGR3	Pearson Correlation	.200**	.252**	.174*	.343**	.345**	.395**	.337**	.188*	1	.227**	.255**	.524**
	Sig. (2-tailed)	.005	.000	.021	.000	.000	.000	.000	.026		.003	.001	.000
	N	197	197	174	203	203	190	175	140	203	165	164	182
LogGR4	Pearson Correlation	.106	.216**	.211**	.263**	.263**	.284**	.447**	.242**	.227**	1	.441**	.533**
	Sig. (2-tailed)	.108	.001	.002	.000	.000	.000	.000	.001	.003		.000	.000
	N	232	236	212	202	240	230	212	174	165	240	194	220
LogGR5	Pearson Correlation	.094	.171*	.228**	.238**	.260**	.296**	.280**	.264**	.255**	.441**	1	.540**
	Sig. (2-tailed)	.162	.010	.001	.001	.000	.000	.000	.001	.001	.000		.000
	N	223	225	202	200	231	231	203	165	164	194	231	209
LogAvGR	Pearson Correlation	.425**	.524**	.509**	.611**	.593**	.618**	.753**	.584**	.524**	.533**	.540**	1
	Sig. (2-tailed)	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	
	N	253	259	228	225	264	254	246	189	182	220	209	265

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Table 2.10. Pearson correlation coefficients generated for repeated measures of log-10 transformed trunk length (TTL1 – TTL6), corresponding growth rates (GR1 – GR5) (highlighted in grey), and growth rate averaged across the six-year study period (AvGR).

Results of linear regression for average *C. chamissoi* growth rate as predicted by trunk length demonstrates a stronger relationship for log-transformed variables ($R^2 = 0.181$, $p < 0.001$) than it does for untransformed variables ($R^2 = 0.092$, $p < 0.001$) (Fig. 2.14). Although the predictive relationship between trunk length and average growth rate is weak, both regressions are significant.

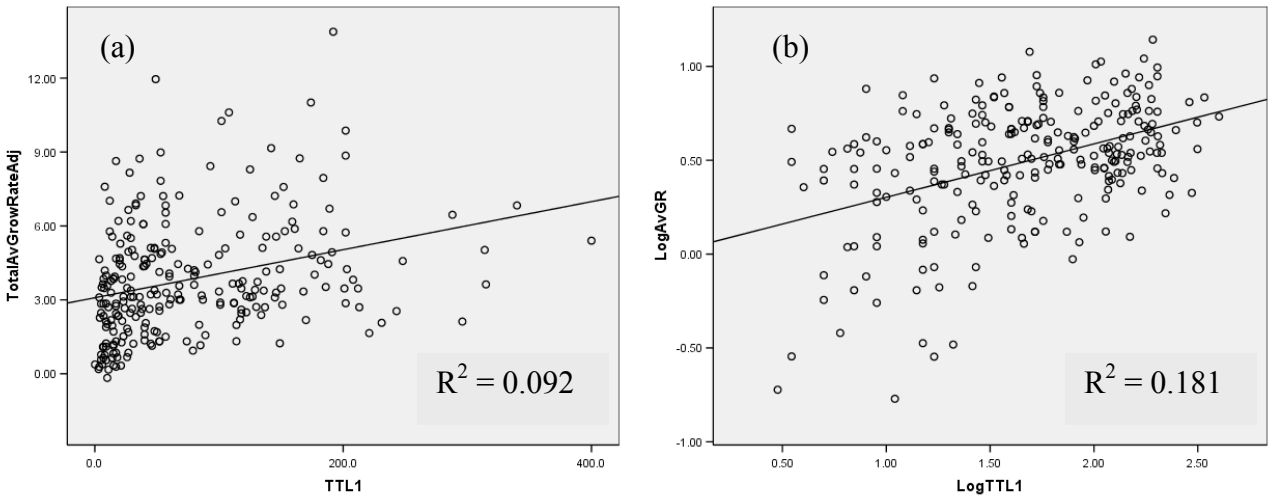


Figure 2.14. Linear regression of average growth rate (cm/yr) (y-axis) by trunk length (cm) at time one (TTL1) (x-axis), for (a) untransformed variables ($R^2 = 0.092$, $p < 0.001$; $y = 0.010 * x + 3.087$), and (b) log-10 transformed variables ($R^2 = 0.181$, $p < 0.001$; $\log(y) = 0.284 * \log(x) + 0.018$).

Linear Regression Model Summary (a)

R	R Square	Adjusted R Square	Std. Error of the Estimate
.303	.092	.088	2.239

The independent variable is TTL1.

Coefficients

	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
	B	Std. Error	Beta		
TTL1	.010	.002	.303	5.056	.000
(Constant)	3.087	.204		15.155	.000

Linear Regression of Log-10 Transformed Variables

Model Summary (b)

R	R Square	Adjusted R Square	Std. Error of the Estimate
.425	.181	.177	.295

The independent variable is LogTTL1.

	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
	B	Std. Error	Beta		
LogTTL1	.284	.038	.425	7.436	.000
(Constant)	.018	.066		.275	.783

However, a logarithmic curve was found to better fit untransformed variables than the linear regression, and resulted in the strongest relationship between average growth rate and measured trunk length at date six (TTL6), ($R^2 = 0.262$, $p < 0.001$), estimated as: $y = -1.040 + 1.181 * \log(x)$ (Fig. 2.15b).

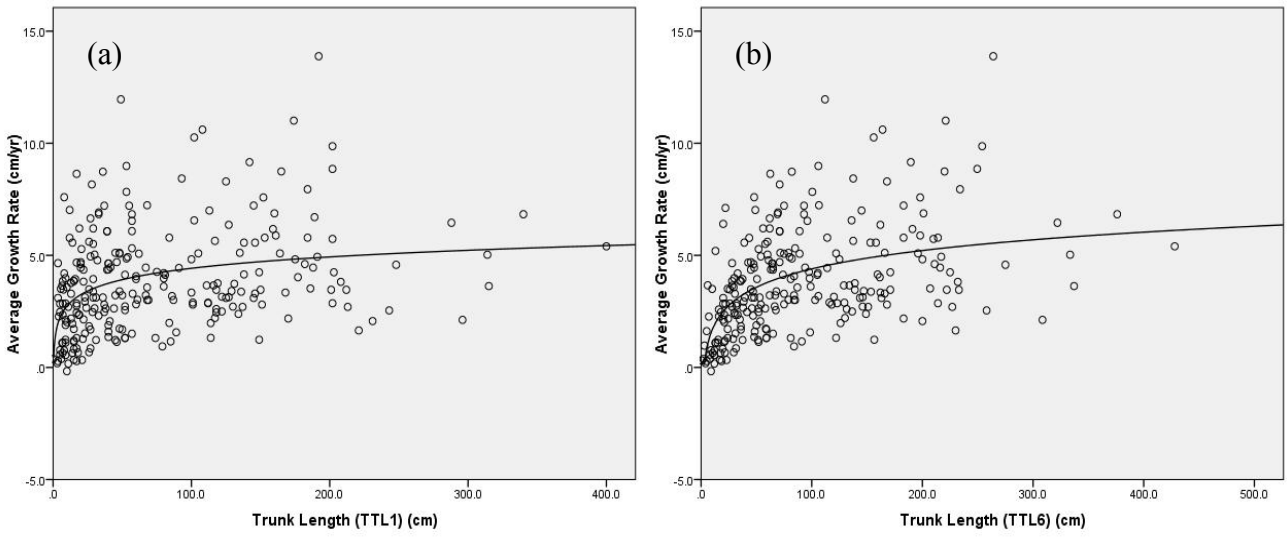


Figure 2.15. Logarithmic regressions for average growth rate (y-axis), as predicted by *C. chamissoi* trunk length at (a) date one (TTL1) ($R^2 = 0.135$, $p < 0.001$; $y = 1.056 + 0.731 * \log(x)$) and (b) date six (TTL6) ($R^2 = 0.262$, $p < 0.001$; $y = -1.040 + 1.181 * \log(x)$).

Logarithmic Model Summary (TTL1)

R	R Square	Adjusted R Square	Std. Error of the Estimate
.368	.135	.132	2.184

The independent variable is TTL1.

Coefficients

	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
	B	Std. Error	Beta		
ln(TTL1)	.731	.116	.368	6.296	.000
(Constant)	1.056	.462		2.286	.023

Logarithmic Model Summary (TTL6)

R	R Square	Adjusted R Square	Std. Error of the Estimate
.512	.262	.259	2.031

The independent variable is TTL6.

Coefficients

	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
	B	Std. Error	Beta		
ln(TTL6)	1.181	.125	.512	9.482	.000
(Constant)	-1.040	.531		-1.959	.051

Despite the significance of the relationship between trunk length and growth rate in all regressions, trunk length was only a weak predictor of growth rate in the strongest result (R^2 = 0.262). Trunk top circumference demonstrated a stronger relationship with average growth rate ($R^2 = 0.529$, $p < 0.001$; $\log(y) = 0.931 * \log(x) - 0.878$) (Fig. 2.16), as did longest frond length ($R^2 = 0.510$, $p < .001$; $\log(y) = 0.991 * \log(x) - 1.75$) (Fig. 2.17).

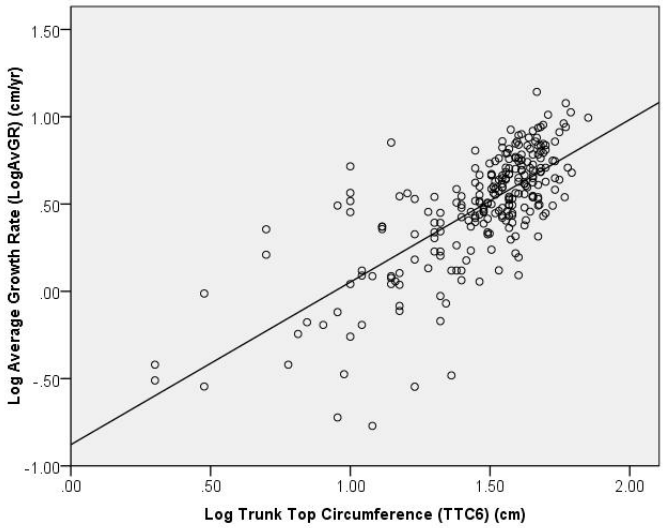


Figure 2.16. Linear regression of average growth rate (cm/yr) (y-axis) by trunk top circumference (cm) at time six (TTC6) (x-axis), for log-10 transformed variables ($R^2 = 0.529$, $p < 0.001$; $\log(y) = 0.931 * \log(x) - 0.878$).

Linear Regression Model Summary

R	R Square	Adjusted R Square	Std. Error of the Estimate
.727	.529	.527	.230

The independent variable is LogTTC6.

Coefficients

	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
	B	Std. Error	Beta		
LogTTC6	.931	.056	.727	16.656	.000
(Constant)	-.878	.083		-10.580	.000

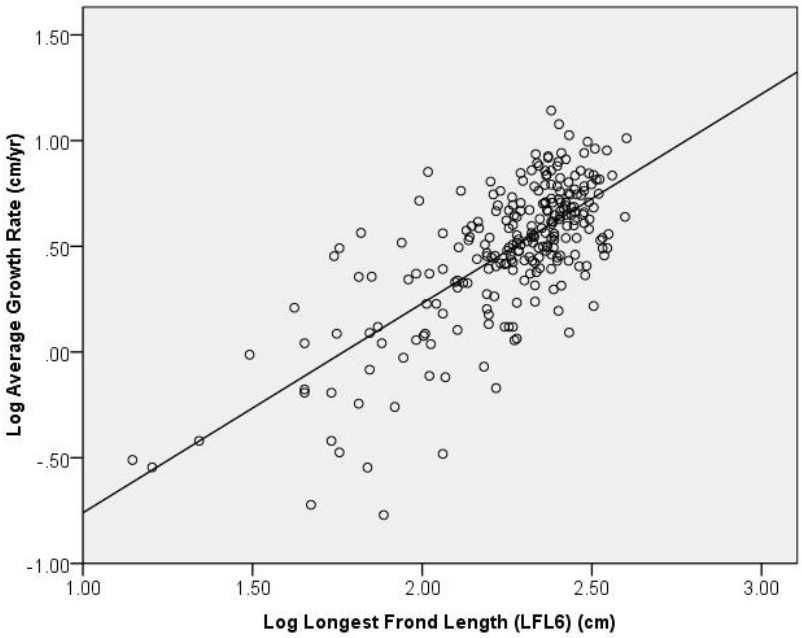


Figure 2.17. Linear regression of average growth rate (cm/yr) (y-axis) by longest frond length (cm) at time six (LFL6) (x-axis), for log-10 transformed variables ($R^2 = 0.510$, $p < 0.001$; $\log(y) = 0.991 * \log(x) - 1.75$).

Model Summary

R	R Square	Adjusted R Square	Std. Error of the Estimate
.714	.510	.508	.234

The independent variable is LogLFL6.

Coefficients

	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
	B	Std. Error	Beta		
LogLFL6	.991	.062	.714	16.051	.000
(Constant)	-1.751	.140		-12.509	.000

SIZE AT FERTILITY

A total of 122 non-fertile *C. chamissoi* were observed across all research sites, with a maximum trunk length of 94 cm. During the study period, there were several ferns that transitioned from infertile to fertile (n = 47), and the minimum trunk length of a newly transitioned fertile fern was 19.5 cm (mean = 40.26 cm). The majority of the tree ferns became fertile when their trunks reached 20 – 40 cm in length (Fig. 2.18).

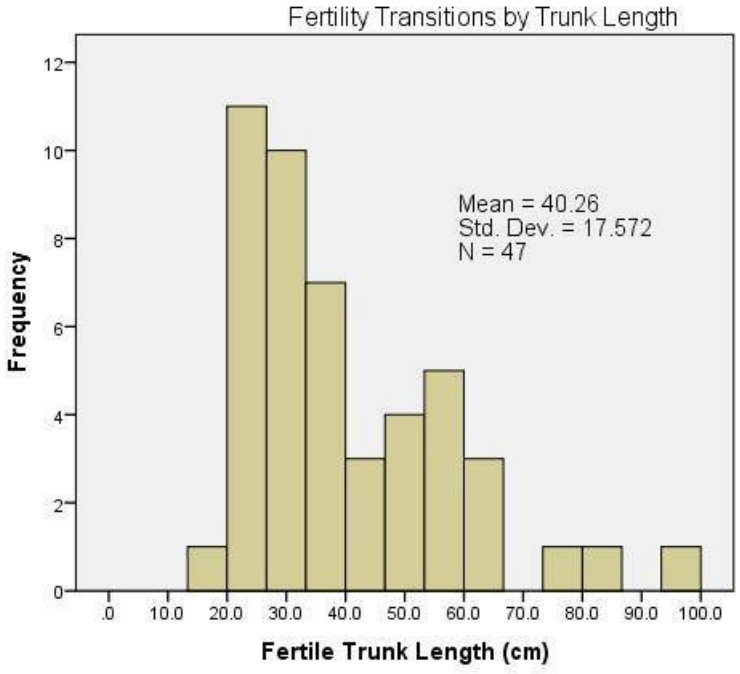


Figure 2.18. Frequency of *C. chamissoi* by trunk length (cm) that became fertile during the study period across all study plots (n = 47).

The average size for newly transitioned fertile ferns was found to vary among study regions, although sample sizes were small for Pahole and Kahana regions (Table 2.11). An additional 109 individuals remained infertile during the study, with a maximum total trunk length (TTL) of 91 cm.

Region	N	Mean	Std. Deviation	Std. Error	Minimum	Maximum
W_Pahole	2	27.500	2.1213	1.5000	26.0	29.0
W_Kahanahaiki	9	37.333	8.8741	2.9580	26.0	53.0
W_Ohikilolo	7	40.429	13.9625	5.2773	21.0	60.0
W_3Points	9	26.444	5.8707	1.9569	19.5	38.0
K_Kahuku	2	25.500	4.9497	3.5000	22.0	29.0
K_Kahana	1	47.000	.	.	47.0	47.0
K_Aiea	5	34.400	12.6610	5.6622	22.0	54.0
K_Lyon	12	59.167	19.9890	5.7703	25.0	94.0
Total	47	40.255	17.5722	2.5632	19.5	94.0

Table 2.11. *C. chamissoi* mean trunk length (cm) that became fertile during the study period by region. W_ indicates study sites located in the Wai‘anae Mountain Range, K_ indicates sites in the Ko‘olau Mountain Range.

NATURAL RECRUITMENT AND MORTALITY

The meta-population abundance by size of all sampled *C. chamissoi* demonstrates a positive skewness, with more numerous individuals of smaller trunk sizes (0 – 50 cm) (Fig. 2.19).

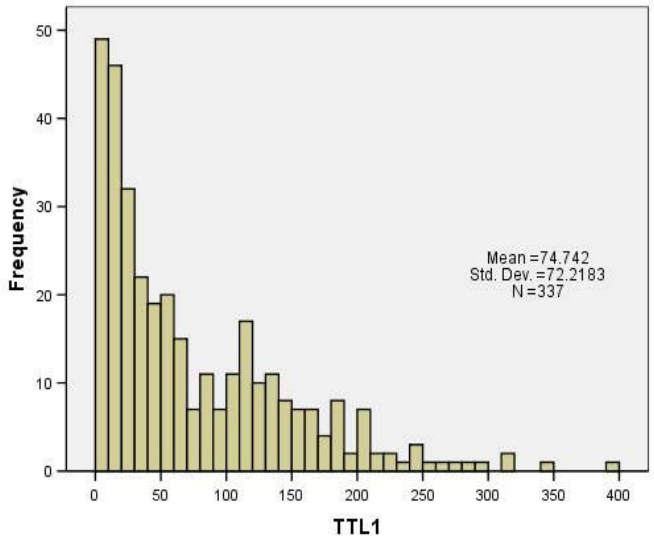


Figure 2.19. The number of *C. chamissoi* measured in all sixteen plots by total trunk length (cm) at first time of measure 2005-2006 (TTL1, $n = 337$).

Across all study plots, there were 14 new *C. chamissoi* individuals which naturally generated during the six year study period, for an overall meta-population recruitment rate of 4.2 %. However, of the new sporelings, three died prior to the last date of measurement, for an observed 21.4 % mortality rate of new recruits. Recruitment occurred in eight of the plots (50% of all measured plots), and K_Kahana demonstrated the highest total number of new ferns, with K_Kahuku second highest, as shown in Figure 2.20(a). The W_Pah2-b site had the highest percentage of recruitment relative to the total population (17%), and K_Kahana demonstrated the second highest percentage (15%), as shown in Figure 2.20(b).

There were a total of 55 tree ferns that died during the study for an observed 16.3 % meta-population mortality rate. The K_Kahuku plot demonstrated the highest total number of dead tree ferns, and K-Lyon the second highest (Fig. 2.20(a)). However, when expressed as a percentage of the initial population as shown in Figure 2.20(b), W_Pah2-b demonstrates the most significant loss of the population (50%), with K_Kahuku demonstrating the second highest loss of approximately 38%. Mortality was found to exceed or equal recruitment in 11 (69 %) of the plots. The remaining five (31 %) plots exhibited no recruitment or mortality, and therefore no change in number of ferns at the conclusion of the study; all of these were located in the Wai‘anae Mountains.

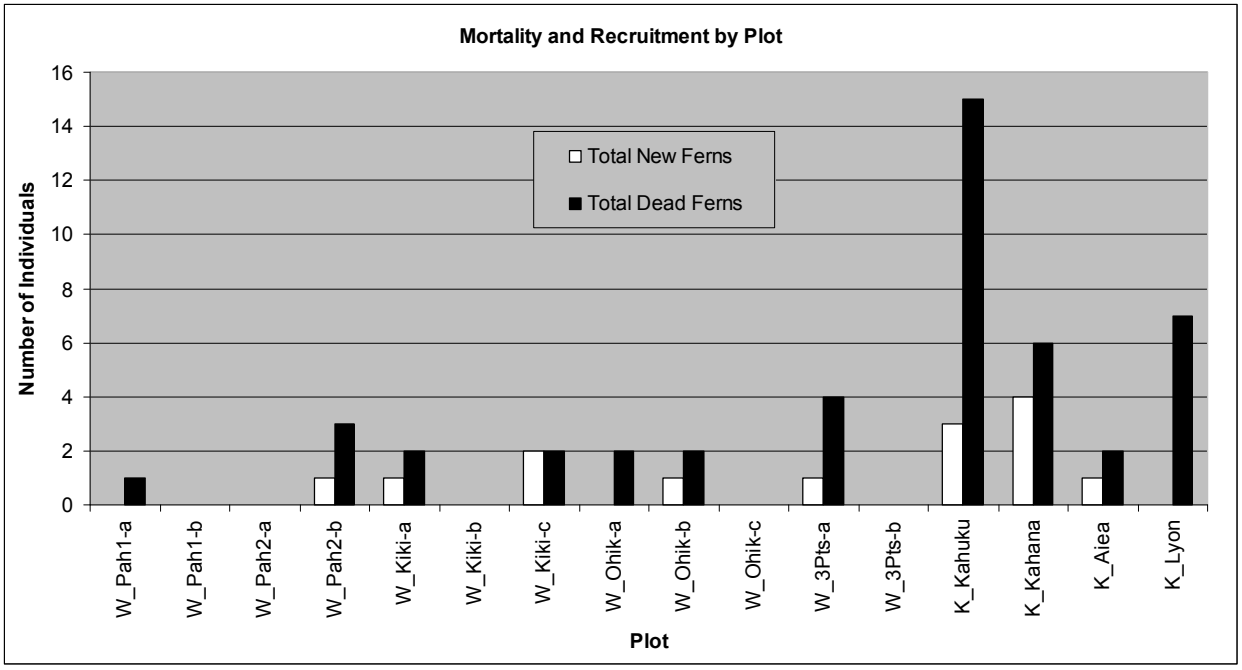


Figure 2.20(a). Number of new individuals (white bars) and number of dead individuals (black bars) as observed per plot (x-axis). *W_ = Waianae Mountain Range; K_ = Koolau Mountain Range; a = inside fence; b = outside fence; Kiki-c = Kahanahaiki outside fence steep site; Ohik-c = Ohikilolo outside fence non-native site.*

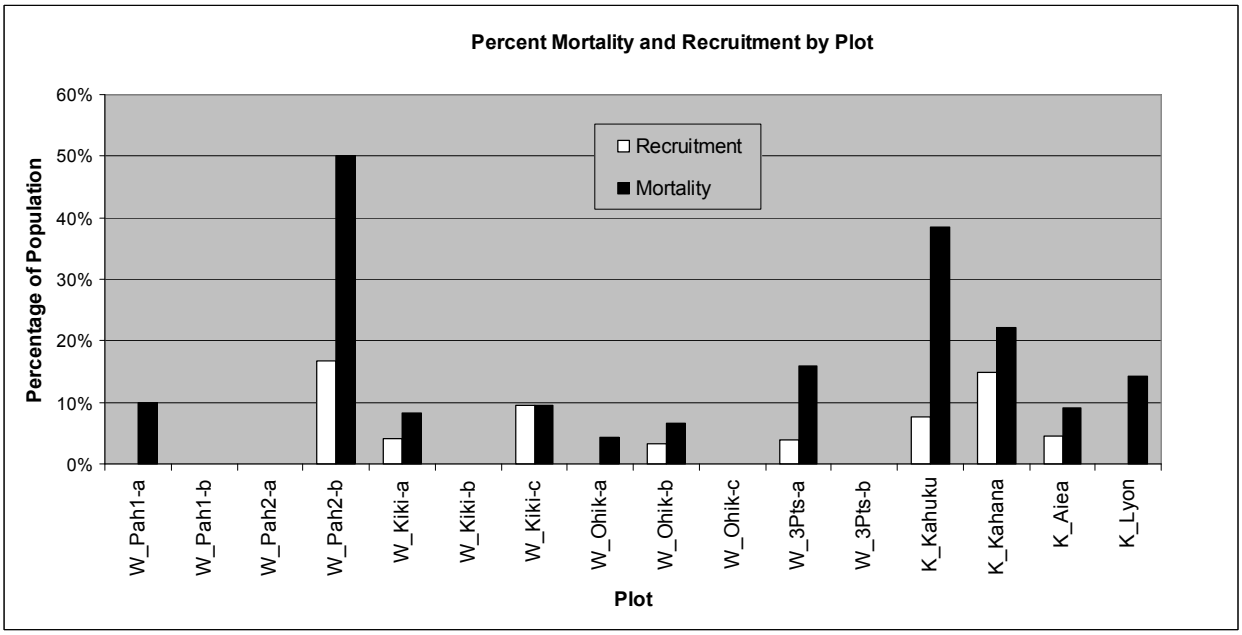


Figure 2.20(b). Recruitment = number of new ferns / total initial ferns (white bars); mortality = number of dead ferns / total initial ferns (black bars). *W_ = Waianae Mountain Range; K_ = Koolau Mountain Range; a = inside fence; b = outside fence; Kiki-c = Kahanahaiki outside fence steep site; Ohik-c = Ohikilolo outside fence non-native site.*

The rate of recruitment did not result in an increase in population size within any of the study plots at the conclusion of the study. There were four plots that did increase in *C. chamissoi* abundance occasionally during the study period, but at the end of the study, mortality resulted in the majority of plots exhibiting an overall decline in abundance, and the remaining plots maintaining no change in abundance (Figure 2.21).

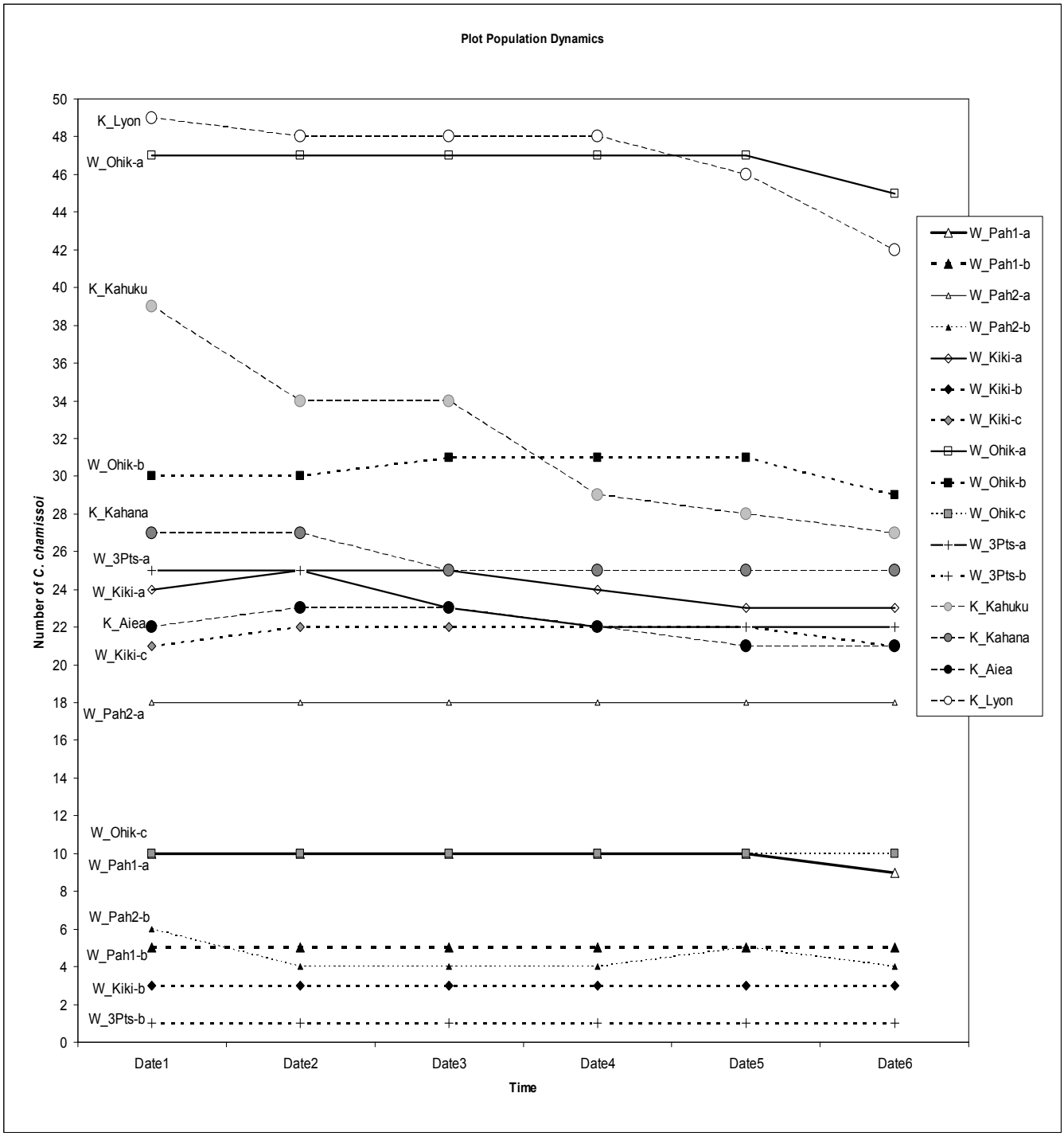


Figure 2.21. Number of *C. chamissoi* (y-axis) by plot (see legend) over entire study period by date (x-axis). Plots in the Waianae Mountains are symbolized as follows: triangles indicate Pahole region, diamonds indicate Kahanahaiki region, squares indicate Ohikilolo region, and crosses indicate the 3 Points region. Circles indicate the four Koolau Mountain plots, all unfenced. *W_ = Waianae Mountain Range; K_ = Koolau Mountain Range; a = inside fence; b = outside fence; Kiki-c = Kahanahaiki outside fence steep site; Ohik-c = Ohikilolo outside fence non-native site.*

The smaller ferns exhibited the highest mortality across the entire study period (Fig. 2.22), with size class one exhibiting 40% of the observed cases (Fig. 2.23).

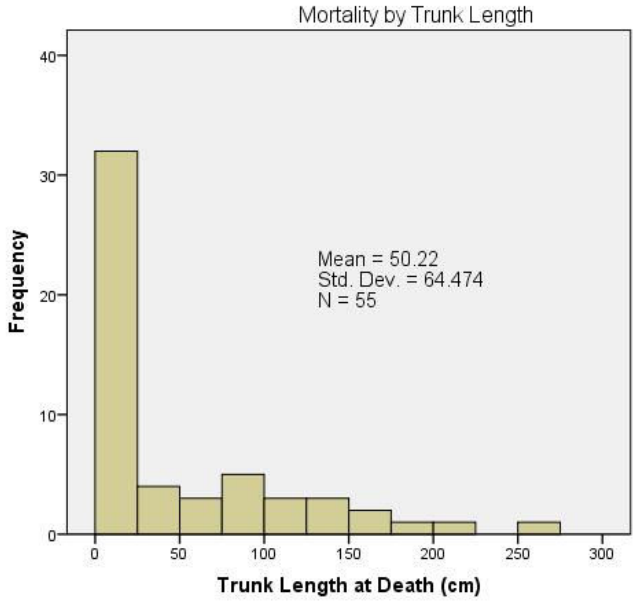
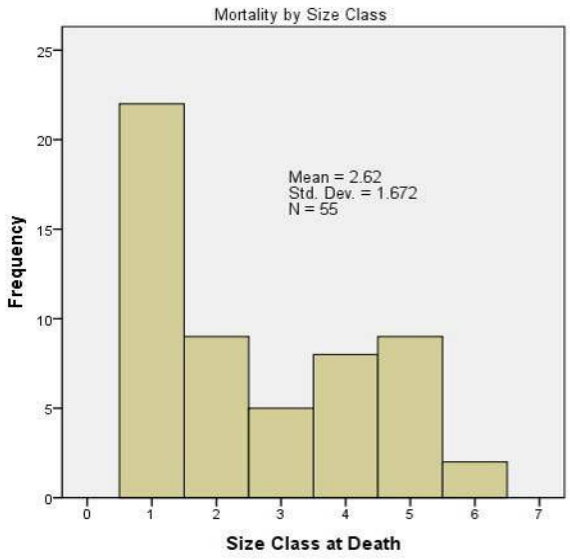


Figure 2.22. Number of *C. chamissoi* individuals observed dead (y-axis) by trunk length at death (cm) (x-axis) for all plots over entire study period.



Size Class at Death	Frequency	Percent	Cumulative Percent
Size 1 (0.1 - 9.9 cm)	22	40.0	40.0
Size 2 (10 - 19.9 cm)	9	16.4	56.4
Size 3 (20 - 39.9 cm)	5	9.1	65.5
Size 4 (40 - 99.9 cm)	8	14.5	80.0
Size 5 (100 - 199.9 cm)	9	16.4	96.4
Size 6 (200 - 299.9 cm)	2	3.6	100.0
Total	55	100.0	

Figure 2.23. *C. chamissoi* mortality statistics by size class, summarized for all plots over the six-year study period.

Of the individuals that died, 67.3% were not yet fertile. Fern mortalities in size class seven (300 - 428 cm trunk length) were not observed during this study. The majority of fern deaths observed during the study period were of unknown causes, and individuals with a trunk length less than 20 cm composed 56.4% of unknown mortalities, with individuals of trunk length greater than 20 cm composing the second highest rate of mortality at 23.6%. Across the six-year study period and all study plots, tree falls accounted for the death of five individuals, and were the highest known cause of mortality (9.1%). Three individuals suffered accidental, human-caused death during non-native vegetation eradication (5.5%), and pig predation was observed to cause the death of two individuals (3.6%).

DISCUSSION

This study includes, perhaps for the first time, observations of tree fern growth before initial development of the trunk, and estimated time to trunk establishment based on nursery growth data. The y-intercept generated from the regression equation of actual, known nursery-grown *C. chamissoi* age as predicted by trunk length was equal to 0.7 years (8.4 months), which agrees with the 6-8 months that was reported for sporeling development immediately after germinating from the gametophyte stage in the laboratory (Lynch 2011). Nursery-grown *C. chamissoi* were able to achieve a trunk height of 10 cm in approximately 3.7 years. In comparison, naturally occurring tree ferns of the same trunk length were estimated to be older, at 8.1 years. These older age estimates for naturally occuring individuals could be due to greater

variability in moisture, light, and soil conditions associated with natural habitats, which likely results in slower growth rates *in situ*. Indeed, observed mean growth rates of nursery-grown *C. chamissoi* were higher for size classes one and two (2.26 cm/yr, and 2.69 cm/yr, respectively), than that observed for *in situ* individuals of similar size (1.34 cm/yr, and 2.44 cm/yr, respectively).

I found that *in situ* tree fern growth rate does increase with size, and smaller individuals do grow quite slowly. The island-wide mean trunk growth rate of *C. chamissoi* was estimated at 3.8 cm/yr, with a range of 1.3 cm/yr for smallest individuals (size class one), and a maximum mean growth rate of 5.6 cm/yr for large individuals (size class six). Differences in growth rate by size class demonstrated a statistically significant, sequentially increasing rate of growth rate with size, with the exception of ferns in the largest size class (size class seven), which slightly declined in growth rate. Size-dependent growth has also been found for other tropical forest tree species (Herault *et al*. 2011), and is thought to increase with tree size due to greater photosynthetic area and increasing access to light (Sterck *et al.* 2003). The relative decrease in mean growth rate for the largest size class of *C. chamissoi* is consistent with natural senescence observed in other tree fern species (Seiler 1981; Nagano and Suzuki 2007; Mehltreter and Garcia-Franco 2008) and in other fern species (Bremer 2004).

Previously published growth rates for *C. chamissoi* on O‘ahu were measured over one year (November 1997 – October 1998) at a mean growth rate of 3.0 cm/yr for individuals with a trunk length between 1.5 m to 2.0 m at Lyon Arboretum (Durand and Goldstein 2001a). My results indicate that *C. chamissoi* of similar size, measured for five years at Lyon Arboretum, grew at a similar, but slightly greater rate of 3.8 cm/yr (n = 28). These slight differences in observed growth rates could be due to any number of differences in site-specific factors, despite

close regional proximity of study sites, such as disturbance history (a large tree fall was observed in my plot during the study period), canopy and understory species composition (Lyon Arboretum has been naturalized by numerous introduced species), forest structure, and light regime. Furthermore, annual rainfall was quite variable among the study periods. Growth data collected over longer time periods may therefore provide a more robust estimation of growth rate as it varies by size and habitat.

The observed mean growth rates for the larger *C. chamissoi* on O‘ahu (size classes four to six: 0.4 – 3.0 m trunk length) ranged between 4.23 to 5.58 cm/yr. These growth rates are similar to those previously observed for *C. glaucum* on the island of Hawai‘i, measured at 5.08 cm/yr (Wick and Hashimoto 1971), and 4.4 to 6.5 cm/yr on younger and older sites, respectively (Walker and Aplet 1994). The congruency of these growth rates may indicate that despite the large differences in substrate age among islands, large *C. chamissoi* and *C. glaucum* individuals grow at comparable rates. Nevertheless, the Hawaiian *Cibotium* appear to be relatively slow-growing species among other tree fern taxa. For example, these growth rates as measured for *C. chamissoi* on O‘ahu are quite a bit lower than those measured for the invasive tree fern *Sphaeropteris cooperi* at 15.4 cm/yr on O‘ahu (Durand and Goldstein 2001a). The authors suggest that the ability of *S. cooperi* to maintain a greater leaf surface area (12 to 18 fronds per fern) than native *Cibotium* spp. (3 to 6 fronds per fern) enables the higher relative growth rate of *S. cooperi* (Durand and Goldstein 2001a). Other relatively fast-growing species, such as *Cyathea caracasana* in Colombia, at 16.8 cm/yr (Arens 2001), and *Cyathea arborea* in Puerto Rico, at 28.6 cm/yr (Conant 1976) have higher growth rates in open habitats or forest gaps. However, higher growth rates have been observed for other tree fern species across both primary and

secondary habitats, such as *Cyathea delgadii* in Costa Rica, at 21.3 cm/yr and 81.9 cm/yr, respectively (Bittner and Breckle 1995).

Despite the use of a method to estimate tree fern age that was based on the variation in growth rate by size, additional factors continue to complicate our ability to determine *C. chamissoi* age with certainty. The oldest wild *C. chamissoi* in this study was measured at a trunk length of 428 cm, and was calculated to be approximately 100 years old. In fact, it is likely that these tree ferns are older, because growth rate calculations were complicated by the gradual decomposition of the older rhizome tissue, a phenomenon which has also been reported by Sharpe and Mehltreter (2010), and resulted in some of the growth measurements to demonstrate decreases in trunk length. These portions of the decomposing rhizome were along the horizontal section of the caudex, at the furthest distance from the growth apex. Growth rate calculations were also complicated by the many tree ferns that fell over, developed new roots, and continued growing upward. Some of these events were caused by falling trees and branches during the study. This seems to be a common growth pattern by *C. chamissoi*, as I observed approximately 46.7 % of the individuals across all plots at the beginning of the study (n = 351) to have significant proportions of their trunks oriented horizontally along the ground, and 4.84 % of measured individuals were observed to fall over during the study. All but one of these survived and continued growing along the main caudex or from lateral shoots. This tipping rate is comparable to the 44% measured for *Leptopteris wilkesiana* tree ferns that were observed to have fallen over and continued growing in primary forests of Fiji (Ash 1986). It is likely that natural causes in addition to tree falls are responsible for the occurrence of this event in the life history of a tree fern, such as high wind events from storms and hurricanes, erosion, flooding, and the gradual force of gravity, which simultaneously acts upon the soil, causing soil creep, and

upon the tall leaf crowns of tree ferns, slowing pulling them both downslope on the steep sides of mountains. In these ways, measurement of *C. chamissoi* growth rates and subsequent age estimation have proven difficult to ascertain due to variable life histories, and long persistence in habitats which have been subject to variable disturbances during their long life span.

When analyzed across all study sites, tree fern trunk length weakly predicted trunk growth rate, similar to the findings of Tanner (1983) for the tree fern *Cyathea pubescens* in Jamaica. Trunk top circumference and longest frond length better predicted *C. chamissoi* growth rate in regression analyses, although both morphological characteristics were still, at best, moderate predictors of growth rate. These findings agree with those of Mehltreter and Garcia-Franco (2008), who also found mean leaf length and trunk height to be significantly correlated, but trunk growth rate to be weakly correlated with trunk height of *Alsophila firma* (Baker) D.S. Conant in Mexico.

Results of mean growth rates for *C. chamissoi* of similar size across all plots indicated regional differences, such that mean growth rate was quite variable among regions, but generally lower for the lower rainfall sites in the western Wai‘anae Mountains as compared to those in the Ko‘olau Mountains. Durand and Goldstein (2001a) did not find substantial differences in *C. chamissoi* growth across sites ranging from 155 m to 1,225 m elevation, but they did document an increase in growth to correspond with an increase in rainfall during the months of April to August (Durand and Goldstein 2001a). I found that *C. chamissoi* individuals of similar sizes, monitored over longer time periods, did demonstrate differences in mean growth rate among regions on O‘ahu, with mean growth rates greatest at Aiea (9.56 cm/yr), and least at Lyon (3.81 cm/yr), both of these sites located in the Ko‘olau range. Observed variations in growth rate among individuals of similar size may reflect variable habitat conditions within and among

populations, and also may reflect larger vegetation patterns of succession or response to changing climate conditions (Oostermeijer et al. 1996; Jansen *et al*. 2012). Additional studies have found tree fern growth rates to vary with successional habitat type, and this may also be a factor influencing *C. chamissoi* growth patterns. Bittner and Breckle (1995) conducted one of the first studies relating tree fern growth rates with habitats, and they found that for two *Alsophila* species and four species of *Cyathea* in Costa Rica, growth rates were indeed influenced by habitat, in that over 2.5 years, individuals within secondary forest grew three times as fast as in primary forest. Similarly, Arens (2001) studied the variations in the growth of *Cyathea caracasana* across a successional mosaic in an Andean cloud forest of Colombia, and determined anatomical and morphological traits to be flexible responses, allowing this tree fern to grow across a range of habitats in various stages of succession.

In my study of *C. chamissoi* growth rates presented here, methods to estimate age based on measured growth rates in natural populations are also complicated by the possibility that some of the individuals observed in the smaller size classes could have generated vegetatively from belowground, and may demonstrate more rapid growth than a newly germinated sporeling from a gametophyte. Therefore, it may be more appropriate to use multiple morphological attributes that are better correlated with growth rate, such as trunk top circumference and longest frond length, in order to gauge the vigor and life history stage of the tree fern.

During the study period, the minimum trunk length of a newly transitioned fertile fern was 19.5 cm (mean = 40.3 cm), and the majority of the tree ferns became fertile when their trunks reached 20 – 40 cm in length. Based on age estimates from mean growth rate by size class, this would indicate *C. chamissoi* reaches maturity between 12.2 and 18.2 years old. This is

quite a long time to survive for reproduction in the dynamic forest understory, especially in areas with grazing and digging ungulates.

Mortality generally exceeded recruitment in ten out of sixteen plots, with the remaining six plots exhibiting no change in overall abundance. The highest rates of mortality for *C. chamissoi* were observed for the smaller sized individuals, with size class one (0 – 10 cm trunk length) exhibiting 40% of the observed mortalities. Of the individuals that died, 67.3% were not yet fertile, indicating that over half of the population died before reproducing during the study period. Of the observed mortalities, it is likely that some of the small individuals were swept away during erosional events, especially in the case of the Kahana plot which was located on a steep slope beneath a military road, which underwent significant grading and road improvements after the initial establishment of the plot.

CONCLUSION

Cibotium chamissoi on O‘ahu demonstrate increasing growth with increasing size, and indeed can be considered slow growing species at an average of 3.8 cm/yr. Frond length and trunk top circumference were better predictors of growth rate than total trunk length, and therefore are suggested for inclusion in measurements of future growth studies of this species. The oldest *C. chamissoi* individual across all study sites was estimated at 100 years, indicating these tree ferns can persist for long time spans, and monitoring their populations may be a useful method in understanding broader patterns in vegetation dynamics. Although recruitment was observed in half of our study sites, the rate of recruitment did not result in an increase in population size within any of the study plots at the conclusion of the study, due to greater rates of

mortality. I found 69% of the study sites to exhibit declines in abundance, and the remaining 31% did not exhibit any change in abundance over the five to six years of monitoring, depending on the study site. Individuals that had not yet reached fertility composed over half of the observed mortalities across all sites. These findings may indicate *C. chamissoi* is gradually becoming reduced in abundance on the island of O‘ahu. Although I was unable to directly measure rainfall at each of the study sites, the general climate record for O‘ahu indicates below-average annual rainfall during the study period, which, in addition to increasing competition with invasive alien vegetation, may explain the reduction in *C. chamissoi* populations over time, and warrants futher investigation.

CHAPTER THREE

GENETIC DIVERSITY OF *CIBOTIUM CHAMISSOI* POPULATIONS ON O'AHU ISLAND, HAWAI'I

INTRODUCTION

Studies of population dynamics have been greatly enhanced in recent years with the theoretical, analytical, and methodological developments of population genetics (Zhang and Hewitt 2003). The major methodological advancements that have contributed to the increase of population genetic studies include the development of polymerace chain raction (PCR) technology, and the discovery of sensitive microsatellite markers, which have become the molecular marker of preference for population genetic studies (Jimenez *et al*. 2010). Microsatellite DNA markers are short tandem repeats (one to six base pairs) of nucleotides found in the nuclear genome of most organisms. They are considered to be advantageous over other molecular markers because they are neutrally inherited, codominant, and can also be used as a powerful tool in the identification of clones (Suvanto and Latva-Karjanmaa 2005). Microsatellite markers occur widely throughout eukaryote organisms, and have high mutation rates while obeying Mendelian inheritance (Zhang and Hewitt 2003). Microsatellites have been usefully applied to facilitate the conservation of biological diversity of rare and endangered species (Avise 1989; Drummond *et al.* 2000; Ellis and Burke 2007; Krishnan *et al*. 2013), and to estimate the gene flow within and among populations of ecologically important species (Crawford *et al*. 2008). They have facilitated the analysis of genetic population structure,

interpretation of recent population history, detection of parentage relationships, and measurement of genetic diversity (Zhang and Hewitt 2003; Jimenez *et al*. 2010).

Historically, ferns were assumed to have low genetic differentiation among populations due to their ability for increased frequency of long distance dispersal (achieved with abundant wind-dispersed spores, and the capacity to establish populations from a single colonizing spore through intragametophytic selfing), and this was thought to result in the majority of genetic variation occurring within fern populations, rather than among them (Soltis and Soltis 1989; Schneller and Holderegger 1996). However, most studies that have utilized microsatellite markers to assess the genetic diversity of pteridophytes have focused on rare species (e.g. Pryor *et al*. 2001; Vitalis *et al*. 2001; Woodhead *et al*. 2003; Woodhead *et al*. 2005; Kang *et al*. 2006; Kang *et al*. 2008; Zhou *et al*. 2008; Jimenez *et al*. 2010; Izuno *et al*. 2012). Rare or restricted fern species have been found to demonstrate low within-population genetic diversity (Zhou *et al*. 2008; Jimenez *et al.* 2010; Izuno *et al*. 2012), although the endangered fern *Adiantum reniforme* var. *sinense* Y.X. Lin (Adiantaceae) in south-central China demonstrated high levels of genetic diversity (Kang *et al*. 2008). This species also demonstrated slightly elevated levels of inbreeding for eight nuclear microsatellites as surveyed across 13 populations (Kang *et al*. 2008), similar to elevated estimated levels of inbreeding found for other restricted fern populations. For example, Zhou *et al*. (2008) reported results of the genetic diversity exhibited across three polymorphic microsatellite loci of the rare tree fern *Alsophila spinulosa* (Hook) Tryon (Cyatheaceae) within a single population in China, and found a noticeable deficit of heterozygotes and overall low genetic diversity, suggesting high levels of inbreeding within the population. In a study of a smaller rare fern, Jimenez *et al*. (2010) utilized microsatellites to assess the genetic diversity of *Dryopteris aemula* in Spain, and found that across five

polymorphic loci for the four populations sampled, inter-population genetic differentiation was high (F_{ST} = 0.520), and therefore the estimated number of migrants per generation was low (Nm = 0.25), with all loci exhibiting a deficit in heterozygotes and significant departure from expected values under Hardy-Weinberg equilibrium. In another study, results of microsatellite analysis of six populations of the alpine lady-fern *Athyrium distentifolium* Tausch ex Opiz (Athyriaceae), classified as a rare plant in Scotland but also more widely distributed in montane areas throughout the Northern Hemisphere, indicated that nine of the ten loci examined demonstrated significant deviation from Hardy-Weinberg equilibrium (Woodhead *et al*. 2003), and significant differentiation was found for the population pairs studied, with 58% of the variation partitioned within populations, and 34% of the variation partitioned between countries (Woodhead *et al*. 2005). Therefore, it appears that despite the ability for long-distance dispersal and increased rates of genetic exchange, microsatellite studies of rare or restricted fern species have demonstrated relatively low genetic exchange among populations, which may result in increased or decreased levels of genetic diversity within populations, depending on the breeding system and distribution of the species (Woodhead *et al*. 2005; Kang *et al*. 2008; Zhou *et al*. 2008; Jimenez *et al*. 2010). This is consistent with previous observations that rare or endemic seed-plant species demonstrate greater population fragmentation, and therefore lower effective population sizes than more widespread species, and this may lead to greater genetic differentiation among populations (Hamrick and Godt 1996).

Previous genetic analyses of fern populations using other molecular methods have provided additional useful insights to the environmental and evolutionary controls on fern population dynamics. In one example, Ranker (1994) found that allozymic variability across 15 loci was higher than expected for a rare species of the small epiphytic fern *Adenophorus periens*

L. E. Bishop, which was sampled from 25 individuals in the Kahaualea State Natural Area Reserve on Hawai‘i Island. Substrate age of the *A. periens* population was estimated to be of approximately 350 – 500 thousand years old, and so it was hypothesized that the sampled population may have low genetic variability due to a limited number of founders, and high levels of inbreeding (Ranker 1994). Contrary to expectations, all of the sampled individuals were found to be heterozygous for at least one locus, indicating that they were all produced from the sexual fertilization between two independent gametophytes, and that the population exhibited no reproduction via intra-gametophytic selfing (Ranker 1994). These results were surprising, given that the genetic variability for this very small epiphytic fern population was greater than expected, a species which today is greatly restricted in distribution and abundance from its previous range. Similarly, Ranker (1992a, 1992b) also found the closely related epiphytic ferns *A. tamariscinus* (Kaulf.) Hook. & Grev. and *A. tripinnatifidus* Gaud. in Hawai‘i to demonstrate intermediate to high levels of genetic diversity as compared to those reported for mainland fern species. However, the most restricted Hawaiian fern species studied, *A. periens*, had significantly higher levels of observed and expected heterozygosities than those of the more widely distributed and common *A. tamariscinus* and *A. tripinnatifidus* (Ranker 1994). It is suggested that *A. periens* has been able to maintain these unexpectedly high rates of genetic variability, despite being locally rare, through its highly outcrossing mating system, and through the incorporation of new genetic mutations (Ranker 1994).

Cibotium chamissoi Kaulf. is a much larger, commonly distributed tree fern on the island of O‘ahu. Distribution is patchy from 150 – 1,200 m elevation in mesic to wet forests, but less common above 800 m, and occasionally it can be found as low as 50 m (Palmer 1994, 2003). Populations may demonstrate high rates of gene flow and therefore little genetic differentiation

among populations, or it is possible that populations may be fragmented and/or geographically isolated to the extent that rates of gene flow are low, and therefore may demonstrate genetic differentiation among populations. Results of demographic analysis as reported in Chapter Two indicate that *C. chamissoi* may be in gradual decline in the majority of sampled populations. The main objective of this study is to determine levels of genetic diversity within natural populations of *C. chamissoi* in order to explore whether populations are primarily outcrossing or inbreeding, and what proportion of the individuals within each population are genetically identical (clones). The *C. chamissoi* on O‘ahu frequently have multiple caudices, and small proliferations have been observed growing as adventitious buds in 68% of large individuals surveyed in a previous study (Arcand 2007), increasing the possibility that populations may be composed of a considerable number of genetically identitical individuals. However, the ability of ferns to reproduce via wind-dispersed spores leads to a heightened capacity for long-distance dispersal, and an increased possibility for frequent inter-population exchanges of genetic material.

OBJECTIVES

The main objective of this study was to utilize microsatellite markers to estimate patterns of genetic diversity in natural populations of *C. chamissoi* in permanent growth plots on O‘ahu across a diversity of habitats. I employ a population genetics approach to address the general question of whether natural *C. chamissoi* populations are genetically isolated, or whether they are able to successfully maintain populations through high rates of genetic exchange and sexual

reproduction. This research will also illuminate whether populations exhibiting no recruitment also demonstrate reduced genetic diversity. Specific research questions were:

i) What are the patterns of genetic diversity within and among populations of *C. chamissoi* on O‘ahu?

H: Because ferns are spore-dispersed via wind, intra-specific evolutionary divergence will not have occurred among populations on O‘ahu despite the geographic lowland barrier and spatial distance between populations in the Wai‘anae and Ko‘olau mountain ranges.

ii) What is the predominant method of *C. chamissoi* recruitment in natural populations: as sporelings (genets) developing from the sexual reproduction of gameotphytes, or ramets developing from vegetative clones?

H: A proportion of C. chamissoi populations will originate vegetatively, but due to the high dispersal ability of spores, it is unlikely that entire populations will represent a single clone.

METHODS

SAMPLE COLLECTION

I collected initial *C. chamissoi* leaf tissue samples from each tagged fern within all permanent plots as measured for the demography study on O‘ahu Island in 2005-2006 (see

Chapter One for map and detailed site descriptions), for a total of 336 ferns sampled. Table 3.1 lists the populations sampled, and includes for each population the number of individuals sampled, whether *C. chamissoi* recruitment has been observed, and geographic site details.

Plot Name	**Plot Code**	N	Recruit-ment	Mountain Range	Fenced	Elevation (m)	Aspect	Slope °	Annual Rainfall (mm)
Pahole In 1	WPah1A	10	N	Waianae	yes	635	NE	34	1383
Pahole Out 1	WPah1B	5	N	Waianae	no	675	NE	24	1513
Pahole In 2	WPah2A	18	N	Waianae	yes	670	NE	34	1425
Pahole Out 2	WPah2B	6	Y	Waianae	no	685	NE	37	1513
Kahanahaiki In	WKikiA	25	Y	Waianae	yes	590	NW	7	1406
Kahanahaiki Out	WKikiB	3	N	Waianae	no	600	NW	19	1384
Kahanahaiki Out Steep	WKikiC	21	Y	Waianae	no	585	NW	34	1384
Ohikilolo In	WOhikA	45	N	Waianae	yes	920	NE	20	1485
Ohikilolo Out	WOhikB	29	Y	Waianae	no	883	NE	32	1476
Ohikilolo Out Weedy	WOhikC	10	N	Waianae	no	867	NW	31	1485
3 Points In	WThreePtsA	25	Y	Waianae	yes	841	NE	13	1764
3 Points Out	WThreePtsB	1	N	Waianae	no	841	NE	22	1764
Kahuku MTA	KKahuN1	38	Y	Koolau	no	337	NE	37	2356
Kahana State Park	KKahaN2	31	Y	Koolau	no	135	SE	27	3812
Aiea	KAieaS1	23	Y	Koolau	no	440	SE	28	3123
Lyon Arboretum	KLyonS2	47	N	Koolau	no	230	NE	29	3836

Table 3.1 Summary description of research plots. N = number of sampled individuals. Mean annual rainfall was interpolated using data provided by *The Rainfall Atlas of Hawai'i* (Giambelluca *et al.* 2011), based on data during the 30-year period 1978 – 2007.

Tissue was collected from healthy looking, fully expanded leaves, and stored individually on silica in plastic ziplock bags labeled with fern ID number. Fresh tissue samples were collected again from each tagged fern within all plots in July-August 2011, excluding the three Ohikololo plots which were re-collected in November 2011. Tissue was collected from the newest/youngest, healthy looking leaf as close to the leaf apex as possible to achieve the maximum amount of green tissue, and stored individually in plastic ziplock bags in the refrigerator at 4°C.

DNA EXTRACTION

Initial DNA extractions were performed from silica dried tissue samples (collected 2005-2006) in the Ranker Lab at the University of Hawai‘i – Mānoa during the winter of 2009-2010 with the GenCatch^{TM} Plant Genomic DNA Purification Kit (Epoch Biolabs). Extracted DNA was eluted in TE Buffer and stored at -20°C, and in July 2010 was sent to the Nevada Genomics Center at the University of Nevada, Reno for sample normalization, PCR, and fragment analysis. For reasons which we were unable to discern, approximately half of the 96 tested samples showed no tagged products, i.e. they failed to amplify. After examining extraction dates and quantitation data, a pattern did not emerge to explain the failed samples, so fresh collections and DNA extractions were performed again for all fern samples.

Fresh leaf tissue samples were collected again in the summer of 2011, and extractions were performed within one to two weeks from date of collection. Genomic DNA was extracted from approximately 0.6 g of fresh leaf material using the CTAB method (Doyle and Doyle 1987), modified by adding 3% PVP-40 and 5mM ascorbic acid (see Appendix 10). Samples were cleaned, dried, eluted in 200ul $2H_2O$, and stored at -20°C. Working samples were then transferred to 96-well plates and shipped on blue ice to the Nevada Genomics Center for sample normalization, optimization, and PCR.

Due to the mortality of some individuals during the study (N = 55), we were unable to re-collect fresh tissue in 2011, and in those cases we attempted to extract DNA again using the CTAB method from any remaining tissue in storage on silica. In some cases, there was no tissue remaining, and these samples were excluded from further analysis. In addition, there were new individuals that grew during the six-year growth monitoring study (see Chapter 2), and leaf

tissue samples were collected from those still alive in 2011, and were also included in the final analysis.

MICROSATELLITE DEVELOPMENT AND MARKER SELECTION

Cibotium chamissoi leaf samples were collected from an individual at Lyon Arboretum, Mānoa valley, O‘ahu in September of 2009, and stored in plastic ziplock bags in the refrigerator at 4°C until genetic extractions were performed a few days later. A voucher specimen was deposited with the Joseph F. Rock Herbarium (HAW) at the University of Hawai‘i at Mānoa (see Appendix 11 for collection label). Nuclear DNA was extracted from fresh leaf tissue using the GenCatch^{TM} Plant Genomic DNA Purification Kit (Epoch Biolabs), and extracted DNA was sent to Genetic Identification Services, Inc. (GIS), Chatsworth, CA, for the identification of polymorphic loci and development of primer sequences. Twelve polymorphic loci, nine monomorphic loci, and three failed loci were identified by GIS (Table 3.2).

Polymorphic Loci*	Monomorphic Loci	Failed Loci
A3*	A107	B1
A8*	A108	B102a
A105	A112(?)	B116
A114*	B112	
A115*	B115	
A118*	B120	
B4*	C11	
B5	C112(?)	
B104	D6	
B121*		
C12*		
C109*		

Table 3.2. Summary of *C. chamissoi* primer test results, as analyzed by Genetic Identification Services; * = microsatellite locus selected for analysis.

Of the 12 polymorphic loci identified by GIS, nine were selected for PCR and fragment analysis at Nevada Genomics Center, based on results of test runs to combine the maximum number of microsatellite markers with the least amount of noise into two multiplex panels. Samples were run in 96-well format on the ABI Prism 3730 DNA Analyzer, with the filter set to G5 to detect fluorescent dyes 6-FAM (blue), VIC (green), NED (yellow) and PET (red). The 500MW size standards were labeled with LIZ. The nine microsatellite loci were amplified in two multiplex panels; see Appendix 12 for the detailed PCR protocols used by Nevada Genomics.

MICROSATELLITE ANALYSIS

Results of PCR amplifications were scored using GeneMapper 4.0 (Applied Biosystems). Alleles were coded as their integer size in base pairs (bp). Three of the microsatellite markers, A114, B4, and C109, did not amplify well and/or were too noisy to interpret, and therefore were not included in further analysis. The remaining six loci (A3, A8, A115, A118, B121, and C12) were retained, and their estimated product sizes and primer sequences are provided in Table 3.3.

Locus Code	Primer Sequence 5'-3'	Product Size (bp)
A3	F: AGC-CTA-CCG-ACA-AGT-GGT-T R: TTC-CCT-GTG-ATG-ATT-GGA-C	ca. 230-260
A8	F: ATA-AGC-CCT-CTT-GCG-TAG-AAC R: AAG-CAC-GAG-CAT-TGT-TTA-GAA	ca. 190-210
A115	F: ATT-CGG-TAG-TTC-TAT-CAC-TTG-C R: ATT-TCT-CAG-CAT-TCT-GTT-TGT-C	ca. 247-260
A118	F: ACC-AAT-GGG-CTA-ACC-ACT-A R: ATT-CTC-ATA-CCC-CTT-GAA-CAC	ca. 230-300
B121	F: AGG-ATG-GTC-TCA-CTG-TGT-GAC R: AAC-CAG-CAG-GTT-ATA-CAA-GGA-G	ca. 250-280
C12	F: CTT-AGC-TTT-GAA-CTT-TGA-CTC-G R: AAT-TTG-CAT-GTC-CAG-GTG-T	ca. 75-270

Table 3.3. Six polymorphic loci used in this study, forward and reverse primer sequences, and approximate product sizes (base pair).

The following parameters were calculated in order to estimate genetic diversity for each locus in each population with GenAlEx 6.5 software (Peakall and Smouse 2006, 2012): number of alleles per locus (A), the observed (H_o) and the expected (H_e) heterozygosities based on Hardy-Weinberg assumptions, the allelic fixation index (F_{IS}), the number of observed genotypes, and the number of private alleles. Each locus was tested by population for deviation from expected genotype frequencies using the Chi-square test of Hardy-Weinberg equilibrium (Hartl 2000).The identification of clones within each population was achieved by performing a multilocus match analysis.

The calculation Wright's F statistic (Wright 1946, 1951, 1965) provides a measure of allele frequency differences across populations, and is based on the assumption of mutation-drift equilibrium. The fixation index F, also known as the inbreeding coefficient, consists of values ranging from -1 to +1. If random mating occurs, values will be close to zero, while inbreeding or

undetected null alleles will be indicated by substantial positive values, and excess of heterozygosity, due to negative assortative mating, or selection for heterozygotes will be indicated by negative values (Peakall and Smouse 2012). The F_{IS} statistic measures the degree of inbreeding, demonstrated by a reduction in heterozygosity of an individual due to non-random mating within its subpopulation, and is expressed as a deviation from the expected under Hard-Weinberg equilibrium (Weir and Cockerham 1984). The F_{IS} statistic has been shown to be independent of the mutation model (Rousset 1996), and was calculated as:

F_{IS} = (Mean H_e - Mean H_o) / Mean H_e

RESULTS

Missing data varied by locus, from a minimum of 3.8 % at A115, to a maximum of 7.6 % at A118 (see Table 3.4).

Locus	A3	A8	B121	A115	A118	C12
Samples Missing Data	25	15	17	13	26	16
Percent Missing	0.073	0.044	0.050	0.038	0.076	0.047

Table 3.4. Percent missing data by locus.

Results of genetic diversity parameters, as analyzed with GenAlEx 6.5, are reported in Table 3.5.

Population	Locus	N	A	H_o	H_e	F_{IS}	Sig	No. Observed Genotypes	No. Private Alleles
WPah1A	A3	10	2	0.100	0.095	-0.053	ns	2	-
	A8	10	4	0.700	0.510	-0.373	ns	4	-
	A115	10	5	0.500	0.700	0.286	ns	7	-
	A118	10	8	0.500	0.825	0.394	*	9	1
	B121	10	5	0.800	0.750	-0.067	ns	7	-
	C12	10	11	0.800	0.870	0.080	ns	10	-
	Mean	**10.0**	**5.8**	**0.567**	**0.625**	**0.045**		**6.50**	
WPah1B	A3	5	4	0.800	0.660	-0.212	ns	4	-
	A8	5	2	0.600	0.500	-0.200	ns	3	-
	A115	5	5	0.400	0.740	0.459	ns	5	-
	A118	5	5	0.600	0.680	0.118	ns	5	-
	B121	5	4	0.800	0.740	-0.081	ns	5	-
	C12	5	8	0.800	0.860	0.070	ns	5	-
	Mean	**5.0**	**4.67**	**0.667**	**0.697**	**0S.026**		**4.50**	
WPah2A	A3	18	6	0.278	0.528	0.474	*	9	-
	A8	18	4	0.722	0.708	-0.020	ns	8	-
	A115	18	5	0.667	0.785	0.151	**	9	-
	A118	17	12	0.471	0.879	0.465	***	11	-
	B121	18	6	0.833	0.748	-0.113	ns	10	-
	C12	18	12	0.889	0.858	-0.036	*	14	-
	Mean	**17.8**	**7.5**	**0.643**	**0.751**	**0.153**		**10.17**	
WPah2B	A3	6	4	0.667	0.514	-0.297	ns	4	-
	A8	6	4	1.000	0.681	-0.469	ns	4	-
	A115	6	5	0.833	0.667	-0.250	ns	4	-
	A118	6	4	0.333	0.625	0.467	ns	4	-
	B121	6	5	1.000	0.736	-0.358	ns	4	-
	C12	6	5	0.500	0.764	0.345	*	4	-
	Mean	**6.0**	**4.5**	**0.722**	**0.664**	**-0.094**		**4.00**	
WKikiA	A3	25	5	0.400	0.439	0.089	ns	7	-
	A8	25	3	0.600	0.595	-0.008	ns	5	-
	A115	25	3	0.640	0.474	-0.349	ns	4	-
	A118	24	11	0.458	0.827	0.446	***	15	3
	B121	25	5	0.760	0.666	-0.140	ns	9	-
	C12	25	15	0.880	0.886	0.006	**	21	1
	Mean	**24.8**	**7.0**	**0.623**	**0.648**	**.007**		**10.17**	
WKikiB	A3	3	3	0.333	0.611	0.455	ns	3	-
	A8	3	2	0.333	0.278	-0.200	ns	2	-
	A115	3	3	0.667	0.500	-0.333	ns	3	-
	A118	3	3	0.333	0.611	0.455	ns	3	-
	B121	3	3	0.667	0.611	-0.091	ns	3	-

Population	Locus	N	A	H_o	H_e	F_{IS}	Sig	No. Observed Genotypes	No. Private Alleles
WKikiB	C12	3	5	1.000	0.778	-0.286	ns	3	-
	Mean	**3.0**	**3.2**	**0.556**	**0.565**	**.000**		**2.83**	
WKikiC	A3	21	6	0.571	0.732	0.220	ns	11	-
	A8	21	3	0.476	0.611	0.221	*	6	-
	A115	21	7	0.905	0.810	-0.118	***	13	-
	A118	20	6	0.700	0.726	0.036	ns	11	-
	B121	21	6	0.714	0.778	0.082	ns	11	-
	C12	19	7	0.579	0.839	0.310	***	12	-
	Mean	**20.5**	**5.8**	**0.658**	**0.749**	**0.125**		**10.67**	
WOhikA	A3	44	7	0.727	0.792	0.082	***	12	1
	A8	44	5	0.727	0.689	-0.055	ns	9	1
	A115	44	4	0.568	0.602	0.057	*	7	-
	A118	43	9	0.674	0.831	0.188	***	14	1
	B121	45	5	0.667	0.613	-0.088	***	7	-
	C12	44	14	0.909	0.898	-0.013	***	20	5
	Mean	**44.0**	**7.3**	**0.712**	**0.737**	**0.028**		**11.50**	
WOhikB	A3	28	6	0.536	0.707	0.242	*	10	-
	A8	29	4	0.655	0.709	0.076	ns	9	-
	A115	29	5	0.586	0.548	-0.071	ns	7	-
	A118	27	8	0.556	0.843	0.341	***	11	-
	B121	29	4	0.724	0.656	-0.103	ns	7	-
	C12	27	8	0.444	0.795	0.441	***	11	1
	Mean	**28.2**	**5.8**	**0.584**	**0.710**	**0.154**		**9.17**	
WOhikC	A3	10	4	0.700	0.655	-0.069	ns	5	-
	A8	10	3	0.700	0.620	-0.129	ns	5	-
	A115	10	4	0.600	0.625	0.040	ns	6	-
	A118	10	5	0.600	0.585	-0.026	ns	6	-
	B121	10	4	0.600	0.535	-0.121	ns	5	-
	C12	10	4	0.100	0.685	0.854	***	5	-
	Mean	**10.0**	**4.0**	**0.550**	**0.618**	**0.092**		**5.33**	
WThreePtsA	A3	24	7	0.250	0.653	0.617	***	10	-
	A8	24	6	0.417	0.547	0.238	***	8	1
	A115	24	6	0.458	0.569	0.194	ns	9	-
	A118	23	17	0.609	0.912	0.333	*	22	1
	B121	23	7	0.826	0.810	-0.020	ns	13	-
	C12	24	19	0.708	0.880	0.195	ns	21	6
	Mean	**24.7**	**10.3**	**0.529**	**0.732**	**0.284**		**13.83**	
WThreePtsB	A3	1	1	0.000	0.000	#N/A		1	-
	A8	1	1	0.000	0.000	#N/A		1	-
	A115	1	1	0.000	0.000	#N/A		1	-

Population	Locus	N	A	H_o	H_e	F_{IS}	Sig	No. Observed Genotypes	No. Private Alleles
WThreePtsB	A118	1	2	1.000	0.500	-1.000	ns	1	-
	B121	1	1	0.000	0.000	#N/A		1	-
	C12	1	1	0.000	0.000	#N/A		1	-
	Mean	**1.0**	**1.16**	**-**	**-**	**-**		**1.00**	
KKahuN1	A3	31	6	0.258	0.601	0.571	***	9	-
	A8	38	5	0.711	0.699	-0.016	ns	12	-
	A115	37	8	0.622	0.760	0.182	ns	16	2
	A118	34	16	0.529	0.839	0.369	***	20	1
	B121	36	10	0.639	0.796	0.197	ns	18	1
	C12	36	16	0.750	0.836	0.103	ns	24	1
	Mean	**35.3**	**10.2**	**0.585**	**0.755**	**0.234**		**16.5**	
KKahaN2	A3	29	10	0.448	0.803	0.441	***	14	-
	A8	29	6	0.552	0.712	0.225	ns	12	-
	A115	29	9	0.655	0.708	0.075	***	10	-
	A118	28	17	0.571	0.889	0.357	***	24	-
	B121	28	8	0.786	0.749	-0.049	ns	12	-
	C12	29	21	0.793	0.922	0.140	**	26	5
	Mean	**28.7**	**11.8**	**0.634**	**0.797**	**0.198**		**16.33**	
KAieaS1	A3	17	7	0.118	0.751	0.843	***	9	-
	A8	21	3	0.429	0.441	0.028	ns	4	-
	A115	21	7	0.571	0.627	0.089	**	7	-
	A118	21	10	0.476	0.805	0.408	**	15	1
	B121	21	7	0.857	0.734	-0.168	ns	11	1
	C12	23	13	0.783	0.826	0.053	ns	16	3
	Mean	**20.7**	**7.8**	**0.539**	**0.697**	**0.209**		**10.33**	
KLyonS2	A3	45	14	0.289	0.789	0.634	***	20	4
	A8	43	6	0.744	0.704	-0.058	ns	11	-
	A115	46	9	0.717	0.737	0.027	***	12	2
	A118	44	21	0.636	0.934	0.319	***	34	-
	B121	44	9	0.773	0.735	-0.052	ns	17	1
	C12	46	25	0.543	0.935	0.419	***	36	4
	Mean	**44.7**	**14.0**	**0.617**	**0.806**	**0.215**		**21.67**	

Table 3.5. Genetic variability of *C. chamissoi* populations at six microsatellite loci. N = sample size per locus, A = allele numbers per locus, H_o = observed heterozygosity, H_e = expected heterozygosity, and F_{IS} = the allelic fixation index. Significance values reported for Chi-square test of Hardy-Weinburg equilibrium: * P<0.05, ** P<0.01, *** P<0.001, ns = not significant.

Across the six microsatellite loci, the mean number of alleles per locus varied from 3.2 (WKikiB) to 14 (KLyonS2), with the single fern existing at WThreePtsB demonstrating the lowest number of alleles, as it was homozygous for all but one locus. Mean expected heterozygosity (H_e) ranged from 0.565 (WKikiB) to 0.751 (WPah2B) in the Wai‘anae Mountain Region, and from 0.697 (KAieaS1) to 0.806 (KLyonS2) in the Ko‘olau Mountain Region. Mean observed heterozygosity (H_o) ranged from 0.529 (WThreePtsA) to 0.722 (WPah2B) in the Wai‘anae Region, and from 0.539 (KAieaS1) to 0.634 (KKahaN2) in the Ko‘olau Region. Results of Chi-square tests of Hardy-Weinberg equilibrium found significant deviations for one to four alleles within the majority of populations, but no population exhibited significant deviations across all six sampled loci.

The mean allelic fixation index (F_{IS}), also known as the inbreeding coefficient, was found to range by population from -0.094 at WPah2B, to 0.284 at WThreePtsA. The majority of *C. chamissoi* populations were found to have mean F_{IS} values close to zero, indicating random mating. However, five populations (WThreePtsA, KKahuN1, KKahaN2, KAieaS1, and KLyonS2) exhibited moderately positive mean F_{IS} values (approximately 0.2 – 0.3).

The mean number of observed genotypes for each population varied from 2.83 (WKikiB; n = 3) to 21.67 (KLyonS2; n = 44.7). Private alleles were detected in nine of the sixteen populations sampled, with the KLyonS2 population having the highest number of private alleles at 11, and plots WThreePtsA and WOhikA both having 8 private alleles each. No private alleles were detected in seven populations, all of which occurred in the Wai‘anae Region.

Results of the multilocus DNA match analysis by population in GenAlEx demonstrated the presence of genetically identical individuals in six plots (37.5 % of surveyed plots) as presented in Table 3.6.

Population	Clone Groups	No. Clones per Group
WPah1A	0	
WPah1B	0	
WPah2A	0	
WPah2B	0	
WKikiA	0	
WKikiB	0	
WKikiC	1	2
WOhikA	13	7, 3, 3, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2
WOhikB	6	4, 3, 2, 2, 2, 2
WOhikC	0	
WThreePtsA	0	
WThreePtsB	0	
KKahuN1	1	2
KKahaN2	0	
KAieaS1	2	2, 2
KLyonS2	1	2

Table 3.6. Number of clones detected by population.

Plot WOhikA demonstrated the greatest number of clone groups (n = 13), and also the greatest number of clones per population, such that the largest group sampled was found to have seven individuals with identical genotypes. Plot WOhikB had the second highest number of clone groups (n = 6), with four individuals of identical genotypes in the largest clonal group. In all populations sampled, we attempted to discern the extent of multi-trunk individuals, in order to avoid repeated sampling, so these individuals were likely connected below ground.

DISCUSSION

Fern populations have traditionally been assumed to have low genetic differentiation among populations due to the assumed frequency of long distance spore dispersal to facilitate

gene flow among populations, resulting in the majority of genetic variation occurring within populations (Soltis and Soltis 1989). Results of this study support this hypothesis, as all sixteen *C. chamissoi* populations surveyed on O‘ahu had individuals that were heterozygous for at least one locus, and mean observed and expected heterozygosities were relatively high across all populations, as was genetic diversity. These observed heterozygosities for *C. chamissoi* on O‘ahu are similar to those observed for the endangered tree fern *Dicksonia sellowiana* (Dicksoniaceae) in Brazil, with reported mean observed heterozygosity across eight polymorphic markers ranging from 0.29 to 0.44, and expected heterozygosity ranging from 0.27 to 0.56 (Nazareno *et al*. 2013).

However, I did find the number of alleles per locus were reduced in smaller *C. chamissoi* populations. For example, the WKikiB population with three individuals had a mean of 3.2 alleles per locus, whereas the KLyonS2 population with 44 individuals had a mean of 14.0 alleles per locus. It was also interesting that the single individual that occurred in the WThreePtsB plot was homozygous for five out of the six loci, and perhaps the low genetic diversity of this lone individual is related to the lack of recruitment observed in this plot. It would be interesting to test this hypothesis in future research using the gametophyte culture experiment methods similar to those of Lloyd (1974). In his study of breeding systems of pioneer ferns occupying lava as compared to that of ferns occupying intermediate or mature rainforest habitat on Hawai‘i Island, Lloyd (1974) found evidence of intra-gametophytic mating as the predominant method in species of pioneer habitats, and inter-gametophytic mating as the predominant method in species of mature forest habitats. Laboratory germination of *Cibotium glaucum* spores collected from ten individuals in the mature forest habitat of Kilauea Forest Reserve yielded gametophytes which, when isolated, ranged in the order that they formed

gametangia, from male to hermaphroditic, female to hermaphroditic, male to hermaphroditic to female, and also dioecious with both male and female gametophytes that ultimately became hermaphroditic (Lloyd 1974). This was thought to provide evidence for the biochemical effect of antheridogen, acting to influence the sexual development of gametophytes (Lloyd 1974). As summed for each of ten *C. glaucum* parent individuals, sporophyte production from these gametophytes ranged from 10.0 % - 73.4 %, with a mean of 41.0 %, and half of the gametophyte families exhibited abortive sporophyte embryos at a mean of four abortive sporophytes per gametophyte (Lloyd 1974). Lloyd concluded that *C. glaucum* is a highly heterozygous species, with relatively high percentages of lethal genes. When the gametophytes lacking sporophyte production were combined in culture with gametophytes from other individuals, and compared to the gametophytes of the same parents in isolation, Lloyd (1974) determined that both of the *C. glaucum* gametophytes in paired cultures produced normal, viable sporophytes, and both isolates were lethal, as demonstrated by abortive zygotes and embryos, and abnormal sporophytic tissue or abnormal frond or root production in sporophytes. This study supports the hypothesis that Hawaiian *Cibotium* have an adaptable breeding system that favors inter-gametophytic mating, promoting high levels of heterozygosity, with a diminished capacity for intra-gametophytic selfing that is genetically controlled by homozygous lethal gene combinations (Lloyd 1974).

In addition to the relatively high levels of genetic diversity observed within most *C. chamissoi* populations, private alleles were observed in nine of the sixteen populations sampled (56.3%), which indicates that some level of genetic differentiation among populations has occurred. The fact that no private alleles were detected in seven populations, all of which occurred in the Wai‘anae Region, may indicate that these populations are part of the same sub-population, and that higher rates of gene flow among these populations has occurred. Similar

results were observed for microsatellites of the flowering, wind-dispersed tree *Metrosideros polymorpha* Gaud. (Myrtaceae; *ʻŌhiʻa*), in that 14 loci exhibited similarity between observed and expected levels of heterozygosity, and two loci exhibited significant deviation from Hardy-Weinberg equilibrium for one population sampled on Hawaiʻi Island (n = 23) (Crawford *et al*. 2008).

Most previous genetic research of small populations has been focused on rare species, although today even common species are experiencing reduction in populations due to the destruction of habitat, fragmentation, and climate change (Kramer and Havens 2009). Therefore, it is important to consider genetic factors when implementing conservation plans in order to maintain and establish populations that will be capable of persisting and evolving in the future (Kramer and Havens 2009). For example, a microsatellite analysis across six polymorphic loci of the endangered, endemic *Coffea commersoniana* (Baill.) A. Chev. of Madagascar revealed that the *ex situ* collection had very low genetic diversity, indicating that all plants sampled (n = 28) were genetically identical, but while the wild populations showed higher genetic diversity, they were found to significantly deviate from Hardy-Weinberg equilibrium with a significant deficiency of heterozygotes (Krishnan *et al*. 2013). When comparing the two wild *C. commersoniana* populations, Krishnan *et al.* (2013) found among-population variation to be 30%, significant at the $P < 0.001$ level, which was considered important for future conservation efforts of this species in preserving genetic diversity of both populations. Conservation recommendations included that they be kept separate for propagation and in reintroduction to the areas where they are both originally found, as the habitats where these populations are currently found also differ in dominant vegetation type and bioclimate (Krishnan *et al*. 2013). However, species with long-distance dispersal abilities may be less sensitive to the negative effects of

habitat fragmentation due to their ability to maintain larger effective population sizes with higher rates of gene flow among fragments (Kramer *et al*. 2008).

The ability of *C. chamissoi* to ramify below ground has been demonstrated in this study, and clones were detected in 37.5% of the surveyed populations. The greatest number of genetically identical individuals occurred in the WOhikA population, which not incidentally is one of the more densely populated sites, and is lacking in sporeling recruitment. This population also demonstrated a relatively low level of allelic diversity (A = 7.1) despite the relatively large population size and number of samples (n = 44).

The current study could be strengthened by combining the genetic diversity analyses summarized here with spatial data. For example, Kang et al (2008) tested populations of an endangered fern (*Adiantum reniforme* var. *sinense*) for isolation by distance (Wright 1943) by plotting pairwise estimates of genetic distance, where $F_{ST}/(1- F_{ST})$, against geographical distance (km) between populations, and found moderate but significant population differentiation that did demonstrate isolation by distance at the whole distribution scale. However, Kingston *et al*. (2004) found no correlation between genetic and geographic distance for the endangered endemic fern *Angiopteris chauliodonta*, sampled from six populations on Pitcairn Island. Further analysis of *C. chamissoi* population differentiation and tests of isolation by distance will be the focus of future investigation.

CONCLUSION

This study supports the hypothesis that Hawaiian *C. chamissoi* populations are able to maintain relatively high rates of genetic diversity through sexual reproduction, and a primarily outcrossing breeding system that appears to favor inter-gametophytic mating, promoting high levels of heterozygosity and genetic diversity within populations. Despite their high dispersal ability, several populations were found to have private alleles, indicating that gene flow among certain populations may be limited. Further analyses are necessary to address whether these population differences in genetic diversity are significant to the extent that forest restoration and conservation efforts should consider supplemental plantings of *C. chamissoi* in areas of limited recruitment.

CHAPTER FOUR

CONCLUSION

The objectives of this study were to determine the population structure, natural regeneration, mortality, growth rates, and genetic diversity of *C. chamissoi* Kaulf. as monitored across a diversity of habitats *in situ* on O‘ahu Island. This research provides a unique case study of the endemic Hawaiian tree fern that is the culmination of six years of work to better understand this species. Results of the nursery-grown *C. chamissoi* growth rate analysis demonstrated that individuals grown under controlled light and moisture conditions (in containers) were able to achieve 10 cm in trunk height in approximately 3.7 years. In comparison, naturally occurring tree ferns were estimated to be 8.1 years old at 10 cm trunk height, using the method of calculating age based on average growth rate it varies with increasing size class, as adapted from Mehltreter and Garcia-Franco (2008). This method may provided a more accurate estimate of tree fern age based upon growth rates, without harming the tree fern, as it incorporates the ways in which growth rate changes over the life of the plant.

My study provides evidence that tree fern growth rate does indeed increase with size, and smaller individuals do grow quite slowly, as we measured individuals 0 – 10 cm in trunk length to grow an average of 1.3 cm/yr. Differences in growth rate by size class demonstrated a statistically significant, sequentially increasing rate of growth rate with size class, with the exception of ferns in the largest size class, which slightly declined in growth rate. These ferns in the largest size class were most likely undergoing senescense. Results of this study calculated a mean growth rate of *C. chamissoi* across all size classes at 3.8 cm/yr, with a range of 1.3 cm/yr for smallest individuals, and a maximum average growth rate of 5.6 cm/yr for large individuals.

These figures agree with others published for *Cibotium* spp. in Hawai‘i, as growth rates for *C. chamissoi* on O‘ahu were previously measured at an average of 3.0 cm/yr for individuals of at least one meter in height (Durand and Goldstein 2001). This observed growth rate is slightly lower than two estimated for *C. glaucum* on Hawai‘i Island, at 4.4 – 6.5 cm/yr (on younger and older sites, respectively, Walker and Aplet 1994) and 5.08 cm/yr (Wick and Hashimoto 1971).

I provide, perhaps for the first time, figures on tree fern growth before initial development of the trunk, and estimated time to trunk establishment based on nursery data. The y-intercept generated from the regression line of actual, known nursery-grown *C. chamissoi* age by trunk length, was equal to 0.7 years, which fits nicely with the 6-8 months that was reported for sporeling development immediately after germinating from the gametophyte stage in the laboratory (Lynch, 2011).

The oldest *C. chamissoi* that was measured *in situ* had a trunk length of 428 cm at the conclusion of the study, and was calculated to be approximately 100 years old. In fact, it is likely that these tree ferns may be even older, because growth rate calculations were complicated by the gradual decomposition of the older rhizome tissue, a phenomenon which has also been reported by Sharpe and Mehltreter (2010), and resulted in some of the growth measurements to demonstrate decreases in trunk length.

During the study period, the minimum trunk length of a newly transitioned fertile fern was 19.5 cm (mean = 40.3 cm), and the majority of the tree ferns became fertile when their trunks reached 20 – 40 cm in length. Based on age estimates from mean growth rate by size class, this would indicate *C. chamissoi* reaches maturity between 12.2 and 18.2 years old. The high rates of mortality observed for immature individuals may warrant conservation concern for the future of C. chamissoi populations on O‘ahu. The measured rates of recruitment and

mortality of *C. chamissoi* during this six-year study indicate that mortality generally exceeds recruitment in ten out of sixteen plots, with the remaining six plots exhibiting no change in overall abundance. The highest rates of mortality for *C. chamissoi* were observed for the smaller sized individuals, with size class one (0 – 10 cm trunk length) exhibiting 40% of the observed mortalities. Of the individuals that died, 67.3% were not yet fertile, indicating that over half of the population died before reproducing.

During the study period, annual rainfall on O‘ahu was significantly lower than the 60-year average, and average air temperature exhibits a significant warming trend. Global circulation models based on projections under various emission scenarios provided by the Intergovernmental Panel on Climate Change (IPCC) predict an increase in atmospheric CO_2 and other greenhouse gasses, raising the global mean temperature and sea level, and influencing storm patterns and precipitation (IPCC 2007). In Hawai‘i, evidence of these changes have been recently documented: temperatures have significantly increased at higher elevations over the last 60 years as measured by multiple weather stations across the state (Giambelluca *et al.* 2008), and sea surface temperatures are also warming, exhibited by widespread coral bleaching and measured warmer temperatures in the oceans surrounding the Hawaiian Islands (Jokiel and Brown 2004). The role of climate change impacts to tropical forests has been assessed in a study of an El Niño associated drought in Malaysia (Nakagawa *et al.* 2000), and as expected, the drought resulted in higher mortalities that differed across taxonomic groups and size class. An increased frequency of El Niño events as predicted with models of climate change may then be expected to result in significant changes in tropical forest size structure, and Condit *et al.* (1996) conclude that a 25-year drying trend associated with climate change in tropical moist forest in central Panama is causing decline and will possibly result in the local extinction of shrubs and

small trees which require moist microhabitats. Further ecosystem-wide effects of warming are anticipated, as an increase in mean annual temperature has been shown to have an increase in soil-surface respiration in Hawaiian tropical montane wet forests (Litton *et al.* 2011), and changes in temperature and precipitation are likely to affect rates of evapotranspiration and streamflow (Safeeq and Fares 2012). A warmer and drier climate may act as a chronic disturbance for moisture-dependent plant species, and in the case of spore-bearing plants like ferns, a decrease in moisture may limit the fertilization necessary to produce new sporophytes from natural gametophyte populations. Although I did not directly measure rainfall or evapotranspiration in my study sites, results of this study indicate that the observed lack of recruitment in *C. chamissoi* populations may be the result of lower than average annual rainfall during the study period.

Results of microsatellite analyses, presented in Chapter 3, indicate that the majority of *C. chamissoi* populations have relatively high rates of genetic diversity, with the exception of the very small populations sampled at WThreePtsB (n = 1) and WKikiB (n = 3). This study supports the hypothesis that Hawaiian *Cibotium chamissoi* populations are primarily outcrossing, and have an adaptable breeding system that favors inter-gametophytic mating, promoting high levels of heterozygosity and genetic diversity within populations. Private alleles were observed in nine of the sixteen populations sampled (56.3%), which indicates that some level of genetic differentiation among populations has occurred. The fact that no private alleles were detected in seven populations, all of which occurred in the Wai‘anae Region, may indicate that these populations are part of the same sub-population, and that higher rates of gene flow among these populations has occurred. The ability of *C. chamissoi* to ramify below ground has also been demonstrated in this study, and clones were detected in 37.5% of the surveyed populations. The

greatest number of genetically identical individuals occurred in the WOhikA population, which incidentally is one of the densest plots, and is lacking in sporeling recruitment. Future analysis to determine the causes of the reduced rates of recruitment observed in this study could address the possible link to climate change, and the likely influence of low annual precipitation on mortality and successful recruitment. Additional factors that likely influence the population dynamics of *C. chamissoi* include the presence and abundance of non-native vegetation, and harvesting by humans.

Future Research: Political Ecology of Tree Ferns in Hawai‘i

Results of this study (Chapter Two) suggest that populations may be vulnerable to harvesting in certain areas, due to their slow growth and limited natural reproduction. Therefore, future research might address the lack of knowledge regarding the current harvesting activities, source locations, and markets for Hawaiian *Cibotium* at local, regional, and national scales. A political ecology framework would be useful to analyze the ways in which tree ferns are used today because it requires the consideration of the various economic, political, social, and cultural explanations that, when combined with historical and ecological analyses, may better explain tree fern population dynamics.

For example, land ownership and land use history are factors to consider which have and continue to influence tree fern populations and their distribution in Hawai‘i. The State of Hawai‘i holds land in public trust, designated as Natural Area Reserves (NARs), where hiking is allowed on established trails, but other public use restrictions are high. These NARs are managed by executive decisions from the Natural Area Reserve Committee, under the State Department of

Forestry and Wildlife (DOFAW), which also manages large forest areas as Forest Reserves, with permitted harvesting and hunting activities allowed for some species. Large areas of montane forest on Oʻahu are also protected under the City and County of Honolulu Board of Water Supply (BOW) as Watershed Reserves, under separate guidelines. Rules and regulations of BOW lands currently reflect a very closed-to-the-public level of protection, although pig hunting is arranged in limited scope for certain areas. National parks and the federal military also have a large presence in Hawaiʻi, and several military training areas and reserves contain surprising amounts of intact forest habitat, which are severely limited to public access. This list here is not comprehensive, but serves to demonstrate the number of land management agencies that operate under different management objectives, and subsequently vary in their restrictions of resource use, and in public access to those resources. In addition, management of these natural areas varies from a passive, hands-off approach, to an active endangered species restoration approach, and these have a multitude of consequences for overall forest health, in terms of native species and watershed/ecosystem conservation.

A few research questions that might be worth asking concerning the current harvesting and use of *Cibotium*, locally "frondly" known as *hāpuʻu*, are provided below. Included are thoughts and observations from my research thus far to begin answering them.

1. What are the current sources of demand for native Hawaiian Cibotium tree ferns, and other non-native tree ferns, in private and commercial economies?

In my time living, and later continuing to visit to conduct tree fern research in Hawaiʻi, I had the opportunity to informally survey neighborhoods for tree ferns planted in yards, visible

from the street. It is very clear that they are a popular landscaping ornamental. For example, in approximately 15 minutes of survey time, I counted 27 native *Cibotium*, planted in the front and side lawns of nine residences in an upper-class neighborhood in Mānoa Valley, located adjacent to state forest land on O‘ahu.

There is a perception of *hāpu‘u* being locally common, and despite what appears to be significant use in landscaping, local demand is perceived as being low to non-existent (anonymous DOFAW forester, pers. comm.). It would be very useful and informative to conduct a survey of orchid and plant nurseries, both to interview the growers on their uses and sources of *Cibotium*, and also to gain an idea for the amount of demand there is in the local market. Are they sold for high or moderate prices? This might influence whether someone would purchase a native tree fern, vs. a non-native species, or alternatively, go out to the rainforest and find one to harvest for free, although possibly illegally depending on the location.

The Australian tree fern is commonly used in landscaping on O‘ahu despite its classification as an invasive species. They can be seen in downtown Honolulu and Waikiki around skyscrapers and in the courtyards of banks and hotels, and also in the yards of homeowners around the island. I personally have a few friends with Australian tree ferns in their yards, and have been told they are desirable due to their higher drought tolerance, taller height, and faster growth. There may also be an element of novelty and luxury associated with their image.

The O‘ahu Nursery Grower’s Association decided a few years ago to <u>not</u> sell the non-native Australian tree ferns. However, a source reports recently that, “a horrible thing!” has been discovered: because of the O‘ahu NGA deciding not to sell Australian tree ferns, some other nurseries decided that they could sell more of them, and so they have increased their stocks or

added Australian tree fern to their inventory. This implies that Hawai‘i residents are unaware of the ecological threat that Australian tree ferns pose to native Hawaiian forest species, or they do not share the same concerns as those within the scientific and conservation communities.

2. What are the spatial and demographic patterns of source and demand, i.e. where are tree ferns harvested from, who is harvesting them, who is buying them, and what do they use them for?

On the islands of Kaua‘i, Maui, Hawai‘i, and O‘ahu, two major retail stores supply native tree fern living logs. The majority of the tree ferns from one store were said to be obtained from two private sources on Hawai‘i Island, according to my phone interview with an employee. In addition, there were several regular sources that offered *C. glaucum* on eBay.com with shipping to anywhere within the United States, and one source offered world-wide shipping. At the time of this writing, a quick search on eBay for Hawaiian tree ferns resulted in four listings. Hawai‘i Botanical Supply offered 10 – 12 inch tall plants in four inch pots, and bidding started at US $9.99, plus shipping for $9.85. These plants were shipped from Hilo, on Hawai‘i Island. An additional seller, Suncatchers of Hawai‘i, offered what looks like approximately one foot cuttings of *Cibotium* trunks (caudices) of larger individuals for $14.95 each, plus shipping for $10.95. These cuttings were sourced from Volcano, on Hawai‘i Island, and were listed (incorrectly) as *Cibotium splendens*. This seller stated that more than 10 individuals would be available for purchase at a time, and also included a statement that reads, “These plants are approved for export from Hawaii to the U.S. Mainland by Hawai‘i Department of Agriculture.” Suncatchers of Hawai‘i also offered the same *C. splendens* in groups of three for $35.99, plus

$14.95 for shipping, and in groups of 10 for $119.99, plus $27.95 for shipping, all from the same Volcano area on Hawai‘i Island. These figures are not intended to represent all of the markets for Hawaiian *Cibotium*, but are provided here to give an idea for the retail values and sources on the current market available to someone with a quick search.

3. If native tree ferns are harvested from public lands, what are the causational justifications and explanations for doing so? Is it for traditional use, or other motivation?

In answering this question, it would be worthwhile to interview and/or survey those who are willing to share harvesting activities, although this might prove to be difficult in cases where no permit has been obtained, or if harvesting is conducted in protected areas. It is evident that people do harvest native tree ferns from state Forest Reserve lands, as I have heard of four different accounts of this. It is unknown whether individuals obtained permission or permits to do so, but I will include here the reports of such activities to illuminate the ways in which tree ferns are currently being used and harvested locally. In one case, an area being actively restored in Mānoa on O‘ahu by a group of volunteers was missing a small *Cibotium* upon their return trip to the site, discovered by evidence the stem had been cut with a saw. The volunteers were disappointed with the loss of a native plant from the area, which is located near a popular hiking trail. In another instance, I was told by a resident of Palolo Valley that people are sometimes seen carrying native tree ferns with them as they exit a popular hiking trail, and these are usually the smaller ones, presumably because they are easier to carry. In the third instance, an anonymous interview subject told me of native tree fern harvesting activities from Forest Reserve land near Volcano National Park on Hawai‘i Island, where individuals without permits

went in with trucks to collect 15 – 20 individuals of intermediate and small sizes on at least three separate occasions in 2009. The fourth account was given by friends of mine who were studying ferns in the Olaʻa Forest Reserve on Hawaiʻi Island several years ago. They encountered a group of local residents that had filled the bed of a pickup with *Cibotium* trunks and croziers, and when asked out of curiosity what they would use them for, stated they were to use as food for their pigs.

The native *Cibotium* are a common species, and especially on the island of Hawaiʻi, they form very dense, tall stands, where harvesting may be possible in a sustainable manner with limited impact to the overall health of the forest. The above anecdotes provide evidence that perhaps local demand for native *Cibotium* is higher than public land managers are aware, but is not intended to criminalize tree fern harvesting. Instead, the take home message is that *Cibotium* are important and desirable plants to people in Hawaiʻi, and harvesting impacts will vary by location. In some places, harvesting may even be desirable to open small gaps for canopy tree seedling release. However, other places may be very susceptible to invasion after harvesting, as congruent with the findings of Buck (1982).

4. What do native and non-native tree ferns represent to the various people who know them and/or use them? Why like them, plant them, or use them?

I have had the pleasure of working with many land managers, scientists, conservationists, students, and public officials in Hawaiʻi during the course of this research, and I asked them to share their thoughts on this question. A few common themes emerge. One respondant said that *hāpuʻu* represents, "A beautiful green umbrella, that canopy layer between the taller trees and

shorter shrubs/herbs," and felt their image is symbolic of the rainforest. Another responded that *hāpu'u*, "…is the magical keystone that creates and maintains complex and unique ecosystems in Hawai'i. An evolutionary gem." Another person suggested that *hāpu'u* represent native healthy forest, with the larger individuals symbolic of older forests. Similarly, the word "maturity" came up to describe the association of these ferns with mature forests. I have come to see them as "the sentinels" of the native Hawaiian forest myself, because in a way they seem to "stand guard" against non-native vegetation.

One respondent titled his response, "Arcane Spooring To Whom It May Concern," and reminded me of the first colonists of Hawai'i naming the *hāpu'u* the "mother of *ohia*," which is, "…quite an accolade, for the *Ohi'a lehua* is the backbone of the Hawaiian forest." He goes on to reminisce about his entomological studies in the tree fern groves on Hawai'i Island:

> I camped in Puna not far from the Pulu Factory ruins to map the forest (in 1978 guided by Ruth Lani Stemmerman, who mastered sandalwood & doctored in Ohi'a ecology). No need to lug air mattresses, as the dry older fronds hung by 100s from the trunks in a vast subcanopy. At dusk, I was delighted that formerly rare twiggy raptorial inchworms climbed out from my comfy cushion in scores to stand up & out, ambushing crickets and gnats till dawn drove them down from the birds and voracious vespids. This teeming scene was paradise enough among the furled fronds for this caterpillar man. On 4 isles fern-notching inchworms secrete themselves between 2 pinnae, carving them customly by 2 teeth into a snug lair. I discovered this novel niche only after camping in a Pahole fern patch and seeing them fleeing the mammalian intrusions across my beloved's neck! Now a Brasil grad seeks new *Haliophyle* noctuid cutworms that evolved on Hawaiian ferns, yet another tale of our ancient herbage to tell.

Thinking of the native tree fern also brought *pulu* to mind of a few people, which is the Hawaiian word for the soft tawny hairs that form at the growth apex and cover emerging fiddleheads. One person responded simply, "I love tree ferns. They're beautiful and simple and raw." And to another, "…they represent birth, rebirth, the spirals, intertwined in and connecting so many things in nature."

The clear theme of the native tree ferns as key species emerges throughout the narratives of scientists, conservationists, and residents of Hawai‘i. *Cibotium* tree ferns have come to be seen as a symbol of the native rainforest, and as they are long-lived, slow growing species, their population dynamics may be considered indicative of larger trends in ecosystem processes. The conservation challenges in Hawai‘i are numerous, and research projects that address forest conservation and management require the consideration of spatial and temporal variations in species growth and persistence across habitats.

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APPENDIX 1: Overstory species diversity by frequency of occurrence.
Presented as percentage of number of plot occurrences / total number of plots (n = 16).

Plot Freq (%) (n=16)	Overstory Species	Family	Life Form	Distribution Status
81	*Metrosideros polymorpha*	Myrtaceae	Tree	Endemic
75	*Psidium cattleianum*	Myrtaceae	Shrub or Small Tree	Naturalized
63	*Acacia koa*	Fabaceae	Tree	Endemic
63	*Antidesma platyphyllum*	Euphorbiaceae	Tree	Endemic
63	*Schinus terebinthifolius*	Anacardiaceae	Tree	Naturalized
50	*Grevillea robusta*	Proteaceae	Tree	Naturalized
38	*Pouteria sandwicensis*	Sapotaceae	Shrub or Tree	Endemic
19	*Elaeocarpus bifidus*	Elaeocarpaceae	Tree	Endemic
19	*Nestegis sandwicensis*	Oleaceae	Tree	Endemic
19	*Psidium guajava*	Myrtaceae	Shrub or Small Tree	Naturalized
19	*Psydrax odoratum*	Rubiaceae	Shrub or Small Tree	Indigenous
19	*Xylosma hawaiiense*	Flacourtiaceae	Tree	Endemic
13	*Aleurites moluccana*	Euphorbiaceae	Tree	Polynesian Introduction
13	*Bobea elatior*	Rubiaceae	Tree	Endemic
13	*Diospyros hillebrandii*	Ebenaceae	Tree	Endemic
13	*Pandanus tectorius*	Pandanaceae	Tree	Indigenous
13	*Psychotria mariniana*	Rubiaceae	Small Tree	Endemic
13	*Psychotria* sp.	Rubiaceae	Shrub or Tree	Endemic
13	*Syzygium sandwicensis*	Myrtaceae	Shrub or Tree	Endemic
6	*Alphitonia excelsa*	Rhamnaceae	Tree	In Cultivation
6	*Alstonia macrophylla*	Apocynaceae	Tree	In Cultivation
6	*Ardisia elliptica*	Myrsinaceae	Shrub	Naturalized
6	*Bobea brevipes*	Rubiaceae	Tree	Endemic
6	*Casuarina distyla*	Casuarinaceae	Tree	In Cultivation
6	*Citharexylum caudatum*	Verbenaceae	Shrub or Small Tree	Naturalized
6	*Cordyline fruticosa*	Agavaceae	Shrub	Polynesian Introduction
6	*Diospyros sandwicensis*	Ebenaceae	Tree	Endemic
6	*Dypsis tsoratensis*	Arecaceae	Palm	In Cultivation
6	*Hedyotis acuminata*	Rubiaceae	Shrub	Endemic
6	*Hedyotis terminalis*	Rubiaceae	Shrub, Liana, Small Tree	Endemic
6	*Hibiscus arnottianus*	Malvaceae	Shrub or Small Tree	Endemic
6	*Hibiscus tilliaceus*	Malvaceae	Shrub or Small Tree	Indigenous
6	*Ilex anomala*	Aquifoliaceae	Tree	Indigenous
6	*Melicope oahuensis*	Rutaceae	Shrub or Small Tree	Endemic
6	*Metrosideros rugosa*	Myrtaceae	Shrub or Small Tree	Endemic
6	*Myrsine lessertiana*	Myrsinaceae	Tree	Endemic

6	*Pinanga coronata*	Arecaceae	Palm	In Cultivation
6	*Pisonia sandwicensis*	Nyctaginaceae	Tree	Endemic
6	*Pisonia umbellifera*	Nyctaginaceae	Tree	Endemic
6	*Pleomele forbesii*	Agavaceae	Tree	Endemic
6	*Psychotria fauriei*	Rubiaceae	Shrub or Small Tree	Endemic
6	*Rauvolfia sandwicensis*	Apocynaceae	Tree	Endemic
6	*Schefflera actinophylla*	Araliaceae	Tree	Naturalized
6	*Simarouba glauca*	Simaroubaceae	Tree	In Cultivation
6	*Syagrus romanzoffiana*	Arecaceae	Tree	In Cultivation
6	*Streblus pendulinus*	Moraceae	Shrub or Small Tree	Indigenous
6	*Syzygium cumini*	Myrtaceae	Tree	Naturalized
6	*Charpentiera tomentosa*	Amaranthaceae	Tree	Endemic

APPENDIX 2: Understory species diversity by frequency of occurrence.
Presented as percentage of number of plot occurrences / total number of plots (n = 16).

Plot Freq (%) (n=16)	Understory Species	Family	Life Form	Distribution Status
100	*Cibotium chamissoi*	Ciboteaceae	Fern	Endemic
88	*Blechnum appendiculatum*	Blechnaceae	Fern	Naturalized
81	*Clidemia hirta*	Melastomataceae	Shrub	Naturalized
75	*Christella parasitica*	Thelypteridaceae	Fern	Naturalized
75	*Nephrolepis exaltata* subsp. *hawaiiensis*	Nephrolepidaceae	Fern	Endemic
75	*Rubus rosifolius*	Rosaceae	Shrub	Naturalized
75	*Sphenomeris chinensis*	Lindsaeaceae	Fern	Indigenous
69	*Alyxia oliviformis*	Apocynaceae	Liana	Endemic
69	*Coprosma foliosa*	Rubiaceae	Shrub or Small Tree	Endemic
69	*Lantana camara*	Verbenaceae	Shrub	Naturalized
63	*Freycinetia arborea*	Pandanaceae	Woody Climber	Indigenous
56	*Carex meyenii*	Cyperaceae	Sedge	Indigenous
56	*Doodia kunthiana*	Blechnaceae	Fern	Endemic
56	*Lepisorus thunbergianus*	Polypodiaceae	Fern	Indigenous
56	*Microlepia strigosa*	Dennstaedtiaceae	Fern	Indigenous
50	*Wikstroemia oahuensis*	Thymelaeaceae	Shrub or Small Tree	Endemic
44	*Paspalum conjugatum*	Poaceae	Grass	Naturalized
44	*Psidium guajava*	Myrtaceae	Shrub or Small Tree	Naturalized
38	*Stachytarpheta dichotoma*	Verbenaceae	Shrub	Naturalized
31	*Athyrium microphyllum*	Athyriaceae	Fern	Endemic
31	*Cocculus trilobus*	Menispermaceae	Liana	Indigenous
31	*Hibiscus arnottianus*	Malvaceae	Shrub or Small Tree	Endemic
31	*Oplismenus hirtellus*	Poaceae	Grass	Naturalized
31	*Psychotria hathewayi*	Rubiaceae	Small Tree	Endemic
31	*Scaevola gaudichaudiana*	Goodeniaceae	Shrub	Endemic
25	*Chamaesyce multiformis*	Euphorbiaceae	Shrub	Endemic
25	*Dicranopteris linearis*	Gleicheniaceae	Fern	Indigenous
25	*Dryopteris fusco-atra*	Dryopteridaceae	Fern	Endemic
25	*Hedyotis terminalis*	Rubiaceae	Shrub, Liana	Endemic
25	*Melicope oahuensis*	Rutaceae	Shrub or Small Tree	Endemic
25	*Melinis minutiflora*	Poaceae	Grass	Naturalized
25	*Phlebodium aureum*	Polypodiaceae	Fern	Naturalized
25	*Psilotum nudum*	Psilotaceae	Fern Ally	Indigenous
25	*Psydrax odorata*	Rubiaceae	Shrub or Small Tree	Indigenous
19	*Acacia koa*	Fabaceae	Tree	Endemic
19	*Ageratina adenophora*	Asteraceae	Herb	Naturalized
19	*Ageratina riparia*	Asteraceae	Herb	Naturalized
19	*Antidesma platyphyllum*	Euphorbiaceae	Tree	Endemic
19	*Ardisia elliptica*	Myrsinaceae	Shrub	Naturalized

19	*Conyza bonariensis*	Asteraceae	Herb	Naturalized
19	*Cordyline fruticosa*	Agavaceae	Shrub	Polynesian Introduction
19	*Deparia prolifera*	Athyriaceae	Fern	Endemic
19	*Diplazium sandwichianum*	Athyriaceae	Fern	Endemic
19	*Dodonaea viscosa*	Sapindaceae	Shrub or Small Tree	Indigenous
19	*Dryopteris glabra*	Dryopteridaceae	Fern	Endemic
19	*Myrsine lessertiana*	Myrsinaceae	Tree	Endemic
19	*Nephrolepis multiflora*	Nephrolepidaceae	Fern	Naturalized
19	*Ophioderma pendulum*	Ophioglossaceae	Fern	Indigenous
19	*Platydesma corunata*	Rutaceae	Shrub	Endemic
19	*Psidium cattleianum*	Myrtaceae	Shrub or Small Tree	Invasive
19	*Psychotria mariniana*	Rubiaceae	Small Tree	Endemic
19	*Sadleria cyatheoides*	Blechnaceae	Fern	Endemic
19	*Setaria gracilis*	Poaceae	Grass	Naturalized
13	*Adiantum hispidulum*	Pteridaceae	Fern	Naturalized
13	*Asplenium horridum*	Aspleniaceae	Fern	Indigenous
13	*Asplenium kaulfussii*	Aspleniaceae	Fern	Endemic
13	*Asplenium nidus*	Aspleniaceae	Fern	Indigenous
13	*Buddleia asiatica*	Buddleiaceae	Shrub	Naturalized
13	*Carex wahuensis*	Cyperaceae	Sedge	Endemic
13	*Charpentiera tomentosa*	Amaranthaceae	Tree	Endemic
13	*Christella dentata*	Thelypteridaceae	Fern	Naturalized
13	*Deparia petersenii*	Athyriaceae	Fern	Naturalized
13	*Elaphoglossum crassifolium*	Lomariopsidaceae	Fern	Endemic
13	*Elaphoglossum paleaceum*	Lomariopsidaceae	Fern	Indigenous
13	*Erigeron karvinskianus*	Asteraceae	Herb	Naturalized
13	*Grevillea robusta*	Proteaceae	Tree	Naturalized
13	*Ilex anomala*	Aquifoliaceae	Tree	Indigenous
13	*Kalanchoe pinnata*	Crassulaceae	Herb	Naturalized
13	*Melicope lydgatei*	Rutaceae	Shrub	Endemic
13	*Metrosideros polymorpha*	Myrtaceae	Shrub or Tree	Endemic
13	*Panicum nephelophilum*	Poaceae	Grass	Endemic
13	*Peperomia* sp.	Piperaceae	Herb	Endemic
13	*Rubus argutus*	Rosaceae	Shrub	Invasive
13	*Schefflera actinophylla*	Araliaceae	Tree	Naturalized
13	*Schinus terebinthifolius*	Anacardiaceae	Tree	Invasive
13	*Selaginella arbuscula*	Selaginellaceae	Fern Ally	Endemic
13	*Setaria palmifolia*	Poaceae	Grass	Naturalized
13	*Smilax melastomifolia*	Smilacaceae	Liana	Endemic
13	*Tectaria gaudichaudii*	Dryopteridaceae	Fern	Endemic
13	*Triumfetta semitriloba*	Tiliaceae	Herb	Naturalized
6	*Angiopteris evecta*	Marattiaceae	Fern	Naturalized
6	*Asplenium acuminatum*	Aspleniaceae	Fern	Endemic
6	*Asplenium normale*	Aspleniaceae	Fern	Indigenous
6	*Asplenium polyodon*	Aspleniaceae	Fern	Indigenous
6	*Bidens torta*	Asteraceae	Herb	Endemic
6	*Bobea brevipes*	Rubiaceae	Tree	Endemic
6	*Cibotium menziesii*	Ciboteaceae	Fern	Endemic

6	*Congea* sp.	Lamiaceae	Herb	In Cultivation
6	*Coprosma longifolia*	Rubiaceae	Shrub or Small Tree	Endemic
6	*Coprosma ochracea*	Rubiaceae	Shrub or Small Tree	Endemic
6	*Coprosma* sp.	Rubiaceae	Shrub or Small Tree	Endemic
6	*Clusia rosea*	Clusiaceae	Shrub or Small Tree	In Cultivation
6	*Cyrtandra* sp.	Gesneriaceae	Shrub	Endemic
6	*Desmodium incanum*	Fabaceae	Shrub	Naturalized
6	*Dianella sandwicensis*	Liliaceae	Herb	Indigenous
6	*Diospyros sandwicensis*	Ebenaceae	Tree	Endemic
6	*Elaeocarpus bifidus*	Elaeocarpaceae	Tree	Endemic
6	*Elaphoglossum alatum*	Lomariopsidaceae	Fern	Endemic
6	*Elephantopus mollis*	Asteraceae	Herb	Naturalized
6	*Erechtites valerianifolia*	Asteraceae	Herb	Naturalized
6	*Gonocormus minutus*	Hymenophyllaceae	Fern	Indigenous
6	*Hedyotis acuminata*	Rubiaceae	Shrub	Endemic
6	*Heliconia caribaea*	Heliconiaceae	Herb	In Cultivation
6	*Heliconia* sp.	Heliconiaceae	Herb	In Cultivation
6	*Hyptis pectinata*	Lamiaceae	Herb	Naturalized
6	*Ilex paraguariensis*	Aquifoliaceae	Shrub	Naturalized
6	*Melicope kaalaensis*	Rutaceae	Small Tree	Endemic
6	*Melicope peduncularis*	Rutaceae	Shrub	Endemic
6	*Montanoa hibiscifolia*	Asteraceae	Shrub	Naturalized
6	*Nephrolepis cordifolia*	Nephrolepidaceae	Fern	Indigenous
6	*Nestegis sanwicensis*	Oleaceae	Tree	Endemic
6	*Oxalis corniculata*	Oxalidaceae	Herb	Naturalized
6	*Pandanus tectorius*	Pandanaceae	Tree	Indigenous
6	*Passiflora edulis*	Passifloraceae	Liana	Naturalized
6	*Perrottetia sandwicensis*	Celastraceae	Shrub or Small Tree	Endemic
6	*Philodendron* sp.	Araceae	Shrub	In Cultivation
6	*Pisonia umbellifera*	Nyctaginaceae	Tree	Indigenous
6	*Pouteria sandwicensis*	Sapotaceae	Shrub or Tree	Endemic
6	*Psilotum complanatum*	Psilotaceae	Fern Ally	Indigenous
6	*Pterolepis glomerata*	Melastomataceae	Herb	Naturalized
6	*Sapindus oahuensis*	Sapindaceae	Tree	Endemic
6	*Solanum* sp.	Solanaceae	Herb	Naturalized
6	*Sonchus oleraceus*	Asteraceae	Herb	Naturalized
6	*Sphaeropteris cooperi*	Cyatheaceae	Fern	Naturalized
6	*Tibouchina urvilleana*	Melastomataceae	Shrub or Small Tree	Naturalized
6	*Xylosma hawaiiense*	Flacourtiaceae	Tree	Endemic

APPENDIX 3. Native and non-native overstory and understory cover by plot.

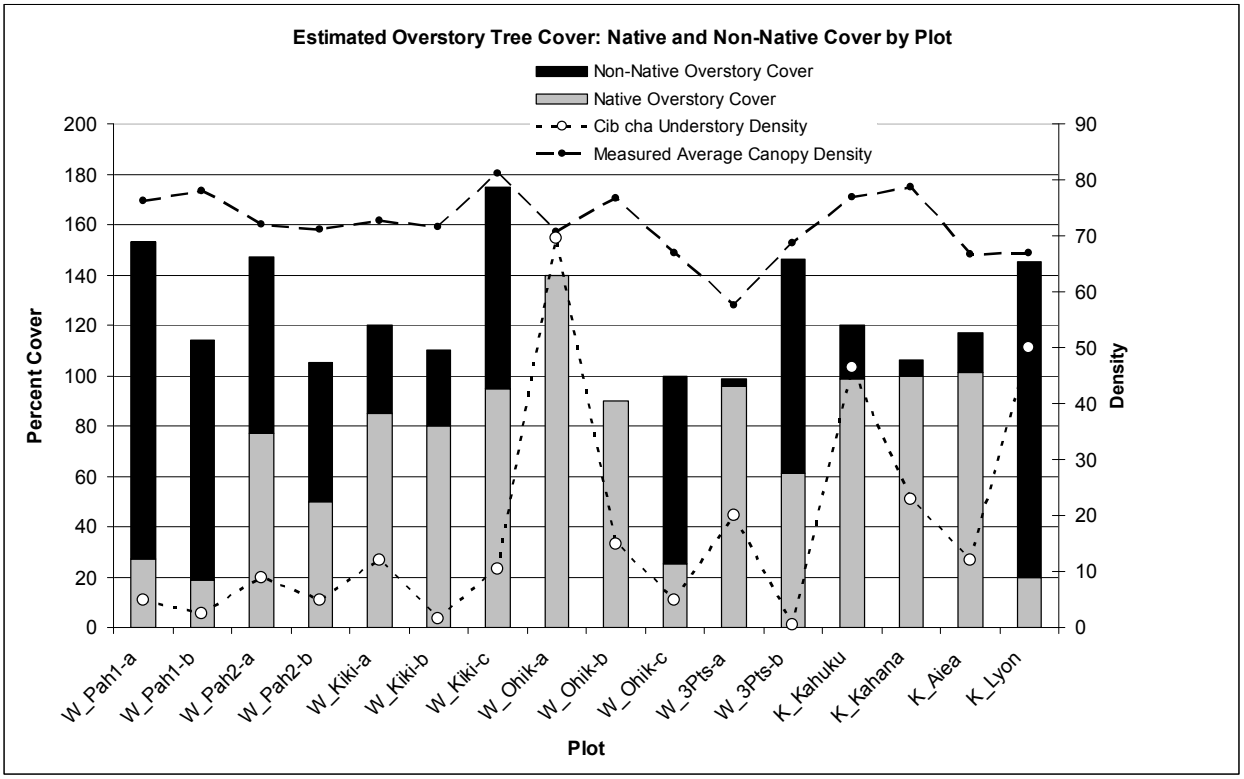


Figure A. Estimated native and non-native tree cover, measured canopy density, and *C. chamissoi* understory density (total individuals / 200 m^2) for sixteen plots at Time 1. *W_ = Waianae Mountain Range; K_ = Koolau Mountain Range; a = inside fence; b = outside fence; Kiki-c = Kahanahaiki outside fence steep site; Ohik-c = Ohikilolo outside fence weedy site.*

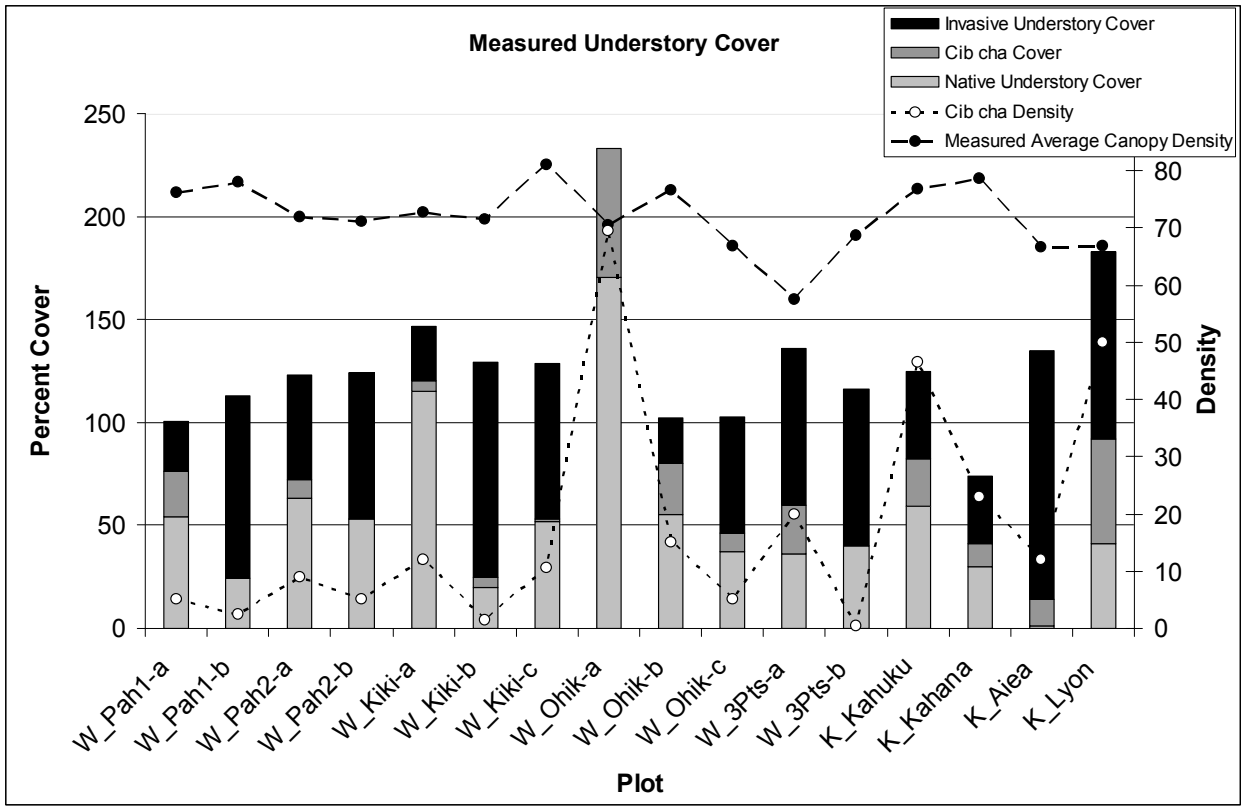
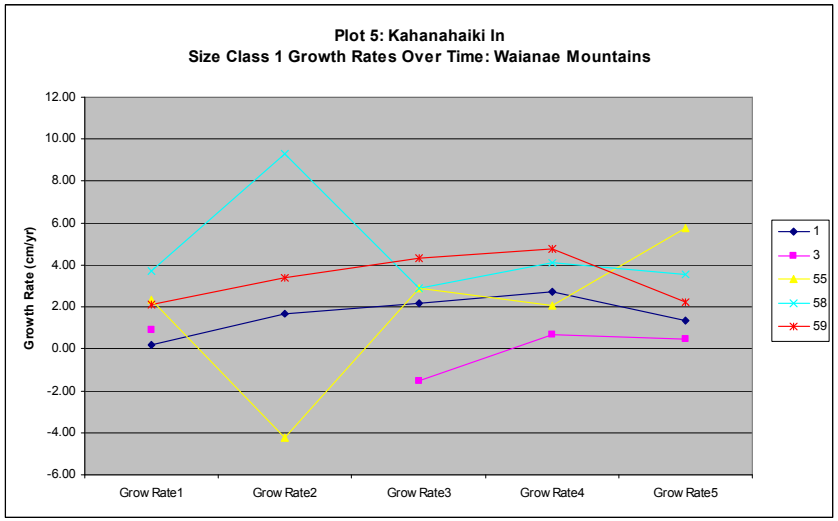
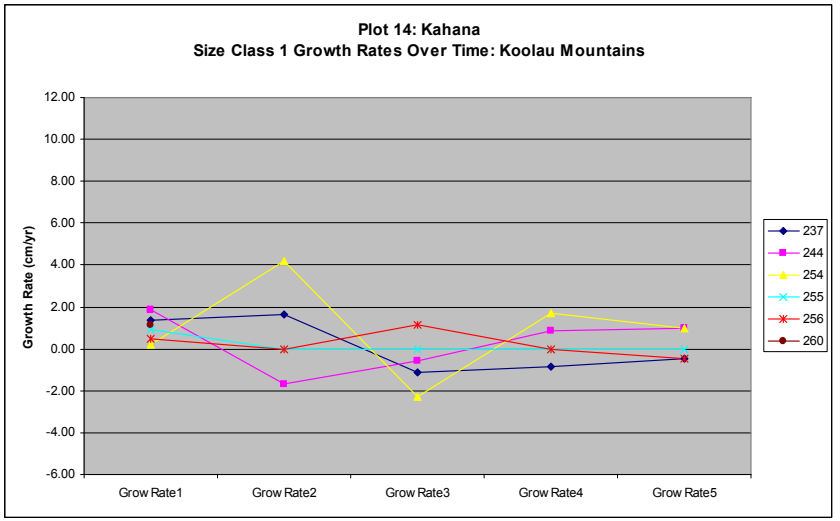
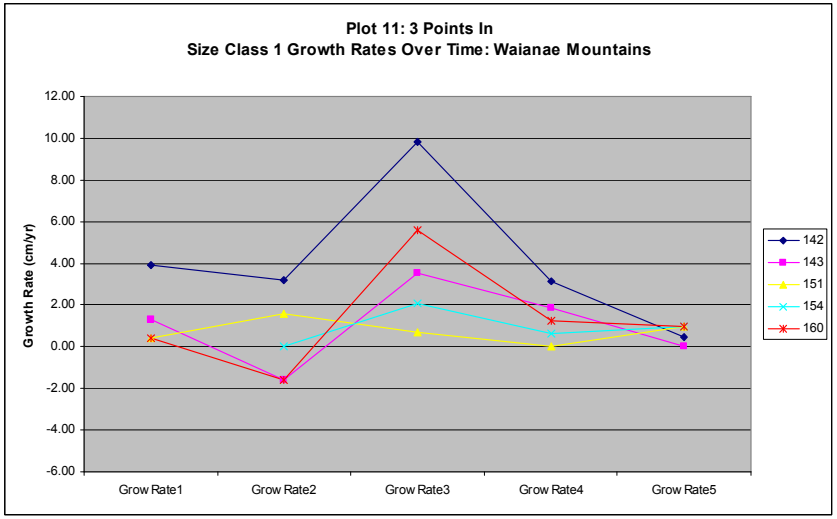


Figure B. Measured native and non-native understory cover, canopy density, and *C. chamissoi* understory density (total individuals / 200 m^2) for sixteen plots at Time 1. *W_ = Waianae Mountain Range; K_ = Koolau Mountain Range; a = inside fence; b = outside fence; Kiki-c = Kahanahaiki outside fence steep site; Ohik-c = Ohikilolo outside fence weedy site.*

APPENDIX 4: Growth rates of individual *C. chamissoi* in size class one.
Indicated by fern ID number (see legends) for four selected plots as calculated between measured times 1 – 5 (x-axis) across the study period.







APPENDIX 5: Median trunk length of *C. chamissoi* by research plot and by region.

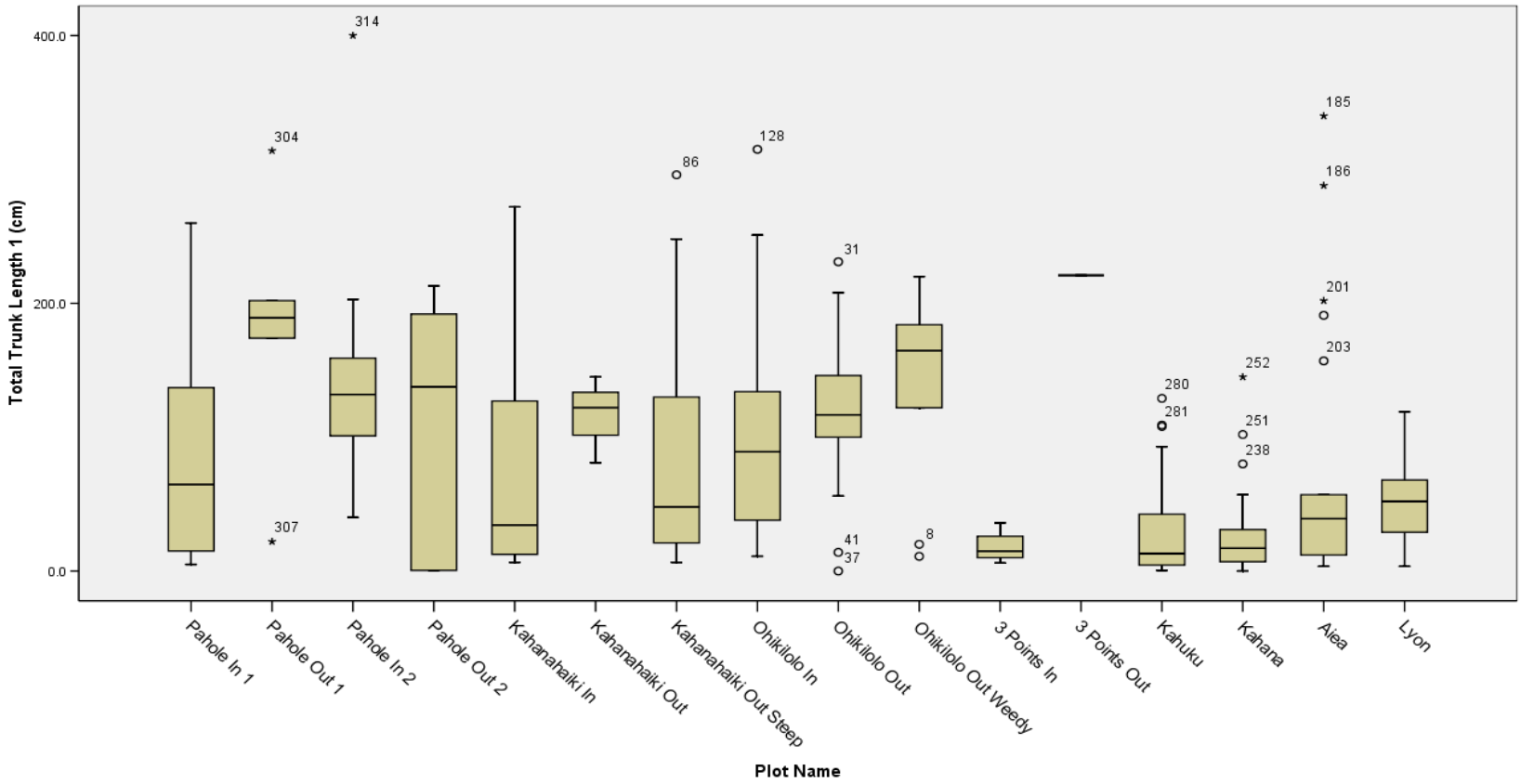


Figure A. Median trunk length by plot. Boxes indicate 50% of cases; central line represents median value, with whiskers indicating max and min values. Circles and stars identify outliers and extreme outliers, respectively.

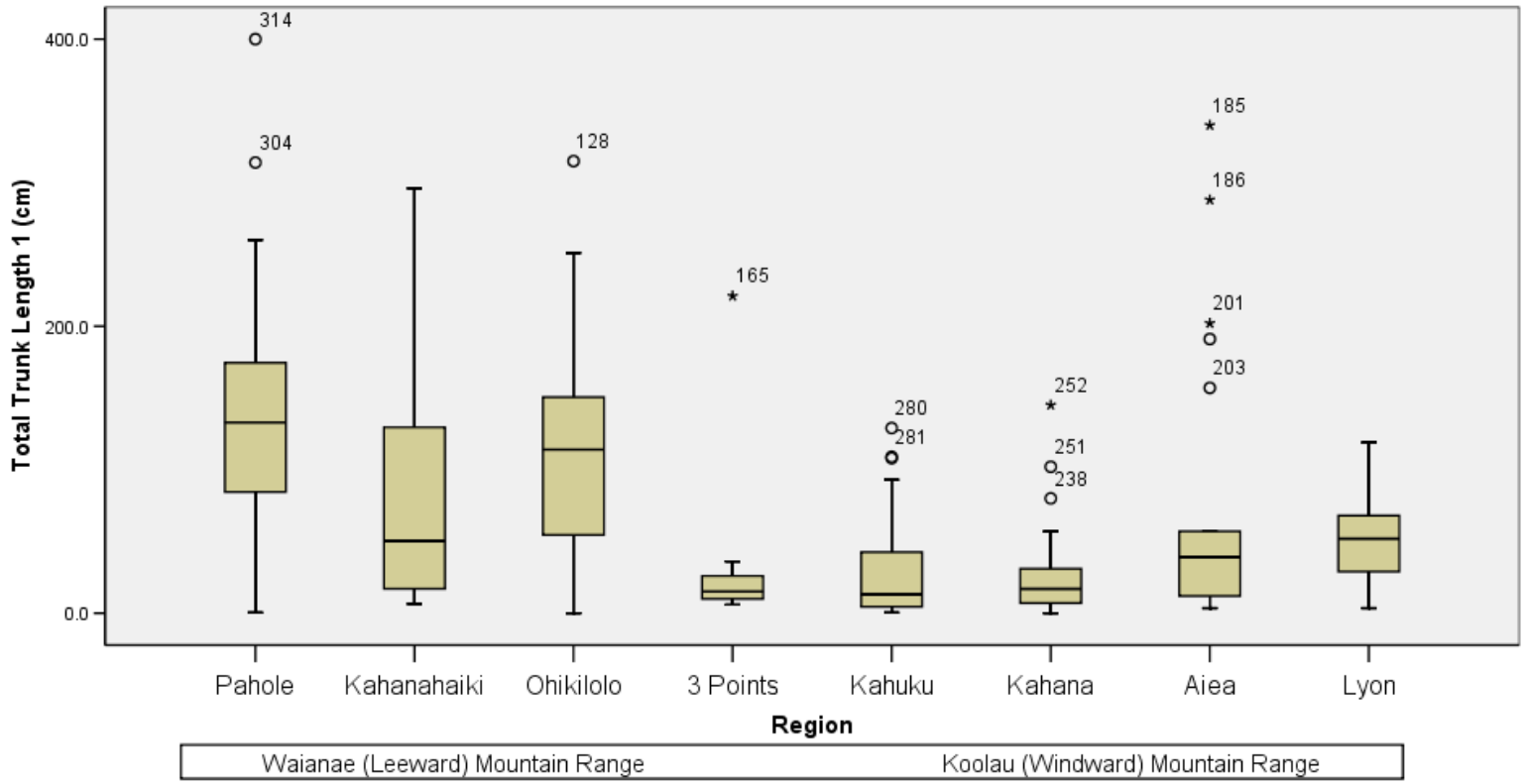
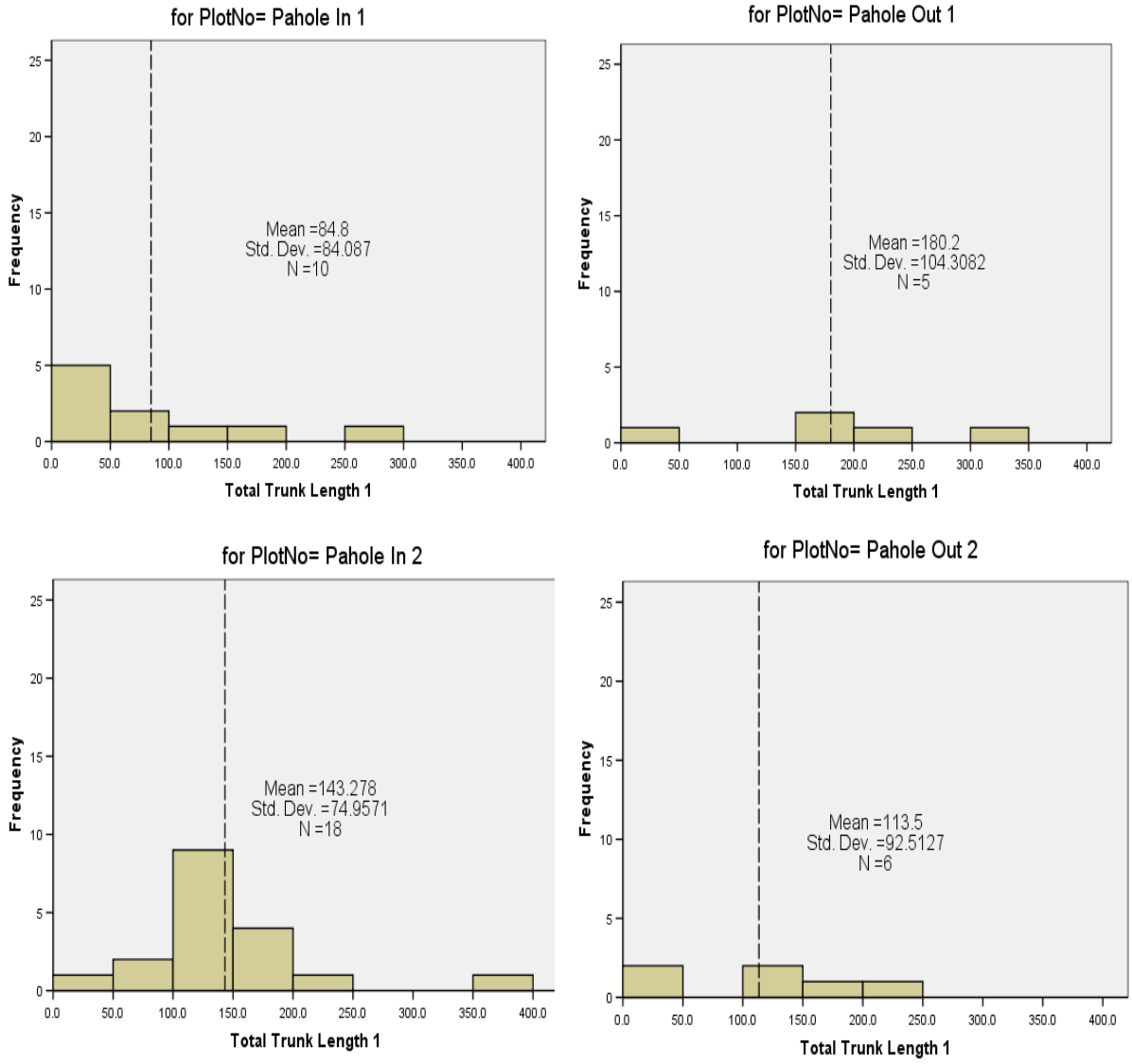
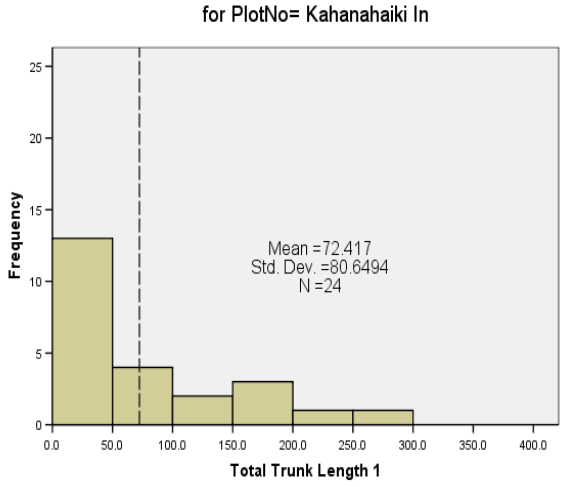
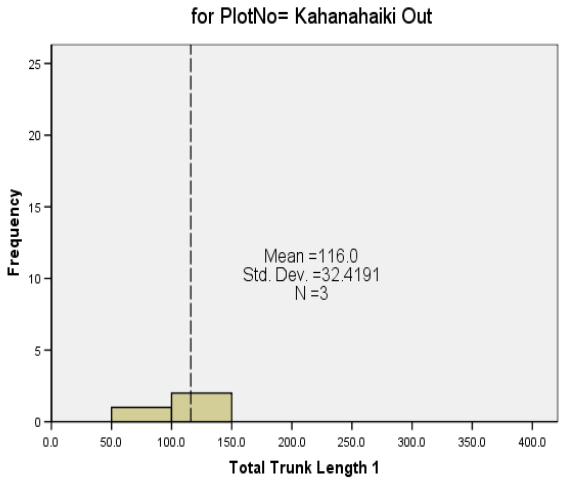


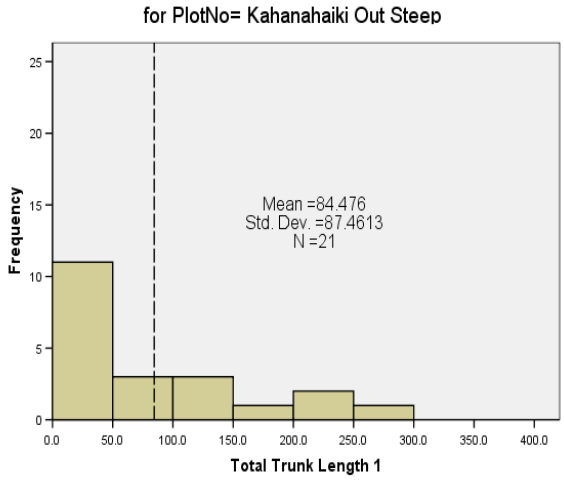
Figure B. Median trunk length by region. Boxes indicate 50% of cases; central line represents median value, with whiskers indicating max and min values. Circles and stars identify outliers and extreme outliers, respectively.

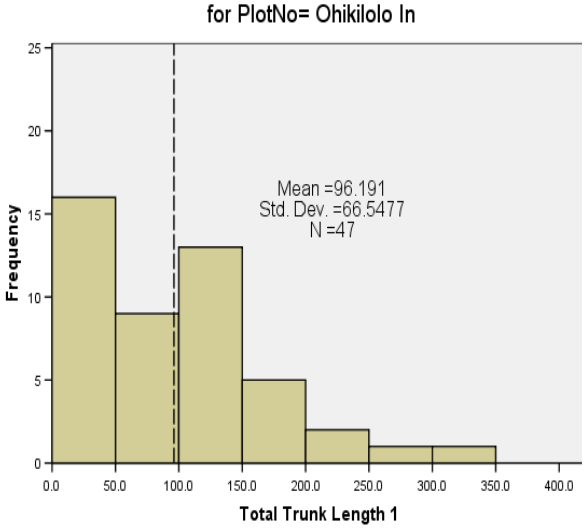
APPENDIX 6: Population structure of *C. chamissoi* by plot.
Frequency of individuals (y-axis), by trunk length (cm) (x-axis).

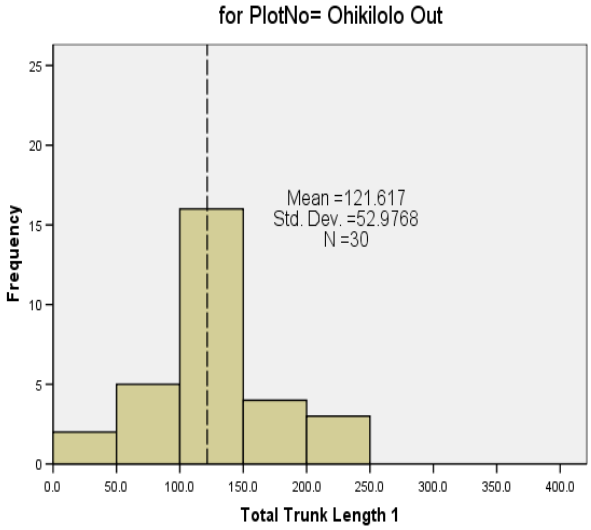


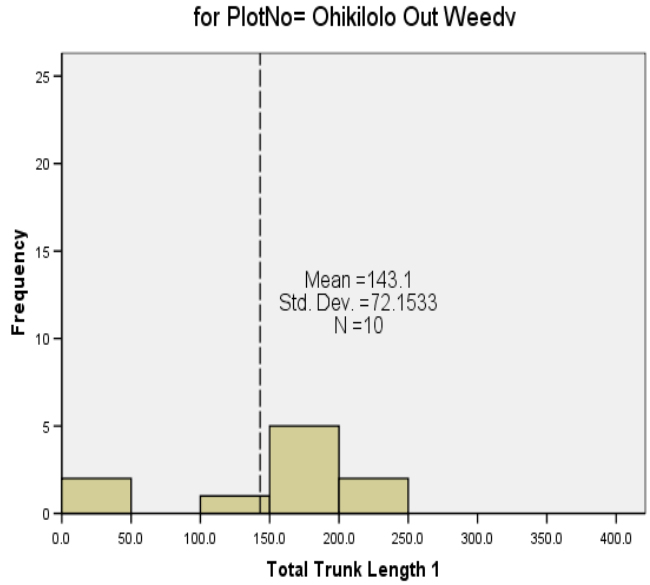

for PlotNo= Kahanahaiki In
Mean =72.417
Std. Dev. =80.6494
N =24
Frequency
Total Trunk Length 1

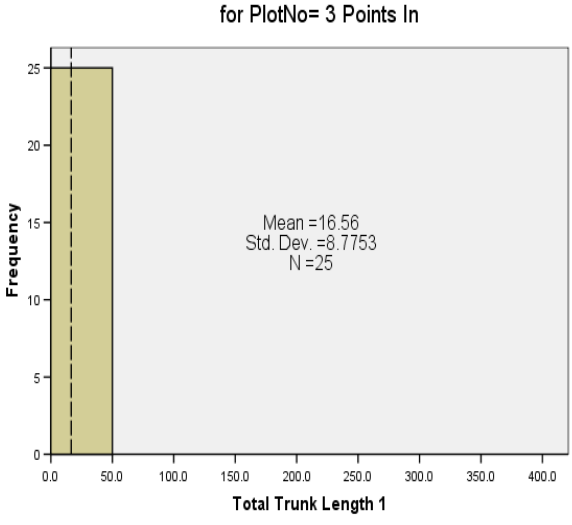

for PlotNo= Kahanahaiki Out
Mean =116.0
Std. Dev. =32.4191
N =3
Frequency
Total Trunk Length 1

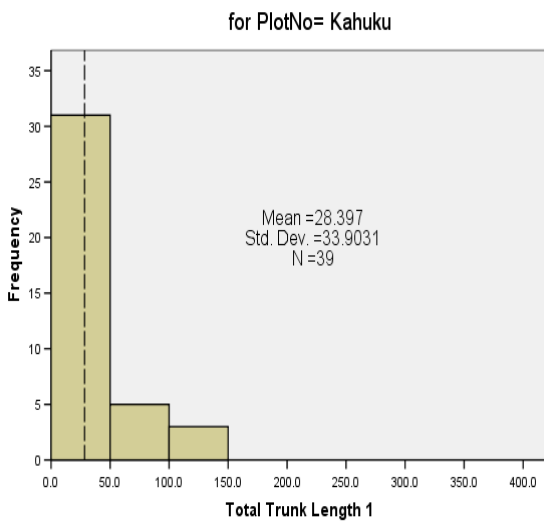

for PlotNo= Kahanahaiki Out Steep
Mean =84.476
Std. Dev. =87.4613
N =21
Frequency
Total Trunk Length 1

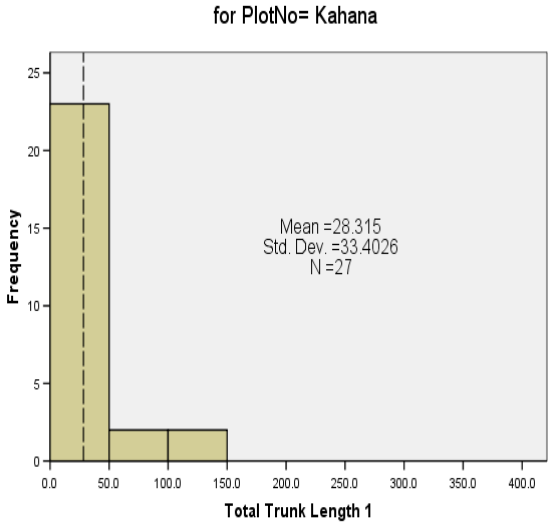

for PlotNo= Ohikilolo In
Mean =96.191
Std. Dev. =66.5477
N =47
Frequency
Total Trunk Length 1

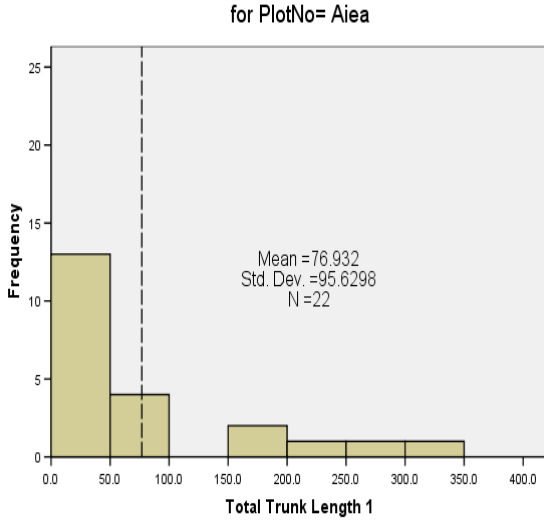

for PlotNo= Ohikilolo Out
Mean =121.617
Std. Dev. =52.9768
N =30
Frequency
Total Trunk Length 1

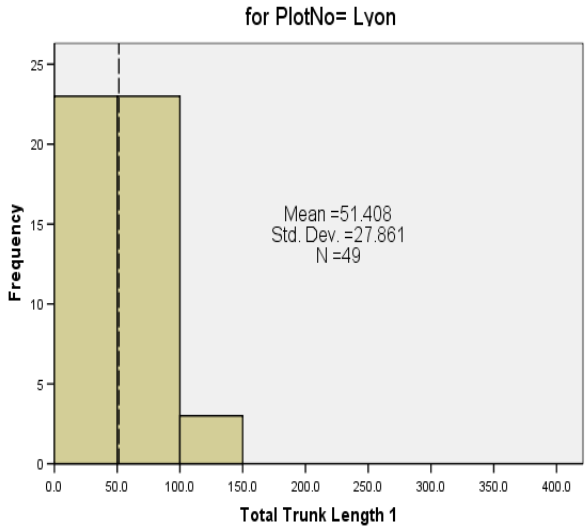

for PlotNo= Ohikilolo Out Weedv
Mean =143.1
Std. Dev. =72.1533
N =10
Frequency
Total Trunk Length 1


for PlotNo= 3 Points In
Mean =16.56
Std. Dev. =8.7753
N =25
Frequency
Total Trunk Length 1


for PlotNo= Kahuku
Mean =28.397
Std. Dev. =33.9031
N =39
Frequency
Total Trunk Length 1

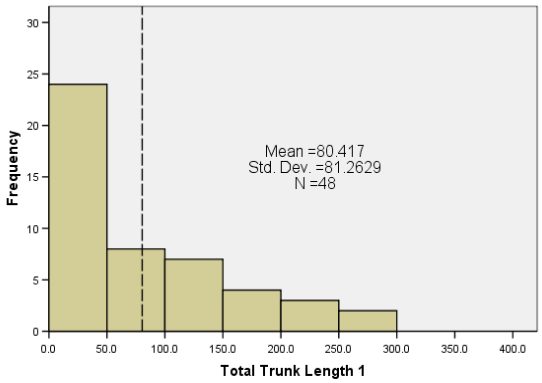

for PlotNo= Kahana
Mean =28.315
Std. Dev. =33.4026
N =27
Frequency
Total Trunk Length 1


for PlotNo= Aiea
Mean =76.932
Std. Dev. =95.6298
N =22
Frequency
Total Trunk Length 1

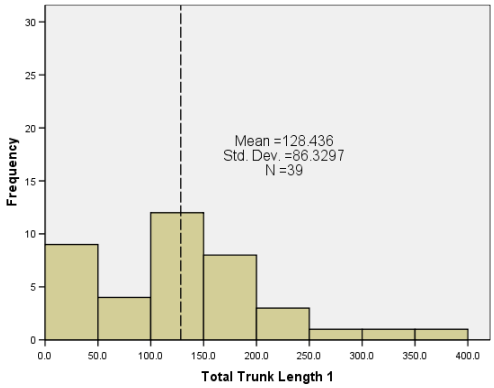

for PlotNo= Lyon
Mean =51.408
Std. Dev. =27.861
N =49
Frequency
Total Trunk Length 1

APPENDIX 7: Population structure of *C. chamissoi* by region.
Frequency of individuals (y-axis), by trunk length (cm) (x-axis).

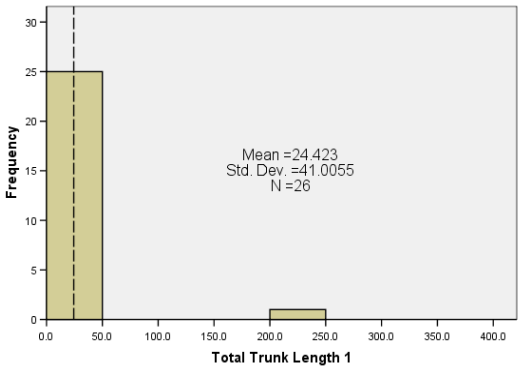
for RegionCode= Kahanahaiki



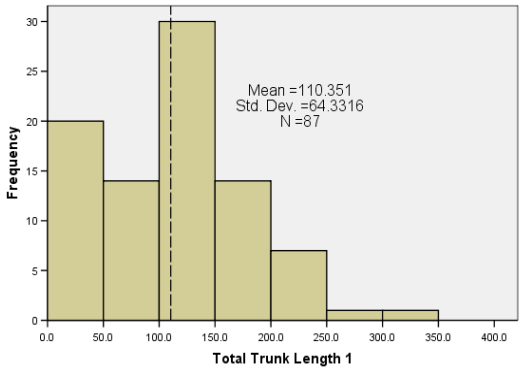
for RegionCode= Pahole



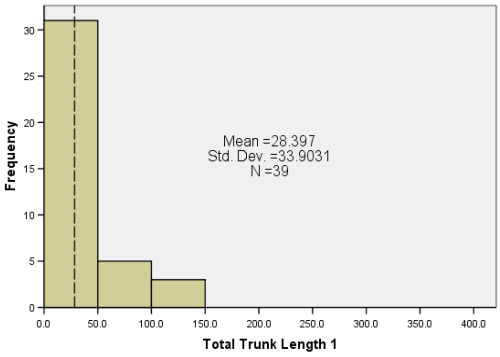
for RegionCode= 3 Points



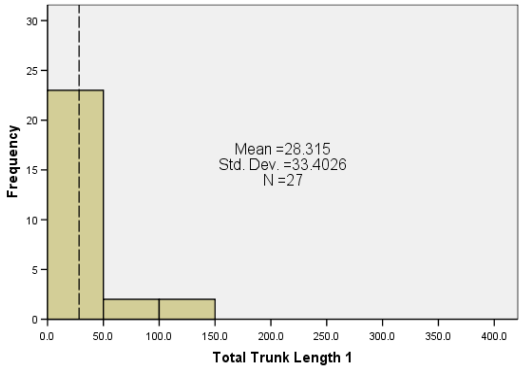
for RegionCode= Ohikilolo



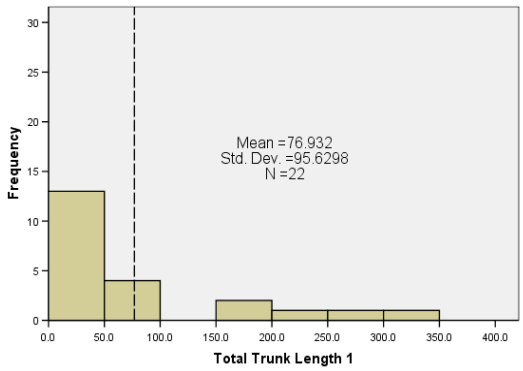
for RegionCode= Kahuku



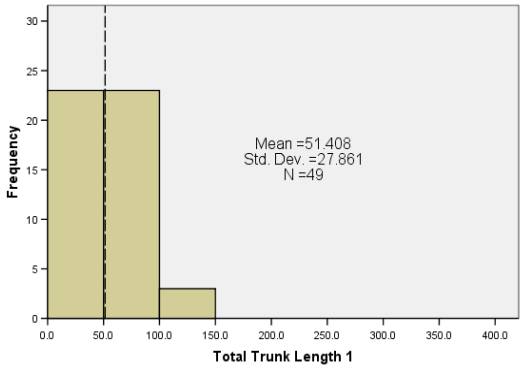
for RegionCode= Kahana



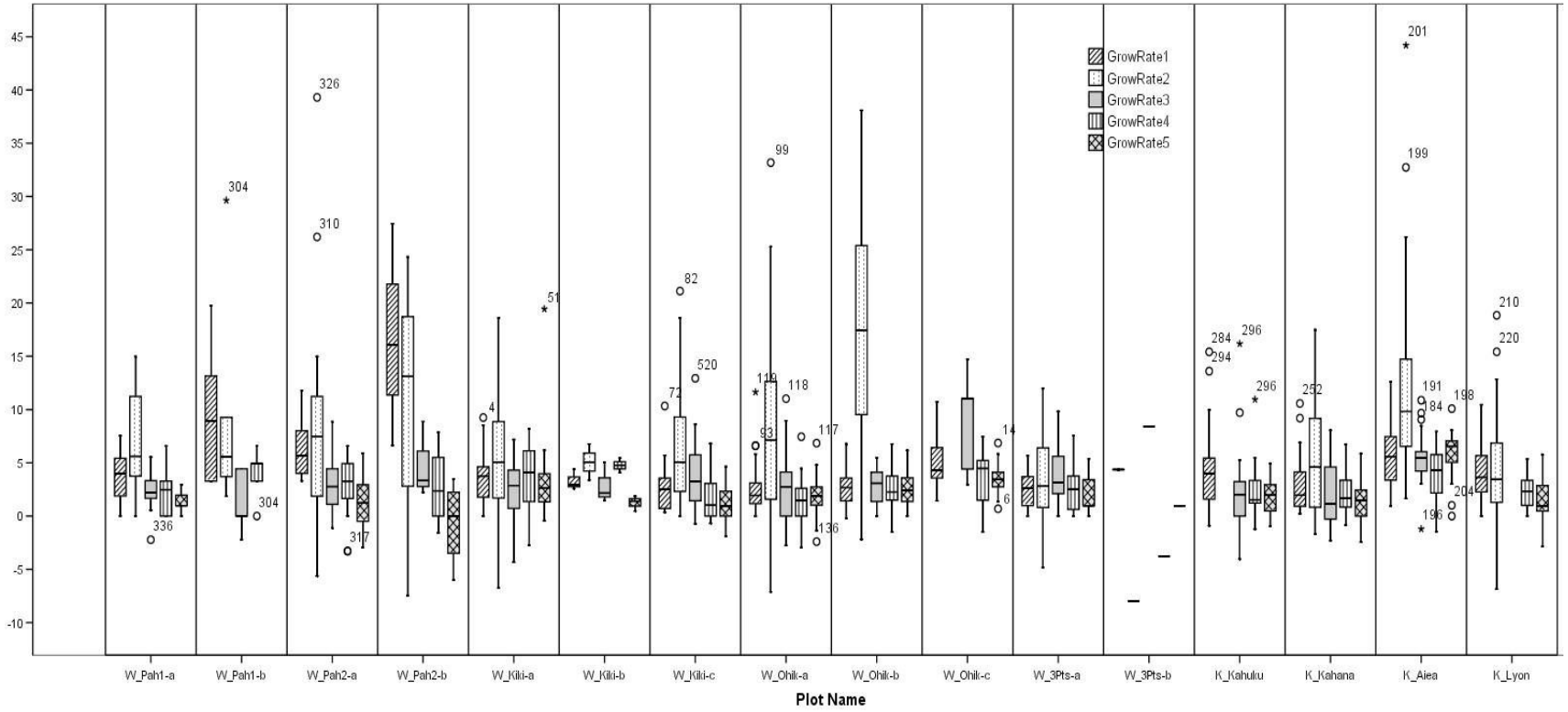
for RegionCode= Aiea



for RegionCode= Lyon

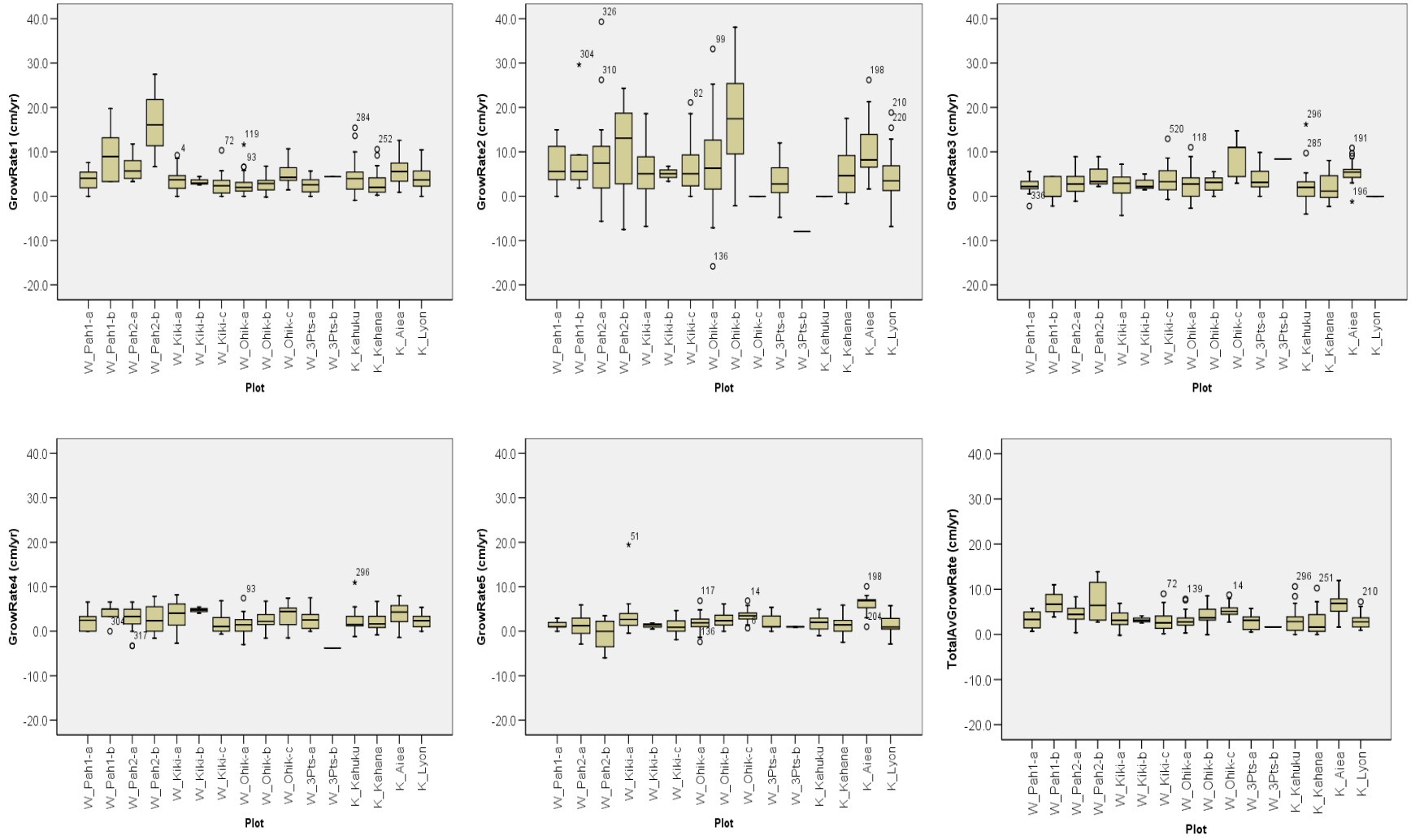


APPENDIX 8: Growth rates 1 - 5 by plot, averaged across all individuals.



Boxes indicate 50% of cases; central line represents median value, with whiskers indicating max and min values. Circles and stars identify outliers and extreme outliers, respectively. *W_ = Waianae Mountain Range; K_ = Koolau Mountain Range; a = inside fence; b = outside fence; Kiki-c = Kahanahaiki outside fence steep site; Ohik-c = Ohikilolo outside fence non-native site.*

APPENDIX 9: Growth rates by plot for each time period.



Boxes indicate 50% of cases; central line represents median value, with whiskers indicating max and min values. Circles and stars identify outliers and extreme outliers, respectively. *W_ = Waianae Mountain Range; K_ = Koolau Mountain Range; a = inside fence; b = outside fence; Kiki-c = Kahanahaiki outside fence steep site; Ohik-c = Ohikilolo outside fence non-native site.*

APPENDIX 10. CTAB procedure for isolating plant DNA.

CTAB procedure for isolating plant DNA (Doyle and Doyle 1987).
Ranker lab June 7, 2011.

DAY 1

PREP:

- Preheat water* bath (55-60°C) located under the hood.
- Preheat water* bath (55-60°C) located on lab bench.
- * Check water level in the water baths.
- Label (near top) & UV glass test tube (1 per plant to be extracted) for 6 min.
- Label & UV plastic test tubes (1 per plant to be extracted) for 6 min.

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1. Heat CTAB buffer to 55-60°C in water bath under the hood.
   WARNING!! CTAB destroys cell walls and membranes. Avoid direct contact with CTAB.

2. In a hot porcelain mortar, add 2-5 ml hot CTAB to 0.5 to 1.0 g (fresh weight) leaf material. 1 g is preferable using 5 mL of CTAB. Grind thoroughly and transfer to clean, labeled, unused glass test tube.
   NOTE: to heat mortar, microwave for 2 minutes.
   NOTE: measure and pour CTAB with graduated cylinder, it is too thick and lumpy to use a pipette.
   NOTE: 2 scoops of sand can be added to aid in grinding.

3. Incubate test tube at 55-60°C for at least 30 min. in water bath (on bench), gently swirl every 10 min.

4. Add 2/3 volume chloroform/isoamyl alcohol (24:1) at room temperature. Cover tube opening with thumb and shake vigorously **under the fume hood**.
   NOTE: Place an empty glove over the tube opening and cover it tightly. Shake tube with the open end facing away from you, than release the glove toward a paper towel that you are holding slightly away from the tube. Chemicals will spray out onto paper towel. Repeat a few times.
   NOTE: move to a new, clean spot on the glove for your next tube.

WARNING!! - a spray of caustic liquid will be released!

(This extraction will separate the DNA into an aqueous fraction and everything else into the chloroform mixture.)

5. Centrifuge at 4000 rpm for 10 min. in the clinical centrifuge.
   NOTE: Make sure spacers are inserted into the centrifuge tube chambers.

6. Carefully remove tubes (*do not disturb layers) from centrifuge and pipette out the top aqueous layer (using a 1000 uL pipette tip) into a plastic tube. Pour remaining waste into the waste container (located under the fume hood). Rinse glass test tubes and place in lab glass waste container.
   NOTE: You want to keep the top layer - don't suck up the other "junk" layers.
   NOTE: When done removing the top layer, use the pipette tip to break up the "junk" sold layer.
   NOTE: make sure the pipette barrel is clean after each tube.

7. Precipitate the DNA by adding 2/3 volume (eyeball measurement is OK) cold isopropanol (-20°C). Place a clean glove on top of tube, hold down firmly with your thumb and invert several times **very**

gently until thoroughly mixed (i.e., count 20 times). Use a clean spot on the glove for the next tube. Store at -20°C at least 30 min. OK if left overnight.
NOTE: Mixing is very important part of this protocol – don't cut corners at this step!
NOTE: Storing overnight is best!

DAY 2

PREP:

- Preheat water* bath (55-60°C) located on lab bench.
- * Check water level in the water baths.
- Label & UV 1.5 mL microcentrifuge tubes (1 per plant to be extracted) for 6 min. (*Make sure these were previously autoclaved as well).
- Ice from Cliff's lab (6^{th} floor).

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8. Remove plastic test tubes from freezer and place on ice. Pour nearly 1.5mL of mixture into a microcentrifuge tube. Centrifuge at 13,000 g in microfuge for 1.0 min. (Spin down 1.5 ml of sample, discard supernatant, add another 1.5 ml of sample, and repeat until entire sample has been processed). Keep test tubes on ice the whole time. (Waste can go down drain) *Keep whitish lump at bottom.

9. After discarding last batch of supernatant, place upside down on paper towel to air dry (1-2 minutes). Then add 1.5 ml cold (-20°C) 76% EtOH/10mM NH40Ac. Keep at -20°C for at least 30 min.
NOTE: Thoroughly wash plastic tubes. These will be re-used.
NOTE: Use glass pipette. Liquid pours out fast!
NOTE: 30 minutes is a minimum, can stay longer.

10. Microcentrifuge for 30 seconds at 13,000 g.

11. Discard EtOH/NH40Ac. Dry pellet in speed-vac on LOW until dry. ~ 45minutes – 1 hr. May even take longer, depending on the speed-vac.

12. Add 250 uL of sterile, HPLC water (Volume may vary depending on amount of initial leaf material used and/or size of DNA pellet. Incubate in water bath at 55-60 ° C until entire pellet (or as much as possible) goes into solution. May take more than one hour.
NOTE: After DNA is in solution you can pipette out a smaller amount of liquid (i.e., into a new (UVed) 1.5ul tube) for a smaller "working sample.

13. Store at -20° C.
or continue to Nanodrop and DNA dilution procedures.

APPENDIX 11. Herbarium label for *C. chamissoi* voucher specimen.

Joseph F. Rock Herbarium (HAW)
University of Hawai`i at Mānoa, Honolulu, Hawai`i

PLANTS OF THE HAWAIIAN ISLANDS
HONOLULU

Cibotiaceae
Cibotium chamissoi Kaulf.
Hāpu‘u

Honolulu, O`ahu: Lyon Arboretum, Valley 4C. Caudex circumference =48cm; caudex length =149cm; stipe length =116cm; blade length =162cm; blade width =150cm. Growing with other C. chamissoi on NNE aspect, slope approx. 30 deg.

Elevation (m): 210
Geo-reference: 21.337930, -157.803820
Coll. Naomi Arcand, NA01
Coll. with:
Date: 2009-09-03
Det: Dr. Tom Ranker
Note: Collector: [The family is now Cibotiaceae] Sheet 1 (of 10) = leaf apex; sheet 9 = basal pinna. Voucher specimen.

APPENDIX 12. *C. chamissoi* PCR protocol as performed by Nevada Genomics.

Protocol for Ferns

Stock Primer Solutions

Resuspend untagged reverse primers in TE at 1mM.

Resuspend tagged primers in TE at 100uM.

Prepare F&R Mixes at 50uM

2 ul	1mM reverse untagged primer
20 ul	100uM forward tagged primer
18 ul	water
(40ul)	mix at 50uM

Prepare Multiplex Panels

Prepare strip tube with F&R at 2uM with a 1:25 dilution to be used in solo primer set runs .

Panel 1

C109	0.75 ul F&R mix
A8	0.5 ul F&R mix
B121	0.5 ul F&R mix
water	23.25 ul
	25 ul

A3 - Solo PCR

A3	1 ul F&R mix
water	24 ul
	25 ul

Run Solo

add to panel 1 for 3730 run

Panel 2

B4	3 ul F&R mix
A115	1 ul F&R mix
A114	0.5 ul F&R mix
A118	1 ul F&R mix
C12	3 ul F&R mix
water	16.5 ul
	25 ul

PCR Mix / Sample

1 ul	primer panel
5 ul	Qiagen Master Mix (Qiagen catalog #206145)
6 ul	per tube
Add 4 ul	DNA @ 5ng/ul

Thermocycler Progams

step	time	temp
1	15min	95C
2	30sec	95C
3	1:30	**Tm***
4	30sec	72C
	40 cylces step 2-4	
5	30min	62C
6	Hold	10C

Tm* Panel 1 @ 64C
A3 @ 58C
Panel 2 @ 60C

Dilutions for 3730 Runs

Panel 1 and A3 are each diluted 1 to 300 and multiplexed for the 3730 run

Panel 2 diluted 1:150

APPENDIX 13. Genotype data for clone identification by population.

Summary of Multilocus Matches Analysis for Codominant Data

Data Sheet	CibotiumFinalGenotypes
Data Title	Cibotium Dataset (CoDom)

No. Loci	6
No. Samples	337
No. Pops.	16

Sorted Multilocus Genotypes with Repeated Matching Multilocus Genotypes Listed First

Sample No.	Population	Region	Genotype	No.	Label
284	KAieaS1	KoolauRegion	0000000000168168g	2	A
283	KAieaS1	KoolauRegion	0000000000168168g	0	A
218	KKahuN1	KoolauRegion	25125118519324924926128523624416 3177g	2	B
217	KKahuN1	KoolauRegion	25125118519324924926128523624416 3177g	0	B
146	WOhikB	WaianaeRegion	25125118519923724527927923623617 4191g	2	C
144	WOhikB	WaianaeRegion	25125118519923724527927923623617 4191g	0	C
123	WOhikA	WaianaeRegion	25125118619323724526128423623616 2194g	2	D
117	WOhikA	WaianaeRegion	25125118619323724526128423623616 2194g	0	D
111	WOhikA	WaianaeRegion	25125119119124924925525523423619 1194g	2	E
106	WOhikA	WaianaeRegion	25125119119124924925525523423619 1194g	0	E
122	WOhikA	WaianaeRegion	25125119119323724925725723823817 4196g	2	F
120	WOhikA	WaianaeRegion	25125119119323724925725723823817 4196g	0	F
76	WKikiC	WaianaeRegion	25125119319324925525727923424817 7199g	2	G
72	WKikiC	WaianaeRegion	25125119319324925525727923424817 7199g	0	G
116	WOhikA	WaianaeRegion	25125318519123724525725723623819 6209g	2	H
113	WOhikA	WaianaeRegion	25125318519123724525725723623819 6209g	0	H
93	WOhikA	WaianaeRegion	25125318519323723725725923623617 6222g	2	I
92	WOhikA	WaianaeRegion	25125318519323723725725923623617 6222g	0	I
162	WOhikB	WaianaeRegion	25125719119923723726326323223617 4174g	3	J
136	WOhikB	WaianaeRegion	25125719119923723726326323223617 4174g	0	J
135	WOhikB	WaianaeRegion	25125719119923723726326323223617 4174g	0	J
142	WOhikB	WaianaeRegion	25126218519123724924826623623813 8209g	4	K
141	WOhikB	WaianaeRegion	25126218519123724924826623623813 8209g	0	K
140	WOhikB	WaianaeRegion	25126218519123724924826623623813 8209g	0	K
139	WOhikB	WaianaeRegion	25126218519123724924826623623813 8209g	0	K
125	WOhikA	WaianaeRegion	25126419319323723726128423423616 2222g	2	L
124	WOhikA	WaianaeRegion	25126419319323723726128423423616 2222g	0	L
105	WOhikA	WaianaeRegion	25127918519323723725127323423617 4211g	7	M
104	WOhikA	WaianaeRegion	25127918519323723725127323423617 4211g	0	M

103	WOhikA	WaianaeRegion	251279185193237237251273234236174211g	0	M
102	WOhikA	WaianaeRegion	251279185193237237251273234236174211g	0	M
101	WOhikA	WaianaeRegion	251279185193237237251273234236174211g	0	M
100	WOhikA	WaianaeRegion	251279185193237237251273234236174211g	0	M
99	WOhikA	WaianaeRegion	251279185193237237251273234236174211g	0	M
98	WOhikA	WaianaeRegion	253260191193237245257257234236194225g	3	N
97	WOhikA	WaianaeRegion	253260191193237245257257234236194225g	0	N
94	WOhikA	WaianaeRegion	253260191193237245257257234236194225g	0	N
133	WOhikA	WaianaeRegion	253264185185237245251261234244168224g	2	O
132	WOhikA	WaianaeRegion	253264185185237245251261234244168224g	0	O
130	WOhikA	WaianaeRegion	253264191193245249261269234236174194g	3	P
127	WOhikA	WaianaeRegion	253264191193245249261269234236174194g	0	P
126	WOhikA	WaianaeRegion	253264191193245249261269234236174194g	0	P
119	WOhikA	WaianaeRegion	253264193193237237251257234244194224g	2	Q
118	WOhikA	WaianaeRegion	253264193193237237251257234244194224g	0	Q
151	WOhikB	WaianaeRegion	255255185193237247266289234238191197g	2	R
150	WOhikB	WaianaeRegion	255255185193237247266289234238191197g	0	R
153	WOhikB	WaianaeRegion	257262185185237237257279232238138174g	2	S
152	WOhikB	WaianaeRegion	257262185185237237257279232238138174g	0	S
318	KLyonS2	KoolauRegion	259259185191237245253265236238144188g	2	T
317	KLyonS2	KoolauRegion	259259185191237245253265236238144188g	0	T
129	WOhikA	WaianaeRegion	260264186193237245255261234234168194g	2	U
128	WOhikA	WaianaeRegion	260264186193237245255261234234168194g	0	U
108	WOhikA	WaianaeRegion	262262187191249257257289236240191191g	2	V
107	WOhikA	WaianaeRegion	262262187191249257257289236240191191g	0	V
155	WOhikB	WaianaeRegion	262262191191237237259259238238138138g	2	W
154	WOhikB	WaianaeRegion	262262191191237237259259238238138138g	0	W
270	KAieaS1	KoolauRegion	273273191191245245242242242236177227g	2	X
269	KAieaS1	KoolauRegion	273273191191245245242242242236177227g	0	X