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UNIVERSITY OF NORTHERN COLORADO

Greeley, CO

The Graduate School

CHARACTERIZING ECOLOGICAL AND GENETIC
PATTERNS OF THE COLONIZING SPECIES
DUBAUTIA SCABRA ON THE
BIG ISLAND, HAWAII

A Thesis Submitted in Partial Fulfillment
of the Requirements of the Degree of
Master of Science

Samantha Kaleigh Naibauer

College of Natural and Health Science
School of Biological Sciences

December 2018

This Thesis by: Samantha Kaleigh Naibauer

Entitled: CHARACTERIZING ECOLOGICAL AND GENETIC PATTERNS OF THE
COLONIZING SPECIES *DUBAUTIA SCABRA* ON THE BIG ISLAND, HAWAII

has been approved as meeting the requirement for the Degree of Master of Science in
College of Natural and Health Sciences in School of Biological Sciences

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ABSTRACT

Naibauer, Samantha Kaleigh. *Characterizing ecological and genetic patterns of the colonizing species Dubautia scabra on The Big Island, Hawaii*. Unpublished Master of Science thesis, University of Northern Colorado, 2018.

The Hawaiian Silversword Alliance (HSA) is a group of approximately 35 endemic species found on the Hawaiian Islands. The HSA is of particular interest due to the vast adaptive responses of its members across the Hawaiian Islands. Each of these plant species is generally limited to a very specific habitat type with a unique morphology and physiology. *Dubautia scabra* is the first angiosperm colonist of new lava flows, arriving in as few as two years after lava cools. The critical role *D. scabra* plays in this ecosystem gives a unique starting point for understanding downstream ecological processes such as seed dispersal, colonization, and ecological succession. The processes of colonization and ecological succession are widely understood by ecologists in habitats like forests and grasslands, but researchers have not yet been able to successfully characterize primary succession on truly new habitats. The goals of this study were to (1) characterize patterns of primary succession in new habitats, (2) determine if there is a correlation between lava flow age and genetic variation among populations, and (3) determine the source and direction of seed dispersal of *D. scabra* populations across the Big Island, Hawaii using genetic analysis. Regression analysis showed a negative correlation ($R^2 = 0.4439$) between lava flow age and plant size, and a positive correlation

($R^2 = 0.7692$) between lava flow age and plant density. The largest individuals were found on lava flows of about 100 years of age, with the most individuals found on lava flows between 40 and 45 years of age. Lava flow age, elevation, aspect, rock type, soil hydrology, and mean annual rainfall were also found to be ecological predictors of plant size and density on lava flows. There was no correlation between genetic divergence and lava flow age within populations found on The Big Island, which suggests frequent gene flow and migration events between all populations. Levels of inbreeding were found to decrease over time, and populations on lava flows of about 150 years of age had the least amount of inbreeding. Overall genetic diversity resembled that of other species within the *Dubautia* genus, but measures of genetic divergence were low between populations of *D. scabra* compared to other species within the HSA. The population at Saddle Road clustered genetically and therefore might be the source population that founded the other populations at Hawaii Volcanoes National Park and Hawaiian Ocean View Estates. Ecological and genetic analysis of organisms such as *D. scabra* give researchers the ability to identify patterns of colonization into new habitats, as well as determine the relationships within and among populations in other systems.

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I would like to take this opportunity to thank a few people that made the completion of this project possible:

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CHAPTER I

INTRODUCTION TO THE STUDY SYSTEM: THE HAWAIIAN SILVERSWORD ALLIANCE AND *DUBAUTIA SCABRA*

Introduction

This research project was a genetic investigation of the Hawaiian endemic species, *Dubautia scabra* (DC.) D.D. Keck (Asteraceae). *Dubautia scabra* is a member of the Hawaiian Silversword Alliance (HSA), which is a group of plants widely known for their ecologically adaptive evolutionary histories. The HSA consists of roughly 30 species (Carr 1985; Witter and Carr 1988; Baldwin et al. 1991) which are thought to have differentiated within the last five million years. Each speciation event was likely due to responses to habitat variation and physiological limitations. *Dubautia scabra* is the first angiosperm colonist of new lava flows, and is one of the few species found on exposed lava rock on The Big Island, Hawaii. As a colonizer of volcanic substrates, *D. scabra* provides a unique window into studying many other ecological processes including seed dispersal and establishment, patterns of colonization, and patterns of primary succession. The main purpose of this study was to characterize patterns of ecological succession on The Big Island after lava flow disturbance. The ecological patterns in this study were determined using multiple ecological and genetic techniques which included: plant density, plant frequency, and microsatellite fragment length polymorphisms. The combination of ecological and genetic techniques allowed inference of the general structure of meta-populations, which included defining source populations, direction

of seed dispersal, and characterizing patterns of colonization and habitat connectivity. These techniques also measured genetic diversity of populations and rates of gene flow among lava flows. This chapter provides a historical background of Hawaii and the study system, as well as provide a brief introduction to some of the techniques used.

Historical Background of Hawaii

The Hawaiian-Emperor Island Chain is group of islands that stretch nearly 3,600 miles in a northwest to southeast orientation in the Pacific Ocean. This large chain consists of three subsections: The Emperor Seamount chain, The Hawaiian Seamount chain, and the Hawaiian Archipelago (Figure 1). In 1963, geologist John Tuzo Wilson hypothesized that the linear pattern of this island chain was due to the Pacific Tectonic Plate moving in a northwest orientation over an either stationary or mobile volcanic hotspot (Wilson 2014). Wilson's hotspot theory also suggested that island formation in this region of the Pacific Ocean is age progressive and has been occurring for about 85 million years (Regelous et al. 2003). Since 1963, most researchers have agreed with Wilson's stationary hotspot theory, which explained the shape and direction of the northern most trail of seamounts and islands left by the hotspot. The oldest and most northern structures in The Hawaiian-Emperor Island Chain, the Emperor Seamounts, are located close to the Aleutian Islands of Alaska. The Emperor Seamounts have a vertical orientation that extends into the west Pacific until they reach the Kuril- Kamchatka Trench, a subduction zone just east of the Russian border (Foulger and Anderson 2006).

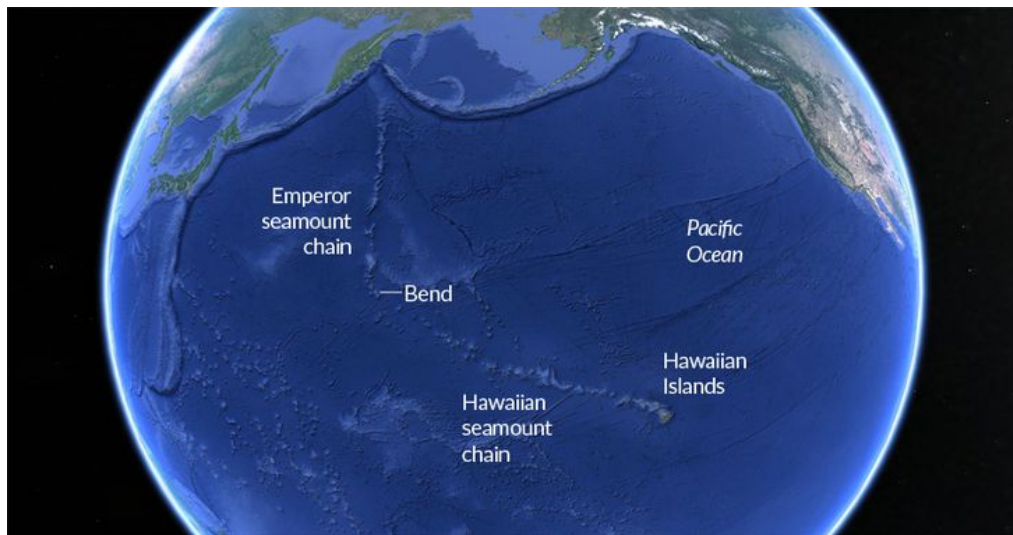


Figure 1. The Hawaiian-Emperor Island Chain. The three subsections from left to right: The Emperor seamounts, The Northwestern Hawaiian seamounts, and the Hawaiian Islands (Kauai, Oahu, Maui- Nui, The Big Island).

Wilson's stationary hotspot theory falls short at explaining the shape and direction of the rest of The Hawaiian-Emperor Island Chain. Centered in the island chain is a sharp bend in the direction of islands. The islands after this bend are referred to as the Hawaiian Seamount Chain, and are mainly small atolls and extinct islands. Wilson's hypothesis argues that an abrupt shift in the Pacific tectonic plate above the hotspot was the cause of the bend. However, more recent studies suggest that such an abrupt shift would not have the force nor rate of motion required to shift the archipelago (Tarduno et al. 2003). It is more likely that plate shifts would have a more gradual change on the island chain (Tarduno et al. 2003). Instead of Wilson's stationary hotspot hypothesis, Wilson's moving hotspot theory suggests that the hotspot under the Hawaiian Islands was moving southward for millions of years (Tarduno and Cottrell 1997; Tarduno et al. 2003; Tarduno et al. 2009; Wilson 2014). During the formation of the Emperor seamounts, the hotspot was moving south very rapidly (over 40 millimeters a year; (Tarduno et al. 2003).

Roughly 47 million years ago, the moving hotspot slowed. Together with the gradual movement of the Pacific Tectonic Plate towards the Kuril- Kamchatka Trench, the bend in the island chain formed (Tarduno and Cottrell 1997; Tarduno et al. 2003). This hotspot has remained relatively stationary since it stopped moving 47 million years ago, and has continued to leave the remaining trail of islands (Tarduno et al. 2003).

The Hawaiian Archipelago is the youngest portion of the Hawaiian-Emperor Island Chain, and consists of the five main islands (Figure 2). The oldest of these islands, Kauai, is estimated to be about five million years old. Oahu is the next island in the chain, and is estimated to be about three million years old. Maui- Nui is an island complex consisting of Lana'i, Maui, and Molokai which ranges in ages from 1.3 to 1.8 millions years old. The Big Island is the largest and youngest island in the archipelago, and is located roughly 2,000 miles southwest of the continental United States. This island is less than 500,000 years old (Witter and Carr 1988; Fleischer et al. 1998).

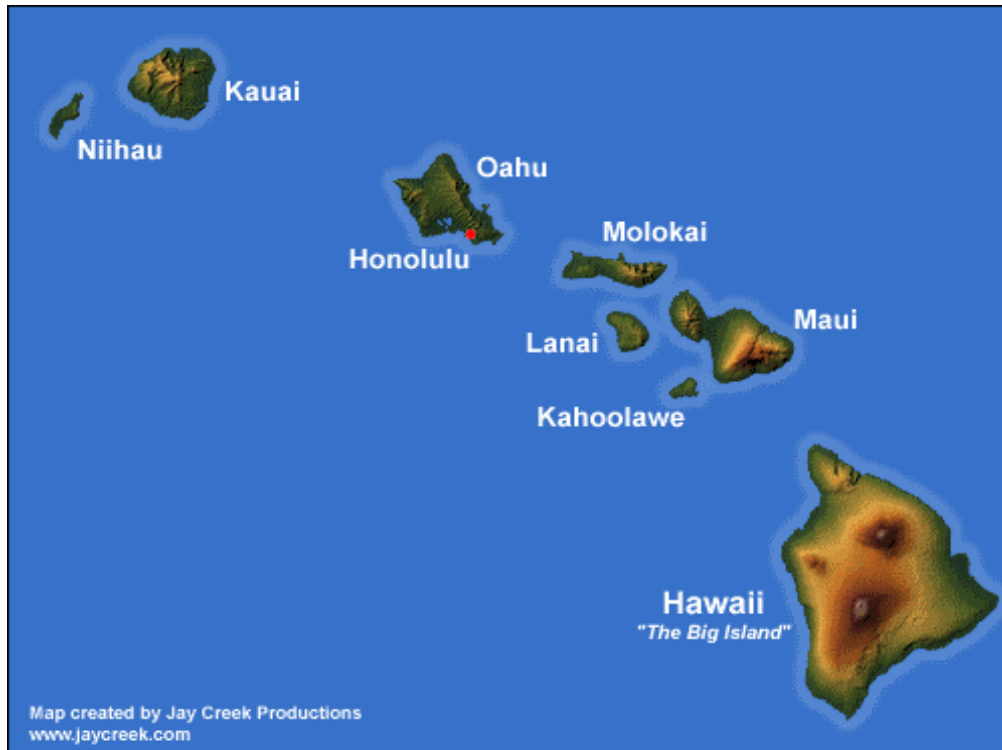


Figure 2: From left to right: Kaua'i, O'ahu, Maui Nui (Moloka'i Maui, Lanai, and Kahoolawi), and Hawai'i (The Big Island).

The volcanic hotspot that created this archipelago is currently positioned just southeast of the Big Island. Because the Big Island is the closest island to the hotspot, numerous active and a few inactive volcanoes still exist on the island. The Big Island has five remaining active volcanoes: Hualalai, Mauna Kea, Mauna Loa, Kilauea, and Lo'ihi (United States Geological Survey: USGS 2017; Figure 3). Hualalai is positioned on the northwest portion of the island, near Kona. Mauna Kea is the highest volcano on the island (13,803 ft.), and its flanks cover most of the northern half of the island (GNIS 2003). Mauna Loa is the largest volcano on earth, and extends to the ocean floor making the summit nearly 56,000 ft. (USGS 2017), with an elevation of 13,679 ft. above sea level. Mauna Loa is also highly active, producing large lava flows around the center of the island. Kilauea is the youngest volcano on The Big Island and is most likely a satellite

of the neighboring Mauna Loa (USGS 2017). Kilauea is positioned on the southeast portion of the island and has continuously been erupting since 1983 (USGS 2017). In early 2018, seismic activity from Kilauea created numerous fissures and eruptions on the eastern side of the island and within Hawaii Volcanoes National Park leading to the destruction of numerous buildings and homes and adding nearly 700 acres of new land to the island (USGS 2018). Lo’ihi is positioned 22 miles off the southeast coast of The Big Island, and the only known submarine volcano in the region (Malahoff et al. 2006). Scientists estimate that the emergence of this new island could take between 10,000 and 100,000 years depending on eruption and growth rate (USGS 2018).

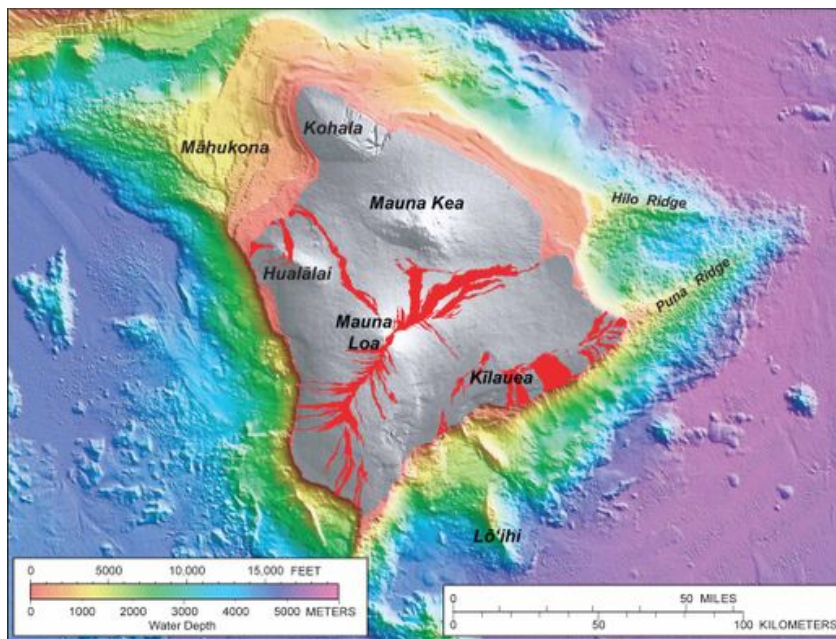


Figure 3: Remaining volcanoes on The Big Island, Mahikona, Kohala, Mauna Kea, Hualalai, Mauna Loa, Kilauea, and Lo’ihi. Mahikona and Kohala are inactive, while Hualalai, Mauna Kea, Mauna Loa, Kilauea, and Lo’ihi are active.

Study System

The Theory of Evolution describes the descent of successive generations through time with small modifications and adaptations, and is one of the key theories to all biological research. Over millions of years, these modifications have led to millions of distinct lineages and species on earth. In many island systems, particularly on the Hawaiian Islands, the evolution of numerous lineages has led to a vast array of unique and endemic organisms. Because the Hawaiian Islands are completely isolated from any other mainland, and were not colonized by humans until around 1,600 years ago, they serve as the perfect natural laboratory to study the evolution of Hawaiian life. Hundreds of studies have been conducted on the Hawaiian islands, and have looked at organisms ranging from flies (Carson 1983; Coyne and Orr 1989), spiders (Gillespie 2005; Croucher et al. 2012), snails (Cowie 1995; Holland and Hadfield 2004), birds (Freed et al. 1987; Lerner et al. 2011), and plants (Helenurm and Ganders 1985; Givnish et al. 2009).

A recurring theme in Hawaiian evolutionary studies revolve around the Progression Rule (Funk and Wagner 1995). The Progression Rule is a simple rule that states that dispersal and colonization in an island chain proceed from the oldest to the youngest island. As an island emerges from the ocean, it is available for colonization from the nearest land mass, like stepping stones across the archipelago. The most recent island formations have been the main Hawaiian Islands; Kauai, Oahu, Maui-Nui, and The Big Island. These islands have been colonized from oldest to youngest island for the past five million years (Baldwin and Sanderson 1998).

Similar patterns of colonization can also be seen in patches of suitable habitat within islands. As volcanoes erupt on land, they change the landscape and create a pathway of new habitat and barren landscapes. Multiple volcanic eruptions over time

connect these lava flows, making a network of hardened and exposed lava all over the surface of the island. Recently formed lava habitats will require species dispersal and colonization in order to initiate successional processes, and undergo thousands of years or erosion and decomposition in order to develop soil and the conditions necessary for complex communities. Ecological succession is a process of change in species structure, habitat structure, and ecological community over time. Ecological succession can be further broken down into two types: primary and secondary. Primary succession is the development of plant and animal communities in an area where no soils have existed before. Secondary succession is the re-establishment of a community where soils and portions of a community have been retained. Secondary ecological succession is more so studied because it is associated with shorter regeneration times, and incomplete destruction of habitat, seed banks, or landscape structure. Volcanic eruptions are high intensity disturbances that alter geologic structures and destroy species and habitat structures. Eruptions create virtually lifeless areas, requiring primary successional processes to reclaim a habitat. It may take thousands of years for barren rock to erode and form soils so that other organisms can persist in these areas. These areas may also have many ecological and habitat shifts within that time. The variation within habitat shifts is quite diverse on the Hawaiian Islands, ranging from barren lava to dry shrub communities to tropical rainforest, depending on time since disturbance, elevation, and mean annual rainfall. The Big Island ranges in elevation from sea level to almost 14,000 ft. on the top of Mauna Kea, while mean annual rainfall might differ as much as 7,000 mm from the northern to the southeastern sides of the island (Giambelluca et al. 2013).

Volcanic eruptions and major changes in geological features have largely shaped the current distribution and evolutionary paths for many organisms on the Hawaiian

Islands. Lava flows may prove to be large physical barriers for some species by restricting individuals or populations to small patches of suitable and undestroyed forest habitat (kipuka's). Over time, these restrictions could lead to genetic bottlenecks, mutations, and eventually speciation. Disturbance followers, however, have the ability to use these networks as "highways", and may use lava flows to access new areas of an island.

Establishment of plant populations in new habitats could take several years and numerous colonizing events (Groom 1998). In many cases, especially among plants, establishment in a new area might reveal un-utilized or under-utilized resources. Depending on the physiological conditions and the plant's ability to adapt to these conditions, speciation might occur. Many types of organisms have also replicated this pattern, including plant species (Percy et al. 2008; Givnish et al. 2009; Ilves et al. 2015), birds (Diamond 1970), Gastropods (Rundell et al. 2004), and spiders (Roesch Goodman et al. 2012). Additionally, the creation of new islands via volcanic activity provides ecologically and geographically distinct habitats and niches. Because many organisms have the ability to adapt to new and ecologically diverse habitats, the Hawaiian Islands have a wide variety of native and endemic organisms. This system provides one of the best arenas to study landscape succession, evolution, and adaptive radiation in the world.

The Hawaiian Silversword Alliance

The Hawaiian Silversword Alliance (HSA) is a small group of plants endemic to the Hawaiian Islands. This group is mostly known for its ability to readily adapt to changing physiological and ecological factors. Adaptive Radiation as the result of this ability, has led to an array of morphologically and ecologically distinct species. Members of the HSA have been under extensive study since the 1950's as researchers tried to

understand the adaptive radiation and rapid evolution that has occurred on the islands (Carlquist 1957, 1958, 1959, 1961; Carr and Kyhos 1981, 1986). One of the first theories proposed about the origination of the HSA suggested that the group had similar bract and flower morphology to the Californian Tarweeds (*Madiinae*; (Carlquist 1959). This theory also suggested the method of dispersal was likely a single colonization event from the mainland (California) to the Hawaiian Islands.

The first genetic evidence of a relationship between the HSA and the Californian Tarweeds was observed by a group of researchers in 1991 (Baldwin et al. 1991). The maternally inherited chloroplast genome was analyzed, and only ten mutations separated the continental *Madiinae* group from the HSA (Baldwin et al. 1991). The small amount of genetic variation suggested that seeds were dispersed from California to Hawaii. It was later estimated that this long-distance colonization event occurred 4.5 to six million years ago to the island of Kauai (Baldwin and Sanderson 1998; Barrier et al. 1999). Since colonization of Kauai, many factors have led to the rapid diversification seen in the HSA, including, the isolation of the Hawaiian Islands, emergence of new islands via volcanic activity, and occupation of unrealized niches. As dispersal from older to younger islands occurs and new habitats are colonized, many individuals have adapted to new habitats and niches in a stepping-stone pattern (Funk and Wagner 1995; McArthur and Wilson 2001).

The HSA is divided into three genera: *Argyroxiphium* DC., *Dubautia* (DC.) D.D. Keck, and *Wilkesia* A. Gray, and is composed of roughly 30 species that are taxonomically recognized today. Each species is generally limited to a specific habitat type and island, and as a result, species have unique morphologies and physiologies (Friar et al. 2006). Members of the HSA range in morphology from lianas and small

cushion plants, to small shrubs and even trees. The most famous of the Hawaiian silverswords are members of the genus, *Argyroxiphium*. These plants have tall, silver, sword like leaves, and are generally found in drier habitats. Other *Argyroxiphium* species known as the Green Swords, have tall, green, sword like leaves, and are more commonly found in wetter habitats. There are only two species within the genus *Wilkesia*, and both are endemic to Kauai. These species are both woody, but do not share similar morphologies or shapes. The genus, *Dubautia*, is the largest and most diverse genus in the Alliance, and is not characterized by leaf shape or form (Baldwin et al. 1991; Garcia-Verdugo et al. 2013). Although many of these species have very diverse plant forms, most of them have numerous, small, orange or yellow flowers.

***Dubautia* Genus**

Dubautia species are located on all islands, with individual species generally being endemic to one or two of the major islands. These species also have the most variation in morphology and physiology within the HSA. Suitable habitats for these species can range from barren lava flows to wet rainforests or the alpine mountain slopes of Mauna Kea. Plant physiologies in various regions of the island must be able to tolerate vast differences in water availability, temperature gradients, and seasonal changes. All members of the HSA have very limited genetic divergence, and about 75% of all possible artificial or natural crosses have been made independent of genus or species. The species within the genus *Dubautia* tend to maintain higher levels of genetic affinity. Limited divergence within this genus has led to numerous hybridization events where habitats overlap, and numerous hybrid species are formally recognized within the genus (Carr and Kyhos 1981; Carr 1985).

Members of *Dubautia* have been separated by two cytotypes according to Carr (1985): *Dubautia* sect. *Dubautia* Gaud., which have 14 chromosomes, and *Dubautia* sect. *Railliardia* (Gaud.) G. Carr, which have 13 chromosomes. The variability in chromosome number is likely due to an aneuploid reduction in one species that colonized a new or emerging island more than one million years ago. Geographically, these cytotypes can be mapped on the Hawaiian archipelago based on island age. Members of *Dubautia* sect. *Dubautia* ($N = 14$) are predominantly found on the oldest islands, Kauai and Oahu, although both *D. laxa*. and *D. plantaginea* have multi-island distributions including young islands. Species within *Dubautia* sect. *Railliardia* ($N = 13$) are found on the younger islands Maui, Molokai, Lanai, and Hawaii, although *D. herbstobatae* and *D. sherffiana* are found on the older island of Oahu. Because the $N=14$ cytotype is the only cytotype found on Kauai, we believe the genus *Dubautia* originated on Kauai with a chromosomal arrangement of $N = 14$.

Ecological differences on various islands are arguably one of the most important factors in the adaptive radiation of *Dubautia*, playing a huge role in what we know about speciation within this genus. Inter-island dispersal across the Hawaiian Islands follows a well-known and described Progression Rule or Stepping Stone Pattern from northwest to southeast (Funk and Wagner 1995). Colonization of new islands results in geographic isolation (allopatry) and exploitation of new niches and ecological variables. These new variables might over time change the morphology and physiology of species, and diversification or speciation may also occur. Typically, there is a burst of speciation after inter-island dispersal when there are many niches to be filled and therefore, ecological opportunity is greatest soon after island colonization. Speciation after dispersal is common in the HSA, and has been documented in variable chloroplast genomes of

numerous members of the Alliance (Baldwin et al. 1991; Baldwin and Sanderson 1998). Within the HSA, speciation has occurred so quickly from the oldest to youngest islands, that there hasn't been enough time for much genetic diversity to accumulate (Witter and Carr 1988; Friar et al. 2006).

Ecological speciation has been documented on all of the major islands within the Hawaiian archipelago. The best studied example of speciation by ecological partitioning has been seen on Maui, where four endemic species, *D. menziesii*, *D. platyphylla*, *D. reticulata*, and *D. waianapanapaensis* were studied (Friar et al. 2006). These species are members of *Dubautia* sect. *Railliardia* and exhibit low levels of genetic differentiation despite statistically different morphological and ecological differences. Morphological and ecological differentiations seem to be maintained in selected populations despite the physical proximity between them. Most noticeably, these plant species seem to follow moisture and elevation clines. This study suggests that speciation is most likely due to habitat variation (dry and wet) and adaptation to different ecological niches over the island. It is possible that physiological constraints and water availability are factors that have aided in the diversification of these species.

Although not as well studied or prevalent, hybridization can also play an interesting role in the differentiation of species with the genus *Dubautia*. The evolutionary history of *D. scabra* has always been unclear as different studies produced conflicting results between nuclear and plastid genomes (Baldwin et al. 1991; Friar et al. 2008). Traditionally, nuclear genomic analysis had suggested that *D. scabra* shares the same nuclear chromosomal arrangement of the Kauai endemic, *D. latifolia* (N = 14). Chloroplast comparisons from another study placed *D. scabra* within the *Railliardia* section (Baldwin et al. 1991). In 2008, another study set out to determine the actual

history of *D. scabra*. Multiple nuclear and chloroplast regions of *D. scabra* were compared to other members of the genus (Friar et al. 2008). These regions showed a mosaic pattern between the two sections of *Dubautia* within the nuclear genome. This mosaic genome suggests a hybrid origin of *D. scabra* between *D. latifolia* (N = 14) and members of *Dubautia* sect. *Railliardia* (N= 13). Hybridization is not unique among plant species, especially among members of the HSA or genus *Dubautia* (Carr 1985; Barrier et al. 1999), with a hybrid history also being implicated for the entire HSA (Barrier et al. 1999). Numerous hybrid species are formally recognized in the HSA, and could be one of the more influential factors driving speciation into specialized ecosystems and niches.

Species of Interest

Dubautia scabra (DC.) D.D. Keck is an endemic species to Maui-Nui and The Big Island, that has two subspecies: *D. scabra* ssp. *scabra* and *D. scabra* ssp. *leiophylla* (A. Gray) G.D. Carr which are probably less than one million years old (Baldwin & Sanderson, 1998). *Dubautia scabra* ssp. *scabra* is commonly thought of as a small shrub that grows in volcanic and rocky substrates, while *D. scabra* ssp. *leiophylla* has longer and more vine-like branches and is found in forested habitats. In his monograph, Carr (1985) separates the species by leaf length and habitat. He suggests that *D. scabra* ssp. *scabra* has leaves that are mostly linear-elliptic to linear-oblong, and are less than 3.5 cm in length. Carr also suggests that *D. scabra* ssp. *scabra* is most commonly found on young and barren lava flows on portions of east Maui and Hawaii. *Dubautia scabra* ssp. *leiophylla* is characterized by narrowly linear to linear-lanceolate leaves that are greater than 3.5 cm in length. *Dubautia scabra* ssp. *leiophylla* is more commonly found in the mountainous and forest regions of Northern Hawaii and Maui. The Manual of the Flowering Plants of Hawaii (Wagner et al. 1999) provides an equally vague description

of the two subspecies, and differentiates them mostly by leaf morphology, but also by location on the island where they are found. The Manual suggests *D. scabra* ssp. *scabra* has leaves usually linear-elliptic to linear-oblong, that are less than 3.5 cm long, and 2-4mm wide. This subspecies is found on portions of east Maui and Hawaii, but it is noted that “In this treatment, the taxonomic disposition of certain East Maui specimens may be rather arbitrary.” *Dubautia scabra* ssp. *leiophylla* has leaves that are linear to linear-lanceolate, 0.08-8mm wide and either more than 3.5 cm long or less than 2 mm wide. According to the Manual, this subspecies can be located on Molokai, Lanai, Maui, and the northern Kohala mountain regions of Hawaii. There is little agreement about the actual distribution of the two subspecies, with herbarium specimens placing both species in close physical proximity with the only separation being habitat. Herbarium collections have documented striking morphological and ecological similarities between the subspecies, including variation in leaf length, growth form, and ability to grow in both barren and forested habitats. *Dubautia scabra* ssp. *scabra* has been known to grow in wet forests, where it takes on a unique vine-like morphology (personal observation). Additionally, previous herbarium collections have been inconsistent, and specimens have been identified solely on morphological differentiation (subshrub vs. vine-like). Our current knowledge of these two subspecies tells us very little about the differences in actual distribution or morphology, and indicates that further research should be done to resolve this taxonomic issue.

Dubautia scabra ssp. *scabra* (hereafter *D. scabra*) is the first angiosperm colonizer of recent lava flows, and is characterized by its small sub-shrub appearance, whorled leaves, and unusual small, white flowers (Figure 4). The other species in the genus are known for yellow/orange flowers. *Dubautia scabra* has been documented as

appearing on lava flows in as little as two years after lava cools (Smather and Mueller-Dombois 1972), and is mostly established within ten years (Smather and Mueller-Dombois 1972; Carr 1985; Friar et al. 2008). This species is found in habitats at an elevation between 2,000 and 8,000 ft. that receives an annual mean rainfall between 2,500 and 6,000 mm (Naibauer unpublished results). *Dubautia scabra* grows well where water collects in the porous volcanic rock, which is important for successful seed establishment (Carr 1985). Once established, plant populations can live for upward of 200 years. Substrates older than 200 years have not been adequately surveyed, and it is not known how long individuals within these populations can live. Older lava flows generally have larger individuals compared to new lava flows (personal observation). It would make sense that those existing individuals are original colonizing members of the lava flow. Additional research should determine if the same colonizing plants continue to grow throughout the lifespan of the population for those 200+ years.



Figure 4. *Dubautia scabra*

The overall life history of *D. scabra* is widely understudied. Little is known about the exact mechanisms of colonization to new habitat, seed recruitment and establishment, pollination, or reproduction. As far as research has been able to tell, seeds are dispersed by wind. Like other asters, a small achene of about 3-4 mm long is attached to tall pappus which is extended into the air. Once a seed is airborne or moved, dispersal must occur to areas with the appropriate ecological conditions including high water availability, and porous volcanic substrate in order to establish (Robichaux 1984). It has been documented that distinct differences in the soil hydrology of recent lava substrates and nearby forests are enough to limit *D. scabra* and other *Dubautia* species from growing in certain conditions where their physiologies are not optimal (Robichaux 1984). Although *D. scabra* is found most commonly on recent volcanic substrates, it has been known to grow in forested habitats. Historically, it was thought that source populations were hidden within the forested regions of Mauna Loa. Upon further investigation, *D. scabra* individuals have sporadically been found on trails, bulldozed roads, and other disturbed areas within forests (personal observation). The method of pollination is also not well understood, but native insects (Roderick 1997), bees (*Hylaeus* spp.; (Robichaux et al. 1998), and Argentine ants (*Iridomyrmex humilis*; (Robichaux et al. 1998) have been affiliated with other members of the HSA. Unlike other members of the HSA, *D. scabra* is the only self-compatible species in the Alliance (Carr 1985; Carr and Kyhos 1986). Because of this, studies have found moderate to high amounts of inbreeding in many of the well-known populations of *D. scabra*. Little is known about how the distribution of suitable habitat impacts the interactions of multiple populations, or gene flow and dispersal. As new volcanic eruptions create suitable habitat, colonization and establishment of new populations should become possible. Lava flows could also act as

corridors between meta-populations, and the source and direction of seed dispersal can be determined.

Suitable habitat is arguably the most important factor for maintaining individuals and populations in an area, and this species is dependent on the availability of collected water in the volcanic substrates (Robichaux 1984). It is likely that once the volcanic substrate breaks down, water drains more effectively, likely contributing to a decrease in seed recruitment and the death of *D. scabra* populations. Additionally, as succession of areas begin to occur, new species will gradually move into the habitat, directly competing with *D. scabra*. Because *D. scabra* is a colonizer of recent lava flows, it has been thought of as necessary for the succession of Hawaiian Island ecosystems by breaking up volcanic substrates into soil. The breakdown of volcanic substrate into soil provides suitable habitat for a number of other species. On older flows, it is not uncommon for these other species to grow directly on top of *D. scabra* (personal observation). Because of the critical role it plays in this ecosystem, *D. scabra* provides a starting point for understanding downstream ecological processes such as introduction of new species and succession. Currently, we have limited knowledge of the overall time and spatial scale of colonization, population establishment, and the later decline of *D. scabra* populations on recent lava flows. Linking spatial and time scales will allow us to make better predictions about the mechanisms of colonization and succession on lava flows and the Hawaiian Islands.

Because succession is such a large component of other ecological systems, this information has many important applications, including: rates of speciation and extinction (Whittaker et al. 2008) and community development. Climate change is one of the largest threats that effects almost every organism on the globe. Suitable habitat for

species can be quite different, but most are shifting to respond to changing conditions. By studying disturbance and ecological succession, we can use patterns learned to predict how certain habitats and regions might react to huge and changing ecological variation. Examining ecological processes is also important related to conservation and rare species management. Once we have documented how disturbance events and species react, we could use knowledge gained from this study to guide conservation practices both on Hawaii and in other regions of the world.

Study Aims and Methods

The main purpose of this master's thesis will be:

- A1 To document patterns of primary succession, colonization, seed establishment, and gene flow of populations with the use of a variety of ecological and genetic techniques.

The processes of colonization and ecological succession are widely understood by ecologists in habitats like forests (Grashof-Bokdam and Geertsema 1998; Guariguata and Ostertag 2001) and grasslands (Collins and Adams 1983; Kahmen and Poschlod 2004), but researchers have not yet been able to successfully characterize primary succession on new habitats. The population structure of species in this area is also important, as it provides a window to study gene flow and evolutionary processes of organisms after volcanic disturbance. *Dubautia scabra* is an ideal focal organism for this work because it is a primary colonizer and initiator of ecological processes on The Big Island of Hawaii.

Ecological Investigations

Patterns and development of a general ecological time scale were obtained through observation and physical measurements following volcanic disturbance. The physical measurements plant size and population density were collected on lava flows of

various ages. As plants and lava flows age, we expected to find an increase in average plant size. Alternatively, we expected that plant density would decrease over time, as larger and more fit individuals would be able to establish and survive longer, while less fit individuals die out. With this information, it was possible to infer a general timeline from population establishment to senescence, and determine the progress of primary succession in lava habitats.

Additionally, documentation of habitat type (nearby forests and disturbed areas) and physical structure (volcanic rock type) were used to determine if there are predictive elements to *D. scabra* habitat. Geographic Information Science (GIS) is a popular tool used in various ecological projects. In the field data points are logged using GPS and can then be used to model spatial and habitat variables. For this project, volcanic rock type, elevation, soil hydrology, annual rainfall, slope, aspect, and age of the lava flow were all calculated in ArcMap (ESRI 2010). Chapter II describes these ecological patterns observed on various lava flows of different ages.

Genetic Diversity and Population Structure

Chapter III describes the spatial components of colonization and gene flow observed through genetic investigation across large areas and populations. This study will use nuclear microsatellites to describe relationships within and among populations. Populations structure should reflect time since colonization, gene flow between populations, and allow us to distinguish between source and sink populations. This genetic investigation should also be able to determine the total genetic diversity between populations from both Maui and The Big Island.

Microsatellites are known, short repeating sequences in the nuclear genome that are highly variable and prone to mutation. All species have these sequences, but usually

contain unique sequences that are specific to the lineage or population from which the individual is found. These genomic regions are most useful for testing genetic relationships among and between populations, and provide information regarding immediate genetic relationships. A lot of variation within microsatellites suggests a distant relationship between individuals or populations, while little variation suggests a close genetic relationship. When there is little genetic differentiation between individuals or populations, microsatellites may not provide the best genetic resolution, however.

Summary

Chapter IV synthesizes the patterns between ecological and genetic components of colonization, seed establishment, and gene flow between populations after a large disturbance. Patterns of genetic diversity will be applied to aspects of the ecological portion of this thesis. It is still unclear if there is a correlation between relative time since disturbance and genetic variation in different regions of The Big Island. Additionally, ecological aspects of habitat preference and structure may have profound effects and many implications on the genetic structure of *D. scabra* populations. This genetic and ecological investigation of the endemic species *D. scabra* will answer questions pertaining to both ecological and genetic patterns on Maui and The Big Island. The main goals of this study were to determine (1) the source and direction of seed dispersal of *D. scabra* populations across the Big Island, Hawai'i using genetic analysis, (2) if there is a correlation between lava flow age, plant density, and genetic variation among populations and (3) the genetic structure of *D. scabra* across the Big Island and Maui-Nui Complex of the Hawaiian archipelago. This system has the potential to provide a wide variety of new information on the overall processes of ecological succession and some of the underlying components associated with it

CHAPTER II

GENERAL TIMELINE AND PATTERNS OF
COLONIZATION, ESTABLISHMENT,
AND SENESCENCE OF
DUBAUTIA SCABRA

Introduction

The Hawaiian-Emperor Island Chain is a group of islands that stretch nearly 3,600 miles in a northwest to southeast orientation in the Pacific Ocean. This large chain consists of three subsections: The Emperor Seamount chain, The Hawaiian Seamount chain, and the Hawaiian Archipelago. The islands within this island chain are age progressive (Wilson 2014), and have been forming for more than 85 million years (Regelous et al. 2003). The oldest and most northern islands in The Hawaiian-Emperor Island Chain, the Emperor Seamounts, are located close to the Aleutian Islands of Alaska. Centered in the island chain is a sharp bend in the direction of islands. The islands after this bend are referred to as the Hawaiian Seamount Chain, and are mainly small atolls and extinct islands. The Hawaiian Archipelago is the youngest portion of the Hawaiian-Emperor Island Chain, and consists of the five main islands. The oldest of these islands, Kauai, is estimated to be about five million years old. Oahu is the next island in the chain, and is estimated to be about three million years old. Maui- Nui is an island complex consisting of Lana'i, Maui, and Molokai which ranges in ages from 1.3 to 1.8 million years old. The Big Island is the largest and youngest island in the archipelago, and is located roughly 2,000 miles southwest of the continental United States. This island is

less than 500,000 years old (Witter and Carr 1988; Fleischer et al. 1998) and is still actively growing.

The volcanic hotspot that created this archipelago is currently positioned just southeast of the Big Island. Because the is Island is the closest island to the hotspot, numerous active and a few inactive volcanoes exist on the island. The Big Island has five remaining active volcanoes: Hualalai, Mauna Kea, Mauna Loa, Kilauea, and Lo'ihi (USGS 2017). Haulalai is positioned on the northwest portion of the island, near Kona. Mauna Kea is the highest volcano on the island (13,803 ft.), and its flanks cover most of the northern half of the island (GNIS 2003). Mauna Loa is the largest volcano on earth, extending from the ocean floor the summit is nearly 56,000 ft. (USGS 2017), with an elevation of 13,679 ft. above sea level. Mauna Loa is also highly active, producing large lava flows around the center of the island. Kilauea is the youngest volcano on The Big Island and is most likely a satellite of the neighboring Mauna Loa (USGS 2017). Kilauea is positioned on the southeast portion of the island and has continuously been erupting since 1983 (USGS 2017). Lo'ihi is positioned 22 miles off the southeast coast of The Big Island, and the only known submarine volcano in the region (Malahoff et al. 2006).

Each volcano is responsible for varying degrees of vegetation damage after eruption, often eliminating whole floras. The mechanisms of ecological succession vary according to the scale and intensity of a volcanic eruption. Ecological succession is also dependent on landscape heterogeneity (how areas of a landscape differ), substrate type, and the availability of colonizers (Clarkson 1990; Tsuyuzaki 2009). Landscape heterogeneity has been known to drive forest ecosystems function, species abundance, and species assemblages on Mexican lava flows (Block and Meave 2017). The assemblage of species found in various ecosystems is also dependent on the number and

type of initial colonizers. Initial colonizers are generally species that were abundant before disturbance, are located in nearby undisturbed habitat, or have seeds that persist in the seed bank. Although seeds do not persist in the seed bank after volcanic disturbance, many species follow disturbance events and readily colonize after disturbance occurs. Lava flows tend to create patchy habitats where colonization from adjacent, undisturbed sites is possible.

Patterns of Colonization

Each volcano on the Hawaiian islands has had different levels of activity, producing variable rates of disturbance, habitat formation, and ecosystem types (Block and Meave 2017). Volcanoes with little or less recent activity have older ecosystems comprised of forests, while volcanoes with more frequent activity are more likely to consist of undeveloped soils unsuitable for plant growth (del Moral and Grishin 1999). Ecological variation is also driven by environmental factors including: elevation, annual rainfall, and time since disturbance. Early ecological studies (Fosberg 1961) documented over 30 ecosystem types present on all the major islands ranging from barren lava flows, bogs, dry shrub communities, to wet forests. On The Big Island, the elevation ranges from sea level to 13,803 ft. on the top of Mauna Kea (GNIS 2003), creating a habitat gradient of nearly 14,000 ft. Mean annual rainfall also plays an important role on the distribution of forests and dry-shrub communities, and the pace of ecological succession. The Trade-winds reach the main Hawaiian islands from the southeast (Timm et al. 2013), depositing more than 700 cm of water on the southeastern side of the island each year (ESRI, 2010; Naibauer unpublished results). This rapid deposition of water on the eastern sides of the island provides enough water for lush rainforests to form, while the northern regions are left dry due to a rain shadow effect (Timm et al. 2013). The frequency and

time and since volcanic disturbance also plays a significant role in the habitat formation of the islands.

Ecological succession is a process of change in species structure, habitat structure, and ecological communities over time. Ecological succession can be further broken down into two types: primary and secondary. Primary succession is the development of plant and animal communities in an area where no soils have existed before, like on volcanic substrates after recent eruption. Secondary succession is the re-establishment of a community where soils and portions of a community have been retained. Succession as a whole process can take thousands of years, with numerous major disturbance events and ecological shifts over that time. The characteristics and patterns associated with ecological succession have been widely understudied. Little is known about the general time line of landscape succession, nor the patterns of species colonization and community development.

Much of the research on the Hawaiian Islands described organisms moving between islands in an age-progressive manner (Rundell et al. 2004; Rubinoff 2008; Garb and Gillespie 2009; Bess et al. 2014). Today, we know this pattern as the Progression Rule (Funk and Wagner 1995), which states that dispersal and colonization in an island chain proceed from the oldest to the youngest island. As an island emerges from the ocean, it is available for colonization from the nearest land mass, like stepping stones across the archipelago. The most recent island formations have been the main Hawaiian Islands; Kauai, Oahu, Maui-Nui, and The Big Island. These islands have been colonized by various organisms from oldest to youngest island for the past five million years (Baldwin and Sanderson 1998).

Metapopulations

Within the Hawaiian archipelago volcanic activity is most prevalent on The Big Island. The Big Island has five active volcanoes that have left a network of exposed and hardened lava flows over the surface of the island (USGS 2017). Volcanic eruptions cause major changes in geological features and have largely shaped the current distribution and evolutionary paths for many organisms on the The Big Island. Lava flows may prove to be large physical barriers for some species by restricting individuals or populations to small patches of suitable and undestroyed forest habitat (kipuka's). Over time, these restrictions could lead to genetic bottlenecks, mutations, and eventually speciation. Disturbance followers, however, have the ability to use lava flow as “highways” to colonize new areas of the island. Metapopulations are multiple subpopulations within a species that are separated by expanses of unsuitable habitat. Population numbers are maintained by constant immigration and emigration from one subpopulation to another. Subpopulations where birth rates exceed death rates disperse surplus individuals to other patches of suitable habitat. These subpopulations are known as source populations. Conversely, subpopulations that have higher death rates than birth rates are known as sink populations. These populations are usually maintained by immigrants coming from a source population.

The Species of Interest – *Dubautia scabra*

Recently formed lava flows are habitat that require species dispersal and colonization in order to initiate successional processes that develop soil and the conditions necessary for complex community development (Jumpponen et al. 1999). *Dubautia scabra* is the first angiosperm colonizer of recent lava flows, and is characterized by its small sub-shrub appearance, whorled leaves, and unusual small,

white flowers. *Dubautia scabra* has been documented as appearing on lava flows in as little as two years after lava cools (Smather and Mueller-Dombois 1972), but populations are mostly established within ten years (Smather and Mueller-Dombois 1972; Carr 1985; Friar et al. 2008). This species is found in recent lava habitats at elevations between 2,000 and 8,000 ft. that receives an annual mean rainfall between 2,500 and 6,000 mm (Naibauer unpublished results). *Dubautia scabra* grows well where water collects in the porous volcanic rock, which is important for successful seed establishment (Carr 1985). Once established, populations can live for upward of 200 years while the volcanic substrate remains intact and continues to hold water.

The overall life history of *D. scabra* is widely understudied. Little is known about the exact mechanisms of colonization to new habitat, seed recruitment and establishment, pollination, or reproduction. As far as research has been able to tell, seeds are dispersed by wind. Like other asters, a small achene of about 3-4 mm long is attached to tall pappus which is extended into the air. Once a seed is airborne or moved, dispersal must occur to areas with the appropriate ecological conditions, including high water availability and porous volcanic substrate in order to establish (Robichaux 1984). It has been documented that distinct differences in the soil hydrology of recent lava substrates and nearby forests are enough to limit *D. scabra* and other *Dubautia* species from growing in certain conditions where their physiologies are not optimal (Robichaux 1984). Although *D. scabra* is most commonly found on recent volcanic substrates, it has been known to grow in forested habitats. Historically, it was thought that source populations were hidden within the forested regions of Mauna Loa. Upon further investigation, *D. scabra* individuals have sporadically been found on trails, bulldozed roads, and other disturbed areas within forests (personal observation). The method of pollination is also not well

understood, but native insects (Roderick 1997), bees (*Hylaeus* spp. (Robichaux et al. 1998), and Argentine ants (*Iridomyrmex humilis*; (Robichaux et al. 1998) have been affiliated with other members of the HSA. Unlike other members of the HSA, *D. scabra* is the only self-compatible species in the Alliance (Carr 1985; Carr and Kyhos 1986), which suggests a single plant could establish a new population. Because of this, studies have found moderate to high amounts of inbreeding in many populations of *D. scabra* (Witter and Carr 1988). Little is known about how the distribution of suitable habitat impacts gene flow and dispersal across multiple populations. As new volcanic eruptions create suitable habitat, colonization and establishment of new populations should become possible. Lava flows could also act as corridors throughout a metapopulation, and the source and direction of seed dispersal can be determined.

Suitable habitat is arguably the most important factor for maintaining individuals and populations in an area, and this species is dependent on the availability of collected water in the volcanic substrates (Robichaux 1984). It is likely that once the volcanic substrate breaks down, water drains more effectively, contributing to a decrease in seed recruitment and the death of *D. scabra* populations within a 200-year period. Additionally, as succession of areas begin to occur, new species will gradually move into the habitat, directly competing with *D. scabra*. Because *D. scabra* is a colonizer of recent lava flows, it has been thought of as necessary for the succession of Hawaiian Island ecosystems by breaking up volcanic substrates into soil. The breakdown of volcanic substrate provides suitable habitat for a number of other species. On older flows, it is not uncommon for these other species to grow directly on top of *D. scabra* (personal observation). Because of the critical role it plays in this ecosystem, *D. scabra* provides a starting point for understanding downstream ecological processes such as introduction of

new species and succession. Currently, we have limited knowledge of the overall time and spatial scale of colonization, population establishment, and the later decline of *D. scabra* populations on recent lava flows. Linking spatial and time scales will allow us to make better predictions about the mechanisms of colonization and succession on lava flows and the Hawaiian Islands.

The initial and secondary stages of primary succession have been widely studied in areas of glacial retreat around the northern hemisphere, including: Norway (Whittaker 1993), coastal Alaska (Boggs et al. 2010), and northwestern Washington state (Jones and del Moral 2009; del Moral et al. 2012). One general patterns of primary succession exists, and includes colonization, population establishment, population decline, and overall increase in species abundance and diversity over time. In other systems, primary succession is generally initiated by a small number of species that have the ability to colonize undesirable habitat. After colonization, these species are able to establish and transform the landscape in some way so that other species might utilize the landscape in later successional stages (ie. Breakup of substrate into soil, protecting seedlings from erosion, creating microsites, etc.). In later stages, overall species diversity and number increase as the initial colonizing species are outcompeted and replaced. In Norway, pioneer grass and wind dispersed species are more likely to be colonizers before shrubs and larger tree species (Whittaker 1993). Population establishment is less predictable, but is mostly effected by stochastic events like favorable environmental conditions and the availability of microsites (del Moral et al. 2012). Population decline and species replacement is dependent on the longevity of each species and favorable environmental conditions.

The purpose of this chapter is to document patterns of primary succession and colonization on The Big Island with the use of a variety of ecological measurements. The processes of colonization and ecological succession are widely understood by ecologists in habitats like forests (Grashof-Bokdam and Geertsema 1998; Guariguata and Ostertag 2001) and grasslands (Collins and Adams 1983; Kahmen and Poschlod 2004), but researchers have not yet been able to successfully characterize primary succession on truly new habitats. *Dubautia scabra* is an ideal focal organism for this work because it is a primary colonizer and initiator of ecological processes on The Big Island of Hawaii.

Methods

Patterns and development of a general ecological time scale can be obtained through observation and physical measurements following volcanic disturbance. The physical measurements plant size and population density were collected on lava flows of various ages. Semi-random transects were placed on recent lava flows of known ages between 1852 and 1984. Each transect consisted of 5, 5X 10 m arrays, and 3 or 4 of those transects were sampled on each lava flow (Figure 5A). The position of each individual located within one of the transects was recorded using GPS, and the width of the plant was measured (Figure 5B). Plant density and average plant size were then calculated based off plant location and from measurements taken in the field. Plant density and size should describe the initial rate and time since colonization, with size indicating the relative age of the plant. As plants and lava flows age, we expect to find an increase in average plant size, and an overall decrease in plant density over time. As larger and more fit individuals mature and survive longer, less fit individuals die out. With this information, we can infer a general timeline from population establishment to senescence, and determine the progress of primary succession in lava habitats.

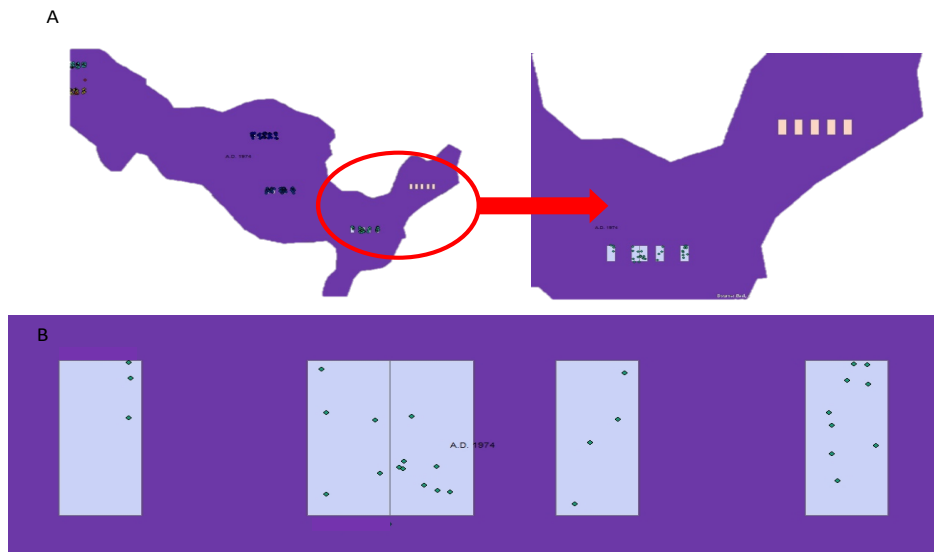


Figure 5. A) Random 5m X 10m plots placed on a lava flow (purple) from 1974. B) Data on size and location were collected for plants located within a plot.

Ecological variables (elevation, mean annual rainfall, soil hydrology, slope, aspect, and lava flow age) and physical structure (volcanic rock type) were documented to determine if there are predictive elements to *D. scabra* habitat. Data pertaining to ecological factors were downloaded from The Hawaii State GIS Project (State of Hawaii, Office of Planning). Volcanic rock type, elevation, soil hydrology, annual rainfall, slope, aspect, and age of the lava flow were all calculated in ArcMap (ESRI 2010). The values associated with each attribute were exported into an Excel file and matched to a corresponding individual found within each transect.

Statistical Analyses

A simple timeline for colonization and landscape development was estimated using linear regression to compare plant density and size to lava flow age. The size of each individual sampled was plotted against the age of the lava flow where it was found to determine which lava flows have the largest individuals. Plant density per meter

squared (m^2) was calculated by dividing the number of individuals found by the number of square meters sampled within that transect. Density per m^2 was then plotted against the lava flow age the transect was located in. This would determine if there is a higher density of plants on recent or older lava flows.

A stepwise regression model in Statistical Analysis Software (SAS) v. 9.4 was used to determine which ecological variables are the best at predicting plant size and density through time. A stepwise regression model is a process that uses predictive variables to determine relationships in a regression model. This process adds variables into the model one at a time to determine which variable's inclusion gives the most statistically significant improvement to the model. This process is repeated until no variable improves the significance of the model. Low p-values suggest a variable is a meaningful addition to the model, while a high p-value indicates the variable provides no prediction and it is not associated with any changes in the model.

Results

The average plant size was calculated and compared to lava flow age in order to determine if there were any relationships between habitat formation and individual plant growth. Data from this study suggests there is a negative correlation ($R^2 = 0.4439$) between plant size and lava flow age, with the largest individuals growing on lava flows of roughly 100 years old (Figure 6). Plant density per m^2 was also calculated in order to infer a general timeline for population establishment and senescence. Plant density is positively correlated ($R^2 = 0.7692$) with lava flow age. Lava flows between 40 and 45 years old have the highest density of plants per m^2 (Figure 7).

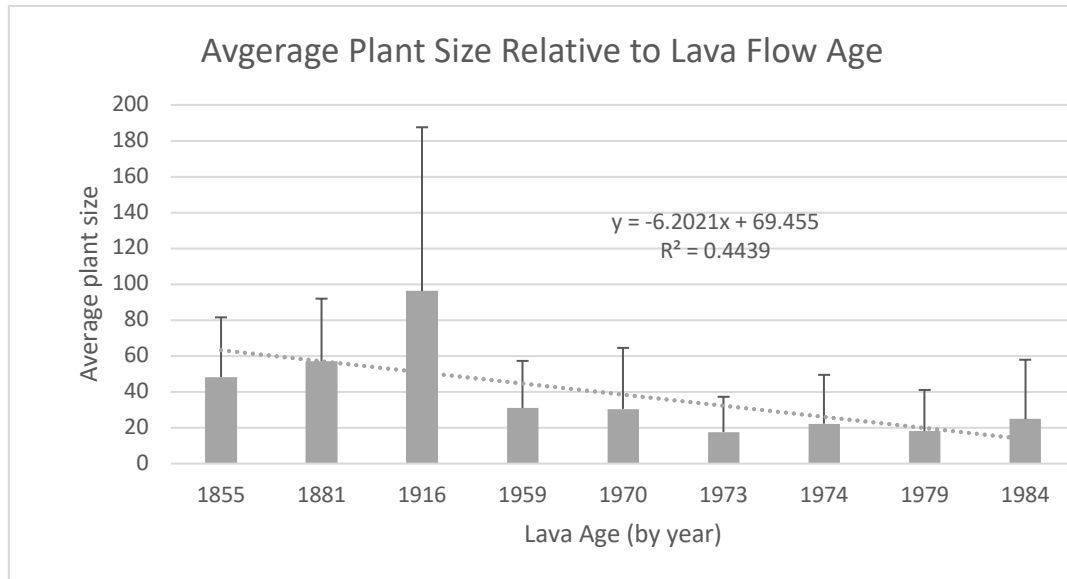


Figure 6. Average plant size (cm) and standard deviation on various lava flow ages. The largest plants are found on lava flows of about 100 years old. $R^2 = 0.4439$.

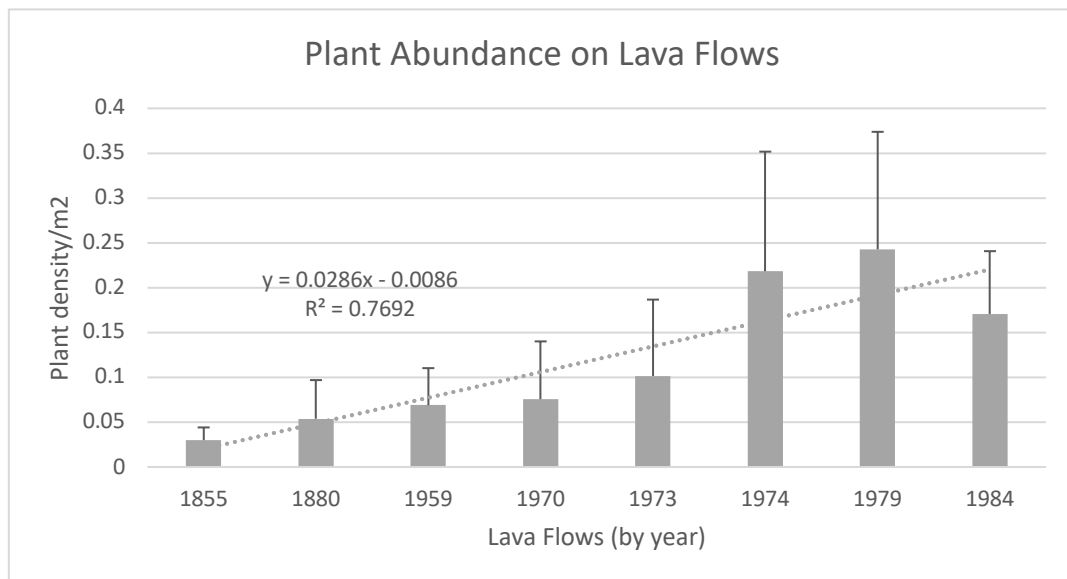


Figure 7. Plant density (per m²) and standard deviation on various lava flow ages. The highest density of plants is found on lava flows of ages between 40 and 45 years old. $R^2 = 0.7692$.

A forward stepwise regression analysis was used to determine which ecological variables were most significant predictors of plant size and density through ecological time. Volcanic rock type, elevation, soil hydrology, annual rainfall, slope, aspect, and age

of the lava flow were all calculated in ArcMap, and used in this analysis. Latitude and longitude for each individual sampled were also calculated, but did not prove to be significant during initial analysis and were omitted. Plant size was best predicted by soil hydrology ($p = <0.0001$), lava age ($p = 0.0152$), elevation ($p = <0.0001$), and rock type ($p = 0.0222$; Table 1). Aspect and mean annual rainfall were not significant for predicting plant size in this system. Fit diagnostics for plant size indicate a few existing outliers within the dataset, but the overall fit of the model is appropriate. Outliers were maintained to capture ecological variation from multiple volcanic regions on The Big Island. The suggested R^2 value is 0.3749 (Figure 6, which is the proportion of the variance in size that is predictable from different ecological variables. All ecological variables tested: lava age ($p = <0.0001$), elevation ($p = 0.0001$), rock type ($p = 0.0032$), soil hydrology ($p = 0.0016$), aspect ($p = 0.0013$), and mean annual rainfall ($p = 0.0129$) were significant for predicting plant density (Table 2). Fit diagnostics for plant density also indicate the presence of a few outliers, but the overall fit of the model is appropriate. These outliers were maintained to account for ecological variation from multiple regions on The Big Island. The regression model suggested an R^2 value of 0.3906 (Figure 8).

Table 1. Summary of forward selection (Size). The variables: soil hydrology, lava age, elevation, and rock type were the only variable that were statistically significant predictors for plant size.

Variable entered	Number Vars In	Partial R-Square	Model R-Square	C(p)	F Value	Pr > F
Soil hydrology	1	0.1802	0.1802	41.7940	34.50	<.0001
Lava age	2	0.0305	0.2107	36.4724	6.03	0.0152
Elevation	3	0.1349	0.3456	6.0851	31.96	<.0001
Rock type	4	0.0219	0.3675	2.8269	5.33	0.0222
Aspect	5	0.0050	0.3725	3.6237	1.22	0.2707
Mean annual rainfall	6	0.0024	0.3749	5.0510	0.58	0.4475

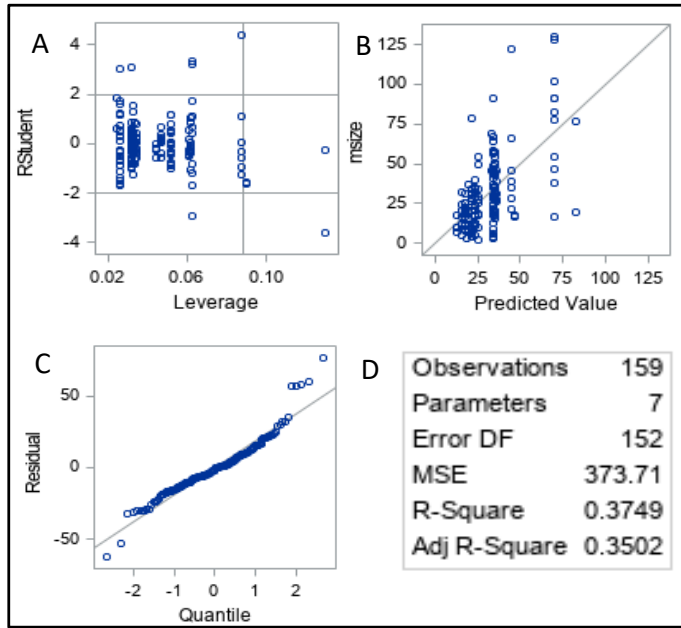


Figure 8. Fit diagnostics for size. A) Studentized residual versus leverage indicates there are a few outliers, but this model explains most of the variability within the dataset. B) Size versus predicted value represents the overall fit of the data within the model. C) Residual versus quantile suggests the spread of residuals is not greater than the spread of the centered fit and is appropriate. D) R-square = 0.3749.

Table 2. Summary of forward selection (plant density). All ecological variable examined were statistically significant for predicting plant density.

Variable entered	Number Vars In	Partial R-Square	Model R-Square	C(p)	F Value	Pr > F
Lava age	1	0.1579	0.1579	51.0319	29.45	<.0001
Elevation	2	0.0752	0.2332	34.6273	15.30	0.0001
Rock type	3	0.0419	0.2751	26.3732	8.96	0.0032
Soil hydrology	4	0.0457	0.3208	17.1916	10.36	0.0016
Aspect	5	0.0445	0.3653	8.3030	10.73	0.0013
Mean annual rainfall	6	0.0254	0.3906	4.0947	6.33	0.0129

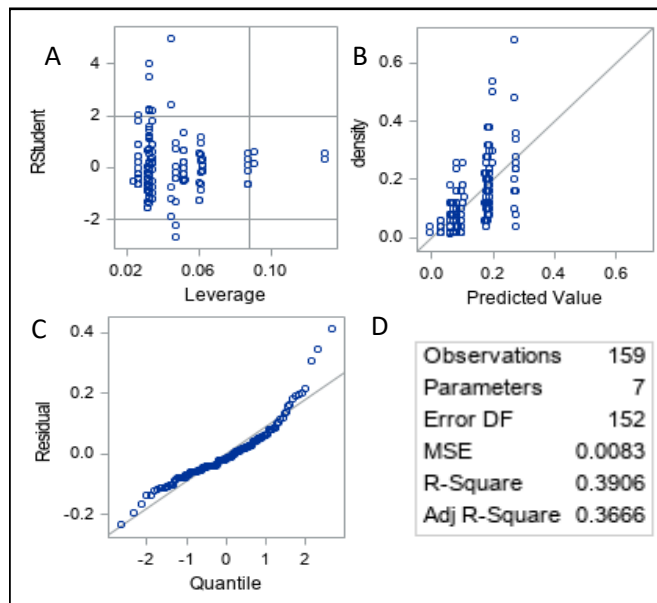


Figure 9. Fit diagnostics for density. A) Studentized residual versus leverage indicates there are a few outliers, but this model explains most of the variability within the dataset. B) Density versus predicted value represents the overall fit of the data within the model. C) Residual versus quantile suggests the spread of residuals is not greater than the spread of the centered fit and is appropriate. D) R-square = 0.3906.

Discussion

Plant size and density on recent lava flows are important factors that help researchers describe the progression of colonization, establishment, and senescence of *D. scabra* populations on The Big Island. Plant size should indicate overall plant age, while plant density in this system should describe the time since colonization, population establishment, and overall decline of plant populations. We expect a larger number of colonizing individuals on recent lava flows, and a smaller number of large established individuals on older lava flows. We found a higher density of individuals of *D. scabra* on recent lava flows ($R^2 = 0.7692$), and larger plants on older flows ($R^2 = 0.4439$). The highest density of individuals occurs on lava flows that are about 45 years old (Figure 7), while the largest individuals are found on lava flows of that are about 100 years old

(Figure 6). After about 50 years, *D. scabra* numbers begin to decline, but established individuals continue to live for roughly another 100 years until they are likely outcompeted or die. Evidence of an increase in vegetation cover could imply progress of primary succession and competition between colonizing and later seral species on lava flows. However, available GIS vegetation information (e.g. normalized difference of vegetation index – NDVI) does not have a resolution adequate enough to determine changes in vegetation cover in habitats with a small area. With the available data, it is likely that changes in soil type, hydrology, and aging lava flows facilitate population decline instead of direct competition with other species. This study also indicates that *D. scabra* may be shorter lived than previously thought, and in most cases, individuals survive for roughly 150 years.

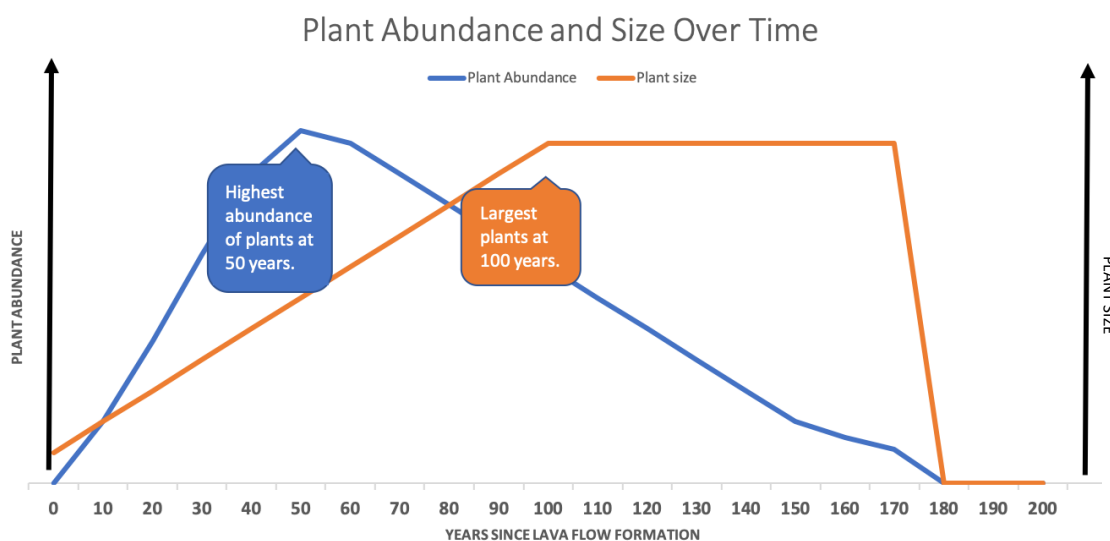


Figure 10. Generalized model of plant abundance over time since colonization. The abundance of *D. scabra* increases until about 50 years where plant density is highest. Populations begin to decline while a few individuals remain until they are outcompeted or die.

Lava age, elevation, soil hydrology, aspect, rock type, and mean annual rainfall were all significant factors for predicting plant size and density on recent lava flows

(Tables 1 and 2). Lava age seems to be one of largest predictors of both plant density and plant size, indicating patterns associated with the time since volcanic disturbance.

Elevation also is significant at predicting size and density. Current predictions place this species on recent lava habitats at elevations between 2,000 and 8,000 ft. (Naibauer unpublished results). Rock type and soil hydrology are important factors that influence water availability within volcanic substrates. Rough and chunky lava (a'a) contains large expanses of air between rocks. This type of lava drains quickly compared to the smooth pahoehoe lava that has less air gaps and holds more water. Large crevices also prevent seeds from germinating and limit successful colonization and establishment on a'a flows.

Dubautia scabra is a species that requires large quantities of water to collect in slow draining volcanic rock. The distribution of this species occurs mainly in areas that receive an annual mean rainfall between 2,500 and 6,000 mm (Naibauer unpublished results). It is a bit surprising to find that mean annual rainfall is only a significant factor in predicting plant density on certain lava flows.

Patterns of colonization and population establishment similar to the one presented here have also been seen in other regions of the world. Primary succession of glacier forelands has been studied in various northern regions, including: Norway (Whittaker 1993), coastal Alaska (Boggs et al. 2010), and northwestern Washington state (Jones and del Moral 2009; del Moral et al. 2012). In most studies, colonization is initiated by a few species. After colonization, overall plant diversity and number increases. Eventually, the initial colonizing species are outcompeted, and later successional stage species become more abundant. For example, after de-glaciation events in Norway, clumps of the initial colonizer, *Saxifraga caespitosa*, were more frequent during the first 30 years of succession (Whittaker 1993). After 30 years, the overall frequency of *Saxifraga*

caespitosa decreased, and larger and more vigorous shrub and tree specimens were most frequent. Alaskan flood plains witness four main vegetation shifts (determined by species dominance) within 100 years after disturbance (Walker et al. 1986). Sand bars were initially established by aspens and then willows, then by alder and finally spruce species as the original colonizing species met individual longevity and greater competition. Following glacial retreat in Glacial Bay National Park and Preserve, Alaska, it took between 137-246 years for alpine forbs and grasses to be dominated by larger shrubs and late-seral trees (Boggs et al. 2010). Relative to these studies, *D. scabra* acts as the initial colonizer on The Big Island to break up substrates and provide habitat for species in later-seral stages. Overtime, *D. scabra* will be outcompeted, and larger shrubs and trees will encroach on newly developed habitat.

Species cycling and landscape succession is an important part of the development on the Hawaiian Islands. After volcanic eruption, barren lava flows are colonized, broken up, and then utilized by numerous species. As volcanoes continue to erupt, new habitats and islands emerge and the process starts again. Each species has an important role in the development and changes within its community. *Dubautia scabra* is one of the first colonizers of barren lava flows, and is one of the only species that can initiate the succession of lava flows. *Dubautia scabra* works by breaking up lava flows into soil for about 150 years before it is outcompeted. Within this time, *D. scabra* also provides habitat and microsites for other colonizing species. As other lava flows become available, *D. scabra* will colonize that new region of the island, and start the long process of primary succession again

CHAPTER III

SPATIAL COMPONENTS OF COLONIZATION AND GENE FLOW IN METAPOPOPULATIONS

Introduction

The Hawaiian Silversword Alliance (HSA) is a small group of plants endemic to the Hawaiian Islands. This group is mostly known for its ability to readily adapt to changing physiological and ecological factors. Adaptive Radiation as the result of this ability has led to an array of morphologically and ecologically distinct species (Friar et al. 2006). Members of the HSA range in morphology from lianas and small cushion plants, to shrubs and even trees (Carr 1985). The HSA is divided into three genera:

Argyroxiphium DC., *Dubautia* (DC.) D.D. Keck, and *Wilkesia* A. Gray, and is composed of roughly 30 species that are recognized today (Carr 1985). The genus *Dubautia* is the largest and most diverse genus in the Alliance. *Dubautia* species are located on all islands, with individual species generally being endemic to one or two of the major islands. These species also have the most variation in morphology and physiology within the HSA. Suitable habitats for *Dubautia* species can range from barren lava flows to wet rainforests or alpine mountain slopes. Plant physiologies in various regions of the islands must be able to tolerate vast differences in water availability, temperature gradients, and seasonal changes (Robichaux 1984).

speciation and interesting patterns within the genetic diversity of the HSA and *Dubautia* (Friar et al. 2006). Generally, after a few successful colonization events, the overall genetic diversity within a population is limited compared to the source population

(Nei et al. 1975). Over time, population growth (Ilves et al. 2015), accumulation of novel mutations (Gompert et al. 2014), and hybridization (Friar et al. 2008) provide the potential for unique evolutionary trajectories (Green et al. 2012). Although the colonizing ancestor of the HSA landed in Hawaii about 5 million years ago (Baldwin and Sanderson 1998), overall genetic variation within the group remains low (Witter and Carr 1988; Baldwin et al. 1991), suggesting there are other ecological factors that also play a large role in species diversification.

Due to the lack of genetic divergence within the HSA, hybridization and ecological differentiation have been the leading drivers of speciation (Friar et al. 2006; Friar et al. 2008; Garcia-Verdugo et al. 2013). About 75% of all possible artificial or natural crosses have been made within the HSA independent of genus or species (Carr 1985). The species within *Dubautia* tend to maintain higher levels of genetic affinity. Limited divergence within this genus has led to numerous hybridization events where habitats overlap, and numerous hybrid species are formally recognized (Carr and Kyhos 1981; Carr 1985). Ecological differences on various islands are arguably one of the most important factors in the adaptive radiation of *Dubautia*, playing a huge role in what we know about speciation within this group. Inter-island dispersal and exploitation of new ecological niches usually results in a burst of speciation. Within the genus, speciation has occurred so quickly that there hasn't been enough time for much genetic divergence to accumulate (Witter and Carr 1988; Friar et al. 2006).

Dubautia scabra (DC.) D.D. Keck is endemic to Maui-Nui and The Big Island, and has two subspecies: *D. scabra* ssp. *scabra* and *D. scabra* ssp. *leiophylla* (A. Gray) G.D. Carr which are believed to be less than one million years old (Baldwin and Sanderson 1998). *Dubautia scabra* ssp. *scabra* is a small shrub that grows in volcanic

and rocky substrates, while *D. scabra* ssp. *leiophylla* has longer and more vine-like branches and is found in forested habitats. The distinctions between species are not well resolved, however. Vague taxonomic and ecological descriptions, and a wide array of phenotypic plasticity have made identification in the field difficult.

Dubautia scabra ssp. *scabra* (hereafter *D. scabra*) is the first angiosperm colonizer of recent lava flows, and is characterized as a small sub-shrub, with whorled leaves, and unusual (for the genus) white flowers. *Dubautia scabra* has been documented appearing on lava flows in as little as two years after lava cools (Smather and Mueller-Dombois 1972), but is mostly established within ten years (Smather and Mueller-Dombois 1972; Carr 1985; Friar et al. 2008). This species is found in habitats at an elevation between 2,000 and 8,000 ft. that receive an annual mean rainfall between 2,500 and 6,000 mm (Naibauer unpublished results). *Dubautia scabra* grows well where water collects in the porous volcanic rock, which is important for successful seed establishment (Carr 1985). Once established, plant populations can live for upward of 200 years.

Current populations of *D. scabra* on The Big Island are scattered over a patchy network of recent lava flows. In general, patchy habitats could create barriers for species dispersal into new areas of suitable habitat leading to genetic differentiation and population structure. *Dubautia scabra* is a disturbance follower, however, and colonization to recent lava flows most likely occurs through immigration in meta-populations. Little is known about how the distribution of suitable habitat impacts

or gene flow and dispersal throughout the meta-population. As new volcanic eruptions create suitable habitat, colonization and establishment of new populations should become possible. Lava flows could also act as corridors between meta-populations, and the source and direction of seed dispersal can be determined.

Chapter III describes the spatial components of colonization and gene flow observed through a genetic investigation across large areas and populations. This study uses nuclear microsatellites to describe relationships within and among populations. Population structure should reflect time since colonization, gene flow between populations, and allow us to distinguish between source and sink populations. This genetic investigation should also be able to determine the total genetic diversity within populations from both Maui and The Big Island.

Methods

Dubautia scabra is a morphologically variable species with a wide distribution on Maui and The Big Island. Plants were sampled from The Big Island from three main volcanic regions; Hawaii Volcanos National Park (HAVO), Hawaiian Ocean View Estates (HOVE), and Saddle Road (SR; Figure 11). DNA was collected from 20 populations, and DNA from three other populations that were sampled for a previous study in the early 2000's from Saddle Road, Hawaii Volcanos National Park, and Maui were included (Table 3; Friar et al., 2008). Samples from Maui were included in order to determine the overall genetic diversity within the species on Maui and The Big Island.

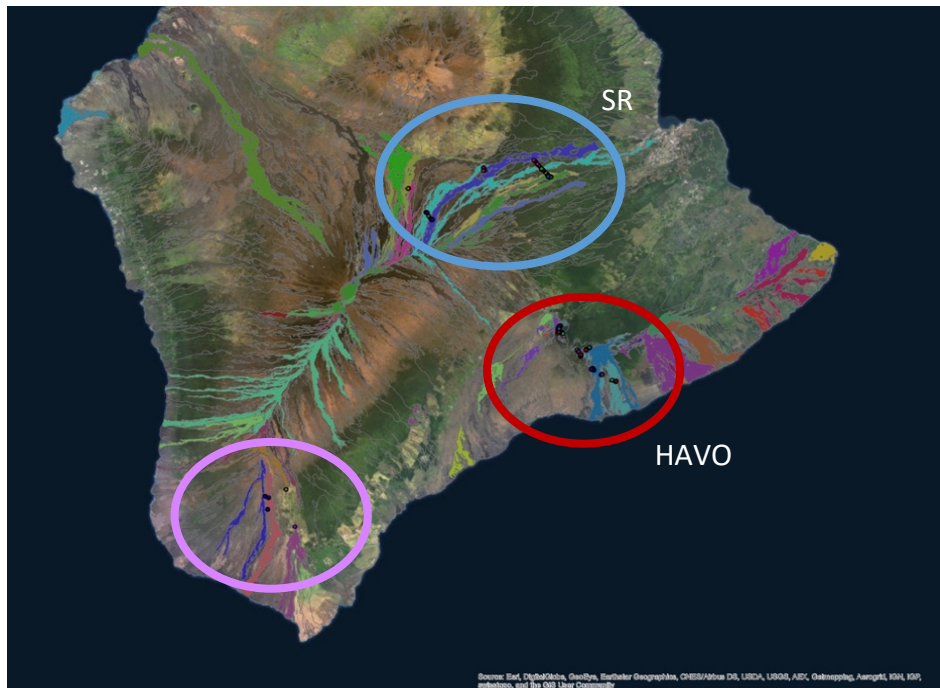


Figure 11. The main volcanic regions on The Big Island. Individuals were sampled from Hawaii Volcanos National Park (HAVO; red), Hawaiian Ocean View Estates (HOVE; purple), and Saddle Road (SR; blue).

Table 3. Individuals were sampled from Hawaii Volcanoes National Park (HAVO), Hawaiian Ocean View Estates (HOVE), and Saddle Road (SR).

Island	Region	Flow Date	Population Name	N
Hawaii	Hawaii Volcanoes National Park	1410-60	HAVO_1410-60-2	11
		1959	HAVO_1959-2	12
		1974	HAVO_1974-2	6
		1974	HAVO_1974-3	6
		1969-71	HAVO_1969-71-1	12
		1969-71	HAVO_1969-71-3	12
		1969-71	HAVO-OLD (Friar et al. 2008)	12
		1972-74	Pu'u huluhulu -3	12
		1972-74	HAVO_1972-74-5	12
		1972-74	HAVO_1972-74-7	12
	Saddle Road	Pu'u huluhulu -1 (Friar et al. 2008)		12
		1855	SR_1855-1	11
		1855	SR_1855-2	11
		1984	SR_1984-1	12
		1880-81	SR_1880-81-2	12
		1880-81	SR_1880-81-3	12
		1880-81	SR_1880-81-4	12
		1935	SR_1935-1	12
		TPR 1852	TPR-1	12
		TPR	TPR-2	12
Maui	Hawaii Ocean View Estates	1916	Kahuku	3
		1887	HOVE_1887	12
	Olinda	Maui (Friar et al. 2008)		12

Deoxyribonucleic Acid (DNA) Extraction

Leaf tissue was collected from the field, frozen, and shipped to the University of Northern Colorado where it was stored at -20°C until the DNA could be extracted. DNA was extracted from 283 individuals using a modified CTAB (Cetyltrimethylammonium Bromide) extraction protocol (Doyle and Doyle 1987). This protocol was modified specifically for members of the HSA due to a high quantity of secondary compounds and phenols present in the leaf tissue (Friar 2005).

Microsatellite Marker Design

Isolation of new microsatellite loci was performed following the subtractive hybridization method of Hamilton et al. (1999) with some modifications using multiple *Dubautia* species; *D. scabra*, *D. ciliolata*, and *D. laxa*. Digested DNA was enriched for eight oligonucleotide repeats (AC)₁₅, (AT)₁₅, (CG)₁₅, (CCG)₁₀, (AAC)₁₀, and (CAC)₁₀. Fragments were cloned using pBluescript II SK-Phagemid vector and the XL1-Blue MRF⁺ bacterial host strain (Agilent Technologies). Color-positive clones were screened for microsatellite regions using a membrane based “dot blot” method (Glenn and Schable 2005) and the Phototope chemiluminescent detection system (New England Biolabs). Positive clones were screened for insert size by PCR using a Master Cycler ProS (Eppendorf). The 20µl reactions contained 1µl template DNA, 0.80µM each of primers T3 and T7 (Integrated DNA Technologies), 1x Thermopol Reaction Buffer (New England Biolabs), 200µM of each dNTP, and 0.20 units of GoTaq Flexi DNA Polymerase (Promega). Clones that exhibited a single amplified band of 400-1000 bp were cleaned using enzymatic cleanup procedure outlined by Fermentas Molecular Biology (Werle et al. 1994) and sequenced using the T3 primer and BigDye Terminator version 3.1 Cycle Sequencing Kit (Applied Biosystems) in 1/16 volume reactions. Sequences were electrophoresed on a 3730 Genetic Analyzer (Applied Biosystems). For inserts containing a di- or tri-nucleotide microsatellite motif, the T7 primer was used to generate a complementary reverse sequence. All sequences were aligned using GENIOUS 8.1.8 (Biomatters Ltd.).

The fragments were analyzed for microsatellite containing three or more repeating units. Of the 20 sequenced fragments, nine were suitable for primer design. Primers were designed using PRIMER 3 (Rozen and Skaletsky 2000). One primer of each pair was

designed with a common tag at the 5' end following the procedure of (Boutin-Ganache et al. 2001). Three common tags were used: M13R (AGGAAACAGCTATGACCAT), T7 (GCTAGTTATTGCTCAGCGG), and CAGT (ACAGTCGGGCGTCATCA). Three of the 9 primers amplified variable loci consistently. Loci were paired and amplified with a common tag containing one of three fluorescent dyes; 6-FAM, PET, or VIC (Applied Biosystems).

Primer Optimization and Polymerase Chain Reaction (PCR)

The nine microsatellite loci designed for this study and an additional eight loci designed for a previous study (Friar et al. 2008) were amplified and optimized in 12 μ l reactions using the Fluorescent Tag Microsatellite PCR Protocol (Glenn and Schable 2005). The primer pairs were optimized for annealing temperature and magnesium concentrations (MgCl₂ or MgSO₄). The reaction mix included 1 μ l genomic DNA, 0.60 μ l non-tagged primer (5 μ M), 0.60 μ l tagged primer (0.50 μ M), 0.70 dNTP mixture (at 2.5 mM), 0.06 μ l Taq Polymerase (Promega, Madison, WI, USA), 2.4 μ l GoTaq Flexi Buffer (Promega, Madison, WI, USA), 0.60 μ l fluorescent tag (5 μ M; M13 tag with 6-FAM, PET, or VIC dye). The remaining 6.04 μ l included various amounts of MgCl₂ or MgSO₄ and diH₂O. Optimized amplification conditions are shown in.

PCR was completed on an Eppendorf Mastercycler ProS (Hamberg, Germany). An initial five-minute denaturing step was followed by 35 amplification cycles. Amplification cycles consist of a 1-minute denaturing step at 95°C, 1 minute of annealing at the primer specific temperatures, and 1 minute of an extension period at 72°C. Once the 35 cycles were complete, the product was held at 4°C. Amplification products were visualized on a 1% agarose gel. The fluorescently labeled PCR products were

multiplexed and analyzed on a 3730 Genetic Analyzer at Arizona State University. Products were loaded along with Hi-Di formamide and LIZ 500 size standard (Applied Biosystems). GENIOUS 8.1.8 was used to score variable fragment sizes. Of the nine microsatellite loci designed for this study, four were variable. Three microsatellite loci from the previous study were variable, resulting in seven variable loci for this research project (Table 4).

Table 4. Primers used for this study. MKMS2, AP3MS3, and 42-2 were developed from Friar et al. 2000. DULA_90BS, DUSC_116S, DUSC_140A, DUSC_140B were developed for the completion of this study.						
Primer	Label	DN A	MgCl₂	Anneal Temp.	Fragment Size (bp)	Repeat Unit
MKMS2	HEX	1ul	MgCl ₂ 3 µl	52.9	137 145	(AG) _n
AP3MS3	6- FAM	1µl	MgCl ₂ 2 µl	57.4	90,92 94	(A) _n
42-2	HEX	1µl	MgCl ₂ 3 µl	55.1	119 124	(AC) _n
DULA_90BS	VIC	1 µl	MgCl ₂ 4 µl	55.1	227, 229,231, 233,235	(TC) ₉
DUSC_116S	PET	1 µl	MgCl ₂ 2 µl	52.9	136, 139, 142, 145	(ACA) ₇
DUSC_140A	6- FAM	1 µl	MgSO 4 2.5 µl	61.7	178 186	(AGATAT) ₄
DUSC_140B	PET	1 µl	MgSO 4 1.5 µl	57.4	122 125	(TAA) ₈

GENALEX ver. 6.503 (Peakall and Smouse 2012) was used to calculate deviation from Hardy-Weinberg Equilibrium (HWE), average number of alleles (A), effective number of alleles (A_e), observed heterozygosity (H_o), expected heterozygosity (H_e), inbreeding coefficient (F_{IS}), and pairwise genetic distance (F_{ST}) between populations.

GENALEX was also used to generate a principle components analysis (PCoA). Principle component analysis is a multivariate analysis that assigns spatial structure to components of biological processes, including genetic diversity.

Population structure was analyzed using the Bayesian clustering analysis software program STRUCTURE ver. 2.3.4 (Pritchard et al. 2000). Burn-in and run lengths of 50,000 replicates were used for each STRUCTURE analysis. The number of inferred populations (K) was determined using STRUCTURE HARVESTER (Earl and vonHoldt 2012). STRUCTURE HARVESTER is a web-based program designed to estimate the rate of change of K as the number of groups is increased from multiple iterations using the Evanno method (Evanno et al. 2005). This method uses an algorithm that compares the rate of change of log-likelihood values between successive K values over consecutive iterations. This allows a K value to be assigned based on the greatest rate of change and the graphs generated indicate which number of genetic groups (K) best fit the data (Earl and vonHoldt 2012).

EDENetworks (Kivela et al. 2015) was used to create a network analysis based off the genetic distance from microsatellite data. Network analysis are used to infer patterns of gene flow among and between populations. Thresholds are generated automatically in order to determine the shortest genetic distance between the most related individuals. EDENetwork does this by calculating which individuals are the most different using a stepwise mutational model, then assigning genetic distance on a scale from 0 to the automatically generated threshold value. A higher threshold suggests there is a high value of genetic relationships and connections between the individuals within the dataset. A lower threshold removes those connections so that only the most related individuals are shown. Threshold may also be set manually by the user in order to

determine which individuals are the most related. The manual thresholds used for this analysis are 0.5.

BayesAss ver. 3.04 was used to infer gene flow between populations (Wilson and Rannala 2003). BayesAss is a Markov chain Monte Carlo (MCMC) analysis that determines the probability of migration rates, an individual's immigrant ancestry, and the generation in which immigration to a new location occurred. Proposed changes for specific parameters such as rate of migration, allele frequencies, and inbreeding coefficients can be fine-tuned in order to extract the most information from the dataset. The number of iterations was set to 50,000,000, while the number of burn-ins was set to 500,000. Every 1,000 iterations were analyzed.

BOTTLENECK ver. 1.2.02 (Piry et al. 1999) was used to detect recent reductions in effective population size. An excess in heterozygosity for rare alleles was assessed by Wilcoxon's sign rank test across all loci within a population (Riley et al. 2010). Populations were considered to have undergone a genetic bottleneck if the probability of the two-phase model (TPM) was <0.05 with 1000 iterations. TPM uses a combination of an infinite allele model (IAM) and stepwise mutation model (SMM) to test for bottlenecks. The TPM model was used following the settings recommended by the authors, where 95% single-step mutation and 5% multiple-step mutations (Piry et al. 1999).

Results

DNA extraction was 100% successful from all sampled populations. Seven microsatellite loci were variable and polymorphic among populations. Locus by population analyses revealed 27 out of 154 comparisons were outside of HWE ($p < 0.01$). Deviation from HWE is expected as *D. scabra* is self-compatible. Additional causes of

deviation from HWE include small populations sizes and overlapping generations. Thirty-six of the 154 comparisons revealed monomorphic loci, indicating that all individuals in a population are identical at one locus.

Diversity statistics are shown in Table 5. The average number of alleles for each population ranged from 1.714 - 2.571. The average number of effective alleles for all regions was 1.481. Observed heterozygosity ranged between 0.167 and 0.393, and was lower than the expected heterozygosity, which ranged from 0.161 - 0.359. The inbreeding coefficient for all populations ranged from -0.153 - 0.339. SR and HAVO show low to moderate levels of inbreeding, while HOVE and Maui do not. Moderate levels of inbreeding are not uncommon in species that are self-compatible, however.

Table 5. Genetic diversity statistics generated for 7 microsatellite regions.

Population	Bottleneck		N	A	A _e	H _o	H _e	F _{IS}
TPR-1		Mean	12	2.143	1.607	0.333	0.324	-0.065
		SE		0.261	0.183	0.081	0.079	0.090
TPR-2		Mean	12	2.429	1.534	0.250	0.315	0.319
		SE		0.369	0.129	0.103	0.067	0.229
SR_1984-1		Mean	12	2.143	1.581	0.286	0.286	0.24
		SE		0.341	0.233	0.140	0.094	0.264
SR_1880-81-4	YES	Mean	12	2.429	1.413	0.214	0.231	0.078
		SE		0.429	0.182	0.098	0.082	0.132
SR_1880-81-3		Mean	12	2.286	1.655	0.250	0.294	0.230
		SE		0.360	0.292	0.114	0.100	0.164
SR_1880-81-2		Mean	12	2.143	1.474	0.210	0.270	0.339
		SE		0.404	0.159	0.093	0.082	0.186
SR_1855-2		Mean	11	2.143	1.503	0.312	0.295	-0.013
		SE		0.261	0.149	0.101	0.068	0.191
SR_1855-1		Mean	11	2.143	1.448	0.208	0.234	0.028
		SE		0.404	0.215	0.068	0.088	0.072
puu_huluhulu-1		Mean	12	2.143	1.602	0.393	0.347	-0.116
		SE		0.143	0.135	0.083	0.057	0.105
SR TOTAL		Mean	11.778	2.222	1.535	0.273	0.288	0.116
		SE		0.319	0.169	0.091	0.074	0.150
HOVE_1887		Mean	12	2.286	1.495	0.274	0.264	0.011
		SE		0.286	0.204	0.099	0.083	0.117
KAHUKU		Mean	3	1.714	1.422	0.286	0.254	-0.153
		SE		0.184	0.141	0.087	0.074	0.114
HOVE TOTAL		Mean	7.5	2.000	1.4585	0.28	0.259	-0.071
		SE		0.235	0.173	0.093	0.078	0.116
MAUI		Mean	10	1.714	1.400	0.167	0.161	-0.084
		SE		0.421	0.311	0.095	0.097	0.041

Table 5. Continued

Population	Bottleneck	N	A	A_e	H_o	H_e	F_{is}
HAVO_1972-74-7	Mean	12	2.286	1.644	0.333	0.346	0.065
	SE		0.286	0.175	0.083	0.074	0.127
HAVO_1974_2+3	Mean	12	2.000	1.456	0.238	0.249	0.298
	SE		0.309	0.185	0.113	0.086	0.246
HAVO-OLD	Mean	12	2.571	1.677	0.298	0.359	0.381
	SE		0.202	0.190	0.110	0.067	0.221
HAVO_1969-71-5	Mean	12	2.286	1.601	0.321	0.333	0.167
	SE		0.286	0.161	0.102	0.073	0.174
HAVO_1969-71-3	Mean	12	2.143	1.483	0.214	0.267	0.255
	SE		0.340	0.180	0.099	0.083	0.204
HAVO_1969-71-1	Mean	12	2.571	1.526	0.226	0.316	0.286
	SE		0.202	0.128	0.096	0.058	0.200
HAVO_1959-2	Mean	12	2.143	1.491	0.167	0.239	0.202
	SE		0.404	0.238	0.077	0.097	0.116
HAVO_1410-60	Mean	11	2.00	1.456	0.299	0.260	-0.139
	SE		0.309	0.162	0.097	0.081	0.038
puu_huluhulu-3	Mean	12	1.714	1.553	0.214	0.215	-0.030
	SE		0.360	0.323	0.120	0.114	0.128
HAVO TOTAL	Mean	10.805	2.190	1.543	0.256	0.287	0.165
	SE		0.258	0.171	0.089	0.072	0.142
		N	A	A_e	H_o	H_e	F_{is}
Grand Total	Mean	11.286	2.035	1.481	0.245	0.248	0.103
	SE		0.067	0.040	0.020	0.017	0.035

Pairwise genetic differentiation (F_{ST}) for each region (Table 6) and pair of populations (Table 7) were calculated in GENALEX. Lower F_{ST} values (<0.05) indicate very little genetic differentiation between individuals or groups. An F_{ST} value of 0.05-0.15 might indicate a small amount of genetic differentiation. Any F_{ST} value between 0.16-0.30 should indicate populations that have moderate to high amounts of genetic differentiation. Some research has suggested that an F_{ST} value > 0.15 supports taxonomic separation of species (Long and Kittles 2009). For populations and regions, low F_{ST}

values (<0.05) are highlighted as green, moderate F_{ST} values have been highlighted as yellow, and High F_{ST} values (>0.14) have been colored red. SR, HOVE, and HAVO are the most genetically similar. Populations on Maui are the most different. Individuals from SR and HAVO generally have lower F_{ST} values indicating less genetic differentiation. Individuals from Maui and Kahuku (HOVE) have moderate to high F_{ST} values.

Table 6. Pairwise F_{ST} values for regions with low values (<0.05) are highlighted in green and high values highlighted in red.

SR	HOVE	HAVO	
0.007			HOVE
0.003	0.013		HAVO
0.161	0.143	0.172	MAUI

Table 7. Pairwise genetic differentiation (Fst) for each pair of populations with all values are color coded: green (<0.05), yellow/orange (0.05-0.30), and red (>0.30).

TPR-1	TPR-2	SR_1984-1	SR_1880-81-4	SR_1855-2	SR_1880-81-3	SR_1880-81-2	SR_1855-1	puu_huluhulu-1	KAHUKU	HOVE_1887	puu_huluhulu-3	HAVO_OLD	HAVO_1974_2+3	HAVO_1972-74-5	HAVO_1969-71-5	HAVO_1969-71-3	HAVO_1969-71-1	HAVO_1959-2	HAVO_1410-60	MAUI
0.053																				
0.061	0.032																			
0.078	0.042	0.025																		
0.039	0.052	0.026	0.037																	
0.067	0.046	0.010	0.045	0.031																
0.087	0.031	0.033	0.021	0.053	0.060															
0.079	0.039	0.023	0.021	0.045	0.038	0.028														
0.021	0.040	0.052	0.063	0.039	0.059	0.067	0.063													
0.148	0.056	0.095	0.097	0.149	0.120	0.073	0.089	0.125												
0.056	0.032	0.007	0.021	0.027	0.015	0.035	0.018	0.047	0.086											
0.102	0.081	0.034	0.053	0.059	0.028	0.082	0.038	0.087	0.166	0.030										
0.030	0.047	0.043	0.043	0.030	0.063	0.049	0.064	0.039	0.105	0.045	0.100									
0.070	0.049	0.025	0.032	0.041	0.041	0.039	0.021	0.060	0.104	0.020	0.041	0.060								
0.051	0.042	0.022	0.033	0.035	0.028	0.039	0.040	0.043	0.099	0.020	0.048	0.030	0.036							
0.025	0.020	0.023	0.038	0.030	0.035	0.040	0.037	0.029	0.069	0.019	0.067	0.024	0.033	0.028						
0.026	0.058	0.051	0.053	0.019	0.062	0.076	0.069	0.036	0.175	0.049	0.094	0.030	0.070	0.052	0.041					
0.019	0.036	0.035	0.043	0.029	0.037	0.062	0.050	0.023	0.108	0.027	0.060	0.031	0.048	0.027	0.021	0.025				
0.077	0.052	0.024	0.036	0.038	0.032	0.044	0.017	0.064	0.111	0.018	0.026	0.072	0.007	0.039	0.038	0.070	0.050			
0.056	0.037	0.009	0.030	0.022	0.021	0.037	0.021	0.047	0.103	0.008	0.043	0.047	0.021	0.029	0.021	0.052	0.035	0.017		
0.283	0.152	0.175	0.227	0.257	0.181	0.180	0.165	0.237	0.121	0.165	0.231	0.250	0.203	0.166	0.181	0.302	0.199	0.202	0.181	

A PCoA for all individuals is shown in Figure 12. The variation represented by Coordinates 1 (22.66%) and 2 (14.95%) are shown. Coordinate 3 is not shown, but represents 13.98% of the variation. The HAVO populations are outline in red, HOVE in purple, Maui in green, and SR in blue. The circles represent 95% confidence intervals. Populations from The Big Island; HAVO, HOVE, and SR do not form distinct clusters, while the individuals from Maui form a tight cluster together that is separated from HAVO and SR.

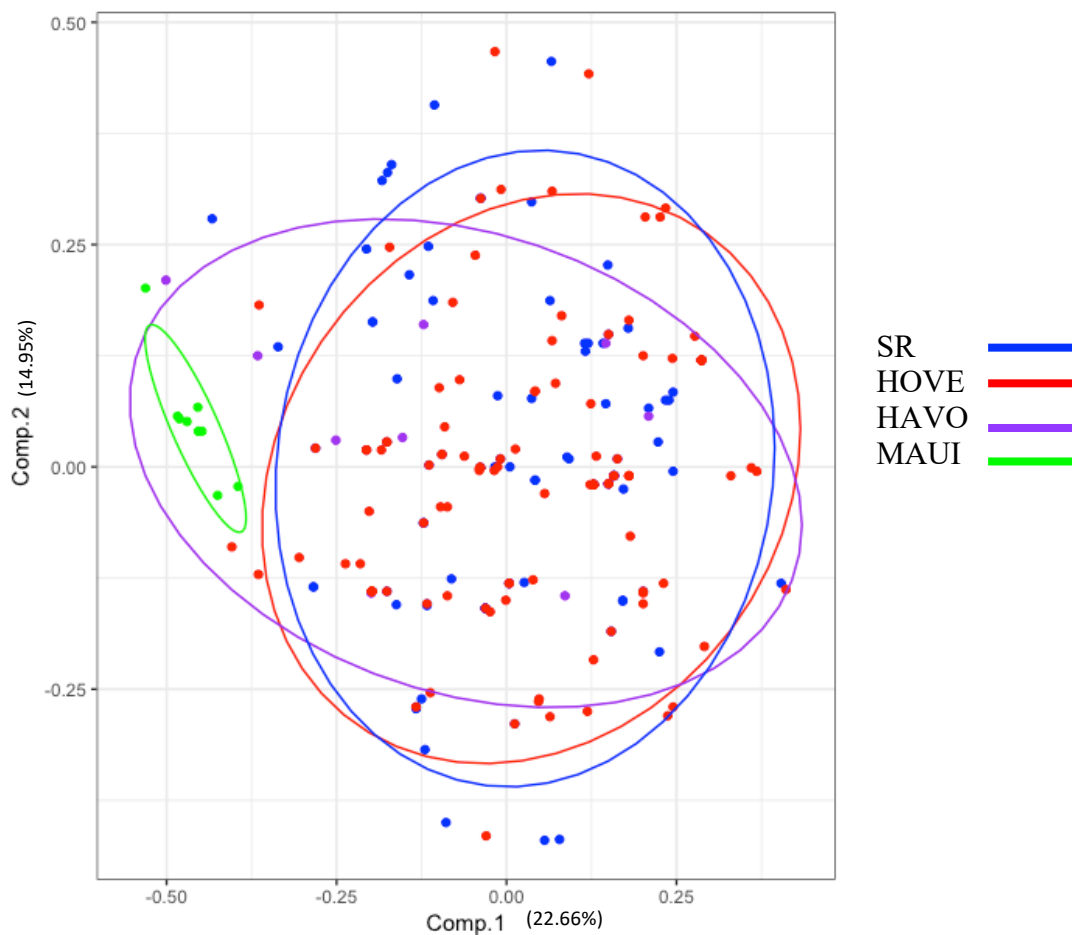


Figure 12. Principle Coordinate Analysis (PCoA) showing the spatial genetic variation between 22 populations on The Big Island and Maui. Coordinate 1 shows 22.66% of the variation, coordinate 2 shows 14.95% of the variation.

Bayesian cluster analyses using STRUCTURE were run for all individuals from the 22 sampled populations. The number of inferred populations (K) were examined from K=1 to K=22 to determine the most likely number of genetic clusters. STRUCTURE HARVESTER determined that K=2 is the most probable genetic assignment for the dataset (Figure 12). The complete STRUCTURE analysis did not split populations by geographical distribution or island (Figure 13). Most individuals were a mix of two genetic assignments, with no real patterns to differentiate between population, or region on The Big Island. Maui stands out as having most of genetic assignment to the blue group, which has a lower frequency in Big Island individuals.

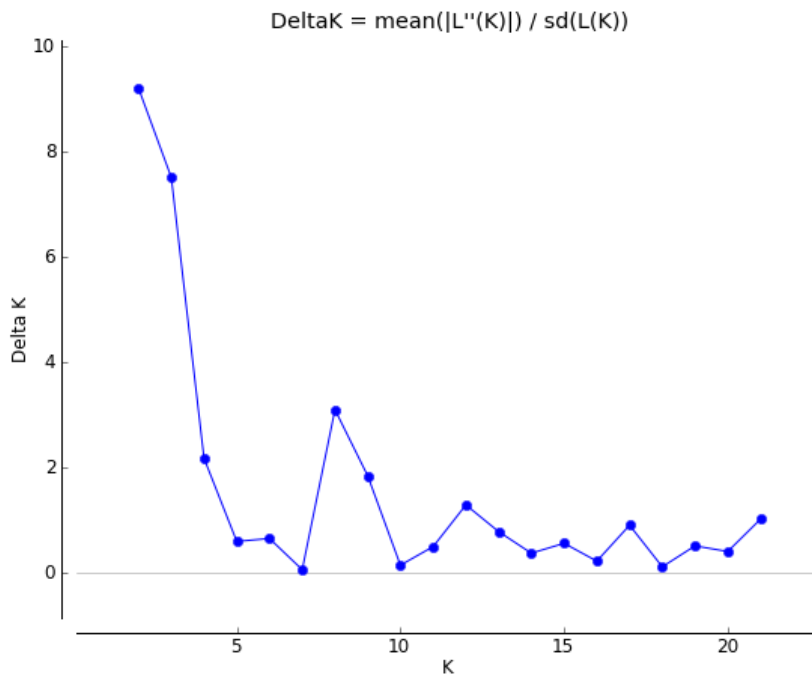


Figure 13. STRUCTURE HARVESTER data indicating the rate of change likelihood calculated using the Evanno et al. (2005) method for each K value assigned. Genetic assignment of K=2 shows the highest probability.

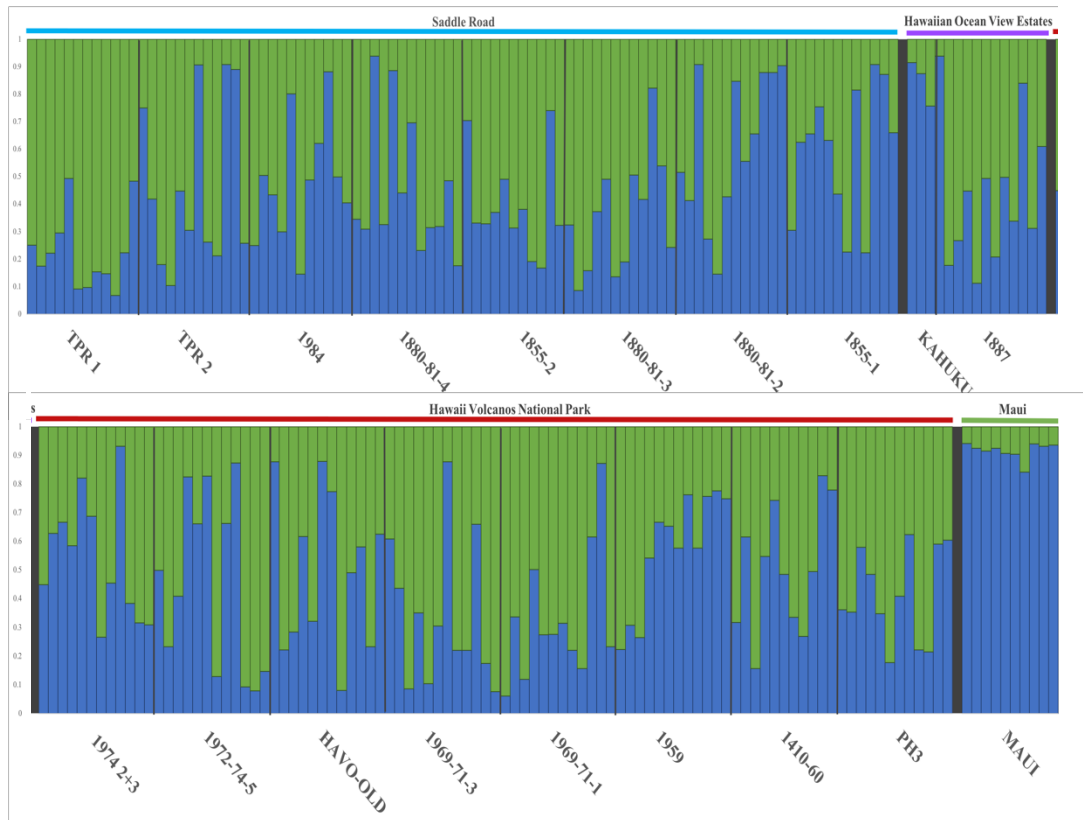


Figure 14. Bar plot images from STRUCTURE analysis indicating inferred population assignment for 283 individuals in 22 populations. $K=2$ was determined as the highest probability of genetic assignment.

Microsatellite data from The Big Island was used to complete EDENetwork analyses in order to determine meta-population dynamics, including source/sink populations and evidence of gene flow between populations. This analysis used seven microsatellite loci to automatically generate a percolation threshold value for relatedness. The automatically generated threshold was 39.5, suggesting that most individuals on The Big Island shared a high degree of genetic relatedness. The threshold was lowered manually to 0.50 in order to determine which individuals are the most related (Figure 15). The colored nodes represent individuals within a population that are connected by edges. Green edges represent high gene flow, while blue edges represent less gene flow. The

populations with the most gene flow are SR-HAVO and SR-HOVE. This could imply that SR acts as a current source population on The Big Island.

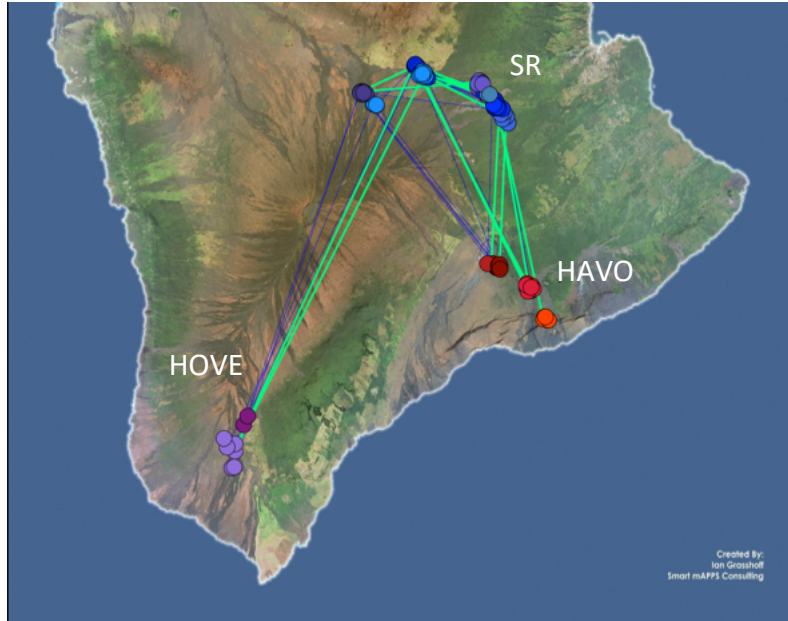


Figure 15. EDENetwork microsatellite analysis of *D. scabra* populations on The Big Island. Percolation threshold = 0.5.

Migration rates inferred by BayesAss suggest that approximately 68% of gene flow is occurring within individual populations (data not shown). The remaining 32% of gene flow was split evenly between other populations, with no bias for region or island. This suggests that the current set of microsatellites do not provide enough data to infer bi-directional rates of migration. The number of migrants (inferred from F_{st}) suggests that a large amount of migration occurs between regions on The Big Island. SR-HOVE has 33.143 migrants per generation. SR-HAVO has 88.350 migrants per generation, and HOVE-HAVO has 19.014 migrants per generation. All regions share less than 2 migration events per generation with populations on Maui.

Evidence of genetic bottlenecks were assessed using BOTTLENECK (Piry et al. 1999). The TPM was used to determine that no recent large reductions in effective population size could only be detected in one population (SR 1880-81-4; Table 5). Patterns of diversity on lava flows of various ages were analyzed in order to determine if flow age impacted genetic diversity within the species. Over time, the number of alleles, number of expected alleles, observed heterozygosity, and expected heterozygosity do not change as lava flows get older (Figure 15). The inbreeding coefficient does tend to decrease over time ($R^2 = 0.2578$), suggesting that some effects of inbreeding are alleviated through additional colonists and gene flow from other populations. The inbreeding coefficient is lowest on lava flows that were formed just over 150 years ago.

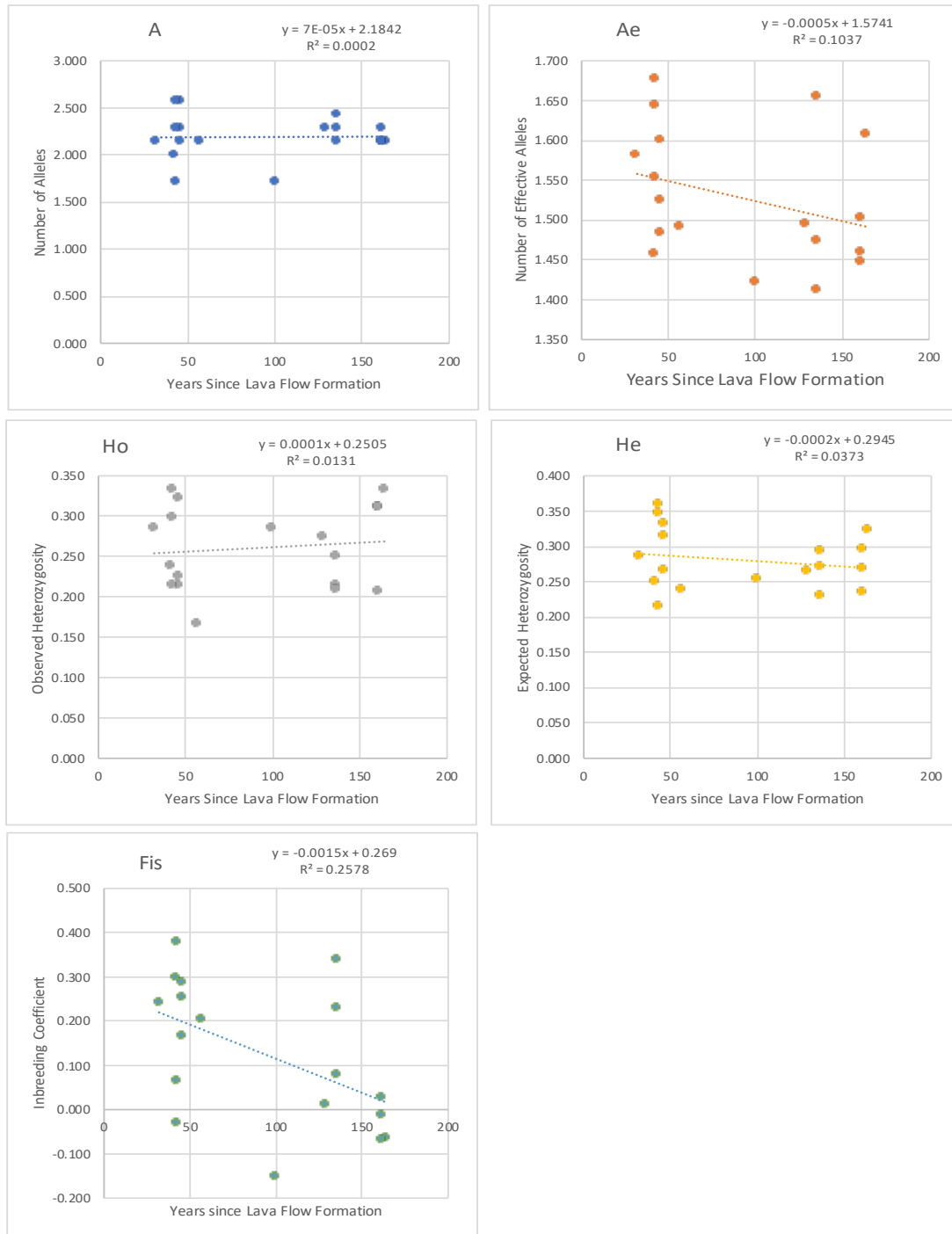


Figure 16. The number of alleles (A), number of effective alleles (Ae), observed heterozygosity (Ho), expected heterozygosity (He), and inbreeding coefficient (F_{IS}) plotted against the number of years since the lava flow was formed for each population. The number of alleles and observed heterozygosity do not increase as populations get older. The inbreeding coefficient does tend to decrease as populations get older ($R^2 = 0.2578$).

Discussion

Dubautia scabra is split into two subspecies, which stretch across a wide distribution on Maui and The Big Island. Three main volcanic regions on the Big Island (SR, HOVE, and HAVO) and one population on Maui were sampled to infer overall genetic structure of *D. scabra*. All major volcanic regions on The Big Island share F_{ST} values that are <0.05 (Table 6), suggesting a low amount of genetic differentiation among populations on the island. Maui is the only region with pairwise F_{ST} values >0.05 , reaching as high as 0.172. This suggests considerable genetic differences exist between islands. However, populations on The Big Island are so closely related, our ability to determine patterns of genetic structure for the whole species becomes limited. STRUCTURE could not differentiate between populations or regions on The Big Island, but did suggest that Maui is reasonably different and likely shares ancestral genetic signal with many individuals on The Big Island. The PCoA also confirms there is little distinction between populations or regions on The Big Island. The population from Maui does form a unique cluster, but all individuals sampled were from the same area. Any genetic differences between populations on The Big Island and Maui can most likely be attributed to the physical distance between the islands, as well as any genetic differences that might occur between subspecies.

Diversity statistics show that there is moderate genetic variability within populations, regions, and islands overall. All populations had similar number of alleles (A) and number of effective alleles (A_e). Observed heterozygosity (H_o) was high in a few populations from SR and HAVO, including; TPR 1 (0.333), HAVO 1972-74-7 (0.333), and Pu'u huluhulu-1 (0.393). Observed heterozygosity was much lower in the Maui population (0.167). We would expect that the population from Maui would have higher

observed heterozygosity compared to regions on The Big Island because it is presumably much older. The averages from all regions for observed heterozygosity and expected heterozygosity were similar. The average value for F_{IS} in all regions was 0.103, and was the highest in populations from SR and HAVO. TPR2 and SR 1880-81-2 had an inbreeding coefficient of 0.333 and 0.339, respectively. HAVO 1974 2+3 and HAVO 1972-74-5 had an inbreeding coefficient of 0.298 and 0.381, respectively. The population at Kahuku (HOVE) was the only population that had a much lower F_{IS} value at -0.153.

The time since colonization can play an important role in the genetic diversity of a species. After a dispersal event to a new island or habitat, only a few genotypes are passed to the new population, creating a genetic bottleneck. Overall genetic variation in founding populations is generally limited and there is usually a decrease in the total number of alleles and heterozygosity (Nei et al. 1975). Over time, genetic variation should accumulate within a new population, creating a unique set of genotypes that differ from the source population. Until genetic variation is able to accumulate, many populations will experience inbreeding. As new populations grow, accumulation of unique mutations increases overall genetic diversity and heterozygosity within populations, reducing the effects of inbreeding. The rate of increase in genetic diversity is usually related to the speed of population growth and the amount of genetic variation carried over from the original colonization (Nei et al. 1975). As far as we can tell, current populations of *D. scabra* are similarly diverse across lava flows of various ages (Figure 15). These populations share considerable gene flow, so detection of any increase in genetic diversity through time is limited by the current microsatellite analysis.

Little genetic divergence over time has also been associated with other members of the HSA, likely due to gene flow and introgression between species. A study using

allozymes (Witter and Carr 1988) found that the HSA underwent a genetic bottleneck after the initial colonization event from California, and still only contains half of the genetic diversity of mainland source species. Over the past 5 million years, genetic divergence has increased as lineages separate across the archipelago. Allozyme data also suggested that additional genetic bottlenecks have been experienced as colonization occurs on other islands or new habitats. For example, populations from younger islands of *D. laxa* and *D. linearis* have lost alleles that are present in populations from older islands (Witter and Carr 1988). Witter and Carr (1988) argue the lack of genetic variability is due to recurring bottlenecks as species colonize new habitats and islands, and insufficient time for new variation to accumulate before the next colonization event.

Other studies, including some that examine the levels of differentiation between *D. arborea* and *D. ciliolata* (Friar et al. 2007; Lawton-Rauh et al. 2007; Remington and Robichaux 2007) have noticed low to moderate genetic differentiation between species within the genus *Dubautia*. *Dubautia arborea* and *D. ciliolata* are generally separated by ecological barriers, but form an unusual hybrid complex where woodlands and shrublands overlap. Low levels of differentiation were observed between the species using AFLP markers and nucleotide sequence diversity from floral regulatory genes (Lawton-Rauh et al. 2007). Moderate levels of genetic diversity have been seen between pure populations of *D. arborea* and *D. ciliolata* using microsatellites (Friar et al. 2007). However, pure and hybrid populations shared common alleles and had similar allele frequency distributions. All of these studies (Friar et al. 2007; Lawton-Rauh et al. 2007; Remington and Robichaux 2007) found overall genetic differentiation between species was minimal, with the most genetic differentiation seen among populations of the same species that were regionally isolated. Remington and Robichaux (2007) speculate that

hybridization between the species is not a recent phenomenon, and has been occurring where populations overlap since lineage divergence. Friar et al. (2007) suggests that little gene flow and almost no introgression between the species could be due to limitation of pollinators, seed dispersal, or localized gene flow between hybrid individuals. Lawton-Rauh et al. (2007) suggests that speciation was most likely driven by differential pressures from the environment and population expansion rather than genetic differentiation. Ecological speciation is a common attribute within the HSA where gene flow is common, and may cause admixture between populations of multiple species (Lawton-Rauh et al. 2003; Friar et al. 2006; Friar et al. 2007; Lawton-Rauh et al. 2007).

Gene flow between populations also plays a significant role in the amount of genetic variation and the distribution of particular alleles. On The Big Island, gene flow among populations is frequent. All F_{ST} values between populations were <0.05 indicating a low level of genetic differentiation and a high level of relatedness. EDENetwork suggests that all populations on The Big Island have a high volume of gene flow (Figure 14). The green edges demonstrated that the most gene flow occurs between SR-HOVE and SR-HAVO. The number of migrants per generation was calculated for each population from F_{ST} (data not shown), indicating that there are 33.143 migrants between SR and HOVE, and 88.350 migrants between SR and HAVO. The one-migrant-per-generation-rule suggests that one migrant per generation is sufficient gene flow to minimize decreases in genetic diversity within subpopulations (Mills and Allendorf 1996), preventing substantial divergence among populations. The direction and rate of gene flow was examined through BayesAss in order to determine which populations are acting as source or sink populations. Migration rates inferred by BayesAss suggest that for all populations approximately 68% of gene flow is within a population, while the

remaining 32% of gene flow was split evenly between other populations, with no bias for nearby region or island. This suggests that gene flow rates among all populations are high and that the current set of microsatellites do not provide enough data to infer bi-directional rates of migration at a fine scale.

Combined data from this study, including F_{ST} , EDENetwork, and the number of migrants per generation is enough to infer that SR is acting as the source population for HAVO and HOVE on The Big Island. SR is centrally located on Mauna Loa, which is also the highest mountain on the island. *Dubautia scabra* is presumed to be a wind-dispersed species, where seeds are likely to be carried downward to populations at lower elevations. Long-distance dispersal from SR to new habitat on The Big Island would not be uncommon, as demonstrated by the number of migrants per generation. HAVO and HOVE were likely colonized and established by seeds dispersed from SR.

Genetic variation after a bottleneck often leaves populations depauperate. Over time, population expansion (Lawton-Rauh et al. 2007; Ilves et al. 2015), accumulation of genetic mutations (Gompert et al. 2014), and genetic recombination (Friar et al. 2008) allow a population to recover. Given enough time, populations that have gone through a bottleneck will accumulate as much genetic diversity as their source populations had when they dispersed (Nei et al. 1975), but many factors will affect how quickly this happens. Diversity statistics relative to this project are not different to those found in other genetic studies of the HSA using microsatellites. Two previous studies (Friar et al. 2006; Friar et al. 2007) show that A_e , H_o , and H_e , range from 1.10- 2.31, 0.047-0.325, and 0.081-0.341, respectively for six other species within *Dubautia*. Friar et al. (2007) does report much higher F_{ST} values among populations of the same species on the same island, however. Different populations of *D. ciliolata* sampled from Maui have F_{ST} values >0.20 .

A high F_{ST} values such as this was only seen between populations found on different islands.

Dubautia scabra populations have a similar genetic variation across its range on The Big Island, due to high levels of gene flow from other populations on the island. Similar genetic variation patterns have also been associated with other species and HSA plant populations on other islands. Although genetic variation has increased from the oldest to youngest islands, species and populations maintain their affinities through hybridization and introgression. Alternatively, species are defined by their ecology and physiological limitation than strong genetic differentiation. In the case of *D. scabra*, recovery of some genetic variation has occurred, but is limited over its distribution.

Diversity statistics show that genetic variation within *D. scabra* populations is similar to other species found within the HSA. Other measure of relatedness, such as measure of genetic divergence, suggest that other species within the alliance have higher F_{ST} values across a much smaller range. Gene flow and migration (seed dispersal) between populations of *D. scabra* are common, and would minimize genetic differentiation across the range. There is some evidence of higher levels of inbreeding on younger lava flows, with overall levels of inbreeding decreasing as lava flows age. There is no evidence of genetic bottlenecks from the initial colonization, nor subsequent colonizations to new volcanic regions. Gene flow between volcanic regions is the highest at SR compared to HAVO or HOVE. It is likely that the source population is SR, and HAVO and HOVE were founded by individuals from SR. Continuous gene flow between populations and regions suggests that all populations on The Big Island are all a part of a much larger meta-population that spans across the whole island.

CHAPTER IV

SUMMARY

This research project is an ecological and genetic investigation of the Hawaiian endemic species, *Dubautia scabra*. *Dubautia scabra* is a member of the Hawaiian Silversword Alliance (HSA), which is a group of plants widely known for their ecologically adaptive evolutionary histories. *Dubautia scabra* is endemic to Maui and The Big Island, and consists of two subspecies, *D. scabra* ssp. *scabra* and *D. scabra* ssp. *leiophylla*. *Dubautia scabra* ssp. *scabra* is commonly thought of as a small shrub that grows in volcanic and rocky substrates, while *D. scabra* ssp. *leiophylla* has longer and more vine-like branches and is found in forested habitats. More importantly, *D. scabra* ssp. *scabra* (hereafter *D. scabra*) is the first angiosperm colonist of new lava flows, and is one of the few species found on exposed lava rock on The Big Island, Hawaii. As a colonizer of volcanic substrates, *D. scabra* provides a unique window into studying many other ecological processes including seed dispersal and establishment, patterns of colonization, and patterns of primary succession.

The main purpose of this Master's thesis was to document patterns of primary succession including: colonization, population establishment, population senescence, and gene flow among populations with the use of a variety of ecological and genetic techniques. The processes of ecological succession and colonization are widely understood by ecologists in habitats like forests (Grashof-Bokdam and Geertsema 1998; Guariguata and Ostertag 2001) and grasslands (Collins and Adams 1983; Kahmen and Poschlod 2004), but researchers have not yet been able to successfully characterize

primary succession of new habitats. The population structure of species in this area is also important, as it provides a window to study gene flow and evolutionary processes of organisms after volcanic disturbance. *Dubautia scabra* is an ideal focal organism for this work because it is a primary colonizer and initiator of ecological processes on The Big Island of Hawaii.

This genetic and ecological investigation of *D. scabra* has answered questions pertaining to both ecological and genetic patterns on Maui and The Big Island. The main goals of this study were to determine (1) if there is a correlation between lava flow age, plant density, and genetic variation among populations (2) the genetic structure of *D. scabra* across the Big Island and Maui-Nui Complex of the Hawaiian archipelago, and (3) the source and direction of seed dispersal of *D. scabra* populations across the Big Island, Hawai'i using genetic analysis. This system has the potential to provide a wide variety of new information on the overall processes of ecological succession and some of the underlying components associated with it.

Major Findings

Ecological Timeline and Patterns

Plant size and density on recent lava flows are important factors that help researchers describe the progression of colonization, establishment, and senescence of *D. scabra* populations on The Big Island. In this system, plant size should indicate overall plant age, while plant density should be consistent with the time since colonization, population establishment, and overall decline of plant populations. We found a higher density of individuals of *D. scabra* on recent lava flows ($R^2 = 0.7692$), and larger plants on older flows ($R^2 = 0.4439$). The highest density of individuals occurs on lava flows that are about 45 years old, while the largest individuals are found on lava flows of that are about

100 years old. After about 50 years, *D. scabra* numbers begin to decline, but established individuals continue to live for another 100 years until they are likely outcompeted or die. This model suggests that individuals and populations of *D. scabra* are shorter lived than previously expected.

Time since disturbance is not the only factor associated with plant size and density. Stepwise forward regression models suggest that: elevation, soil hydrology, aspect, rock type, and mean annual rainfall are other ecological factors that play a significant role in predicting size and density on recent lava flows. Together, these findings suggest that colonization and population establishment on recent lava flows is also dependent on other ecological variables.

Genetic Diversity and Structure

Examination of genetic divergence, diversity, and variability of *D. scabra* were completed using a variety of genetic techniques. Genetic divergence across volcanic regions on The Big Island is low, indicating a high degree of gene flow and relatedness among populations on The Big Island. Genetic divergence between the islands, however, is high. Physical distance between populations and limited gene flow are likely to have contributed to considerable genetic differences between populations on different islands. Overall genetic diversity is moderate within populations and regions on The Big Island with the most genetic diversity seen within populations, not among populations. Limited genetic diversity between populations indicate similar levels of genetic variability across The Big Island due to gene flow. Additionally, genetic diversity does not appear to increase over time as lava flows age.

Gene Flow in a Metapopulation

Similar levels of genetic variation found within and between populations of *D. scabra* is a function of gene flow through a dynamic metapopulation. EDENetwork and estimates of number of migrants per generation suggest there are high volumes of gene flow between all major regions on The Big Island. The most gene flow comes from the populations around Saddle Road, located centrally on The Big Island. Combined data from multiple analyses suggest that the Saddle Road region is the current source population within the larger metapopulation. This region exhibits the most seed dispersal to other regions on The Big Island, including, Hawaii Volcanoes National Park and Hawaiian Ocean View Estates.

Summary

Patterns of colonization and population establishment were documented within the larger meta-population of *D. scabra* on The Big Island. Recent lava flows are colonized by many individuals. Over time, only a few of those individuals will grow and establish populations where physical and environmental conditions are appropriate. After about 100 years, population numbers begin to decline as volcanic substrates are broken down, and the processes of primary succession continue.

Various volcanic regions on The Big Island share low genetic divergence, similar levels of genetic diversity, and a high levels of gene flow. Genetic divergence is low across all populations of *D. scabra*, and genetic diversity is similar in all populations on The Big Island. High amounts of gene flow suggest that all sub-populations within this meta-population have a high degree of relatedness. The populations around Saddle Road share the most genetic relatedness with the other regions and populations on the island. Numerous analyses suggest that Saddle Road is the current source population on The Big

Island, and has founded other populations including Hawaii Volcanoes National Park and Hawaiian Ocean View Estates.

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