

**DOCUMENTING ACOUSTIC VARIABILITY AMONG WINDWARD HAWAI'I
'AMAKIHI (*Hemignathus virens virens*) POPULATIONS ON HAWAI'I ISLAND**

A THESIS SUBMITTED TO THE GRADUATE DIVISION OF THE UNIVERSITY
OF HAWAI'I AT HILO IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF

MASTER OF SCIENCE

IN

TROPICAL CONSERVATION BIOLOGY AND ENVIRONMENTAL SCIENCE

July 2014

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Acknowledgments

Funding for this research was provided by a National Science Foundation Centers for Research Excellence in Science and Technology (CREST) grant (0833211). Research permits were provided by the United States Fish and Wildlife Service (USFWS), the Hawai‘i State Department of Forestry and Wildlife (DOFAW), and the University of Hawai‘i.

I would like to first send a gracious amount of gratitude to my advisor Dr. Patrick J. Hart for continued encouragement, professional leadership, research guidance, and unconditional support throughout the entire process. This journey would not have been possible without his influence. I would also like to thank Dr. Eben H. Paxton for his education on avian biology and bird banding, statistical guidance, and continued comradeship. Dr. Adam Pack, I thank you for your advocacy and encouragement throughout my journey. I also thank Steve Kendel of the USFWS, ‘Iwikauikaua Joaquin, Keala Kanaka’ole, and Mililani Browning of Kamehameha Schools, research technicians Angela Beck, William Ray, Danielle Himelsbech, McKayla Meyer, and Tishanna Ben, and bird banders Nolan Lancaster and Sonia Levitt for all their help through this voyage.

Last but never the least, I thank my family for always supporting me no matter the circumstances and always having faith. I thank you, Alyson Panem, for your endless support and belief in my abilities, encouragement in the toughest times, and standing by my side no matter the costs. Mahalo Ke ‘Akua Mana Loa a me Nā kūpuna

Abstract

Vocalizations play a fundamental role in many aspects of a bird's life, including territoriality, mate choice, individual recognition, and predator avoidance. This is the first study to investigate how vocalizations differ across populations of a Hawaiian honeycreeper. I recorded song from 329 adult male Hawaii 'Amakihi (*Hemignathus virens virens*) in five populations across an elevational gradient on the windward side of Hawaii Island during the 2011-2013 breeding season and used sound analysis software to examine how eight song components vary within and among populations. Our results demonstrated that song varies greatly among populations with greatest divergence and lowest song variability in the low elevation population, where 'Amakihi have recently recovered from a disease-induced bottleneck. I also tested predictions of the Acoustic Adaptation Hypothesis (AAH) by examining how song characteristics vary between open and closed understory vegetation. 'Amakihi in open understory sang at higher frequencies relative to neighboring 'Amakihi in dense understory, thus providing moderate support for the AAH. This study demonstrates that both isolation and habitat heterogeneity has led to localized structuring of song over relatively short geographic distances.

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Introduction

Avian vocalizations are fundamental in numerous aspects of bird life. Male birds demonstrate their reproductive qualities, physical strengths, and mating skills to conspecific females using elaborate and complex songs to attract and stimulate a receiving female (Kendeigh 1941, Greig & Pruett-Jones 2010, Kroodsma & Byers 1991). Songs are also used generally by males to defend feeding and mating territories from intruding males (Nowicki et al. 1998) or to broadcast territory boundaries. Shorter vocalizations, such as calls and shorter songs, serve to broadcast an individual's location to its mate, offspring, or other individuals in its area. Bird song has also been suggested as the primary means of informational transmission through meme flow both within and across generations (Laiola 2008, Lynch 2006).

Passerine Song Learning

The process of song learning in birds has been well documented. Juvenile birds learn and produce a collection of sounds and vocalizations that are functional both within the communication systems of related individuals and also other community members. Most birds undergo critical vocal learning and developmental stages in their early adolescent months that are vital to their reproductive and survival success as an adult. These birds include the oscine songbirds, parrots, and hummingbirds (grouped as “songbirds” from here on). All other birds, primarily sub and non-oscine birds such as chickens, doves, and ducks, have genetically inherited vocalizations that are fixed at birth and are not learned.

The song learning period may be broken into two phases of song development: the sensory acquisition phase and the sensorimotor phase. The sensory acquisition phase

is a period of intense listening where juveniles sample the song repertoires from adult neighboring tutors, absorbing the information and song structures surrounding them. Here, the songs of tutors as well as the song types of the tutors from past learning periods are preserved in receptive juveniles. Later in the sensorimotor phase, the young bird will produce a generic subsong, which is practiced until finally a stable stereotyped song is delivered containing elements adopted from tutor birds. This newly adult bird, like birds before him, will one day provide elements for another attentive young bird learning his song. As generations persist, local song types continue to transition through the song learning process and thus become preserved within a population, similar to the process in which genetic alleles are preserved in a population over time.

Studies have also revealed the importance of social interactions and tutor influence during the critical learning stages of juvenile songbirds (Baptista and Petrinovich 1984). Baptista and Petrinovich (1984) demonstrated that juvenile White-crowned sparrows (sp.) learned more readily from live birds than taped tutors. They were also able to show that white-crowned sparrows were still able to learn song even if the tutor was not the same species, provided the tutor was live and could provide the social aspect lacking in tape tutoring. Since then, the importance of social interaction in birdsong learning has been widely accepted, however it is difficult to clarify the precise aspects of social interactions that influence the learning process.

Population Dialect Formation: Influence of Song Learning

The rules of sharing, learning, and preservation of song types within a population can eventually lead to the formation of variation between populations and localized dialects, similar to accent formation in the human language. Pioneering research by

Morton (1987) demonstrated geographic song variation across the range of the Carolina Wren (*Thryothorus ludovicianus*) that could be attributed to geographic isolation of the populations. This finding suggests that within each population, local dialects accumulate and are shared among the individuals in each group via the learning process. Dialect formation can also be facilitated from copying inaccuracies that may occur during song transmission from a tutor to the receptive juvenile, analogous to novel alleles formed through genetic mutation. Sharing processes during song learning allow these to mistakes accumulate over generations. As they accumulate locally, they become fixed in a population's pool of song types, thus facilitating the formation of song variation among populations.

Once a population dialect is formed, song variation among can be maintained by signal affectedness, summarized by the Honest Convergence (HC) model introduced by Rothstein and Fleischer (1987). The model assumes the ability of a male songbird to sing the correct local dialect is a reliable, honest signal of his high social status and male quality within that population. These high quality, honest "accents" or dialects that are unique to the population are maintained through sexual selection by local females. The local song variant is labeled "honest" because the vocalization is either too difficult to be copied immediately by intruding males or because few or all males have little to no contact with it as a juvenile (Rothstein & Fleischer 1987). Male birds that are unable to deliver the honest dialect belonging to a population are either intruding or young males. Rothstein and Fleischer (1987) supported their hypothesis by demonstrating in the Brown-headed Cowbird (*Molothrus ater*) that a male's possession of the locally appropriate song type was a reliable indicator of its age, which is a major correlate of a male's mating success and possibly its quality as well. Local dialects can therefore act as

a population's recognition characteristic and can influence the chance of successful copulation from females.

Population Dialect Formation: Population Size, Meme Flow, and Bird Song Culture

Dynamic processes of geographical isolation and differences in population size, coupled with song learning, are also known to drive song variation in songbird species (Parker et al. 2012, Valderamma et al. 2012). The effects are similar to traditional population ecology and genetics, where larger populations exhibit higher genetic variation, isolation limits gene flow, resulting in localized variation, and contracting populations exhibit bottlenecked variation (Smith & Wayne 1996). Here, the basic unit of transfer is genes, which are transferred through successful mating events. In the transmission of birdsong, however, the basic unit of transfer is called a 'meme' (Dawkins 197xxx). Meme units were first identified as the individual syllables of songs by Mundinger (1980) and later extended by Lynch et al. (1989) as song units of any size that are capable of transfer between birds. The non-genetic transfer of memes occurs through song learning when song is shared from an adult tutor to a receptive juvenile bird.

Song variability, like genetic variability, is highly dependent on population size (Valderamma et al. 2012, Parker et al. 2012). Larger populations contain a higher availability of song types for learning birds to sample from, similar to gene pools. Also, novel song types are more likely to arise in larger populations due to increased chances of mutation in song elements during learning events (Lynch 1996). Conversely in small populations, higher song sharing, lower availability of novel song types, and increased meme drift reduces acoustic variability (Valderamma et al. 2012, Parker et al. 2012). For example, in North Island Kokako (*Callaeas wilsoni*) in New Zealand, overall song

variability, which includes number of song types, song complexity, and phrase diversity, was positively correlated with population size (Valderamma et al. 2012). Population growth rate also correlated positively with population repertoire size and singing rates, suggesting that vocal changes in small populations could affect population establishment and growth (Valderamma et al. 2012). Laiola & Tella (2006) also demonstrated that habitat patch size is able to influence song variability. Repertoire size of the Duponts Lark (*Chersophilys duponti*) was reduced in smaller habitats compared to large repertoires observed in populations surviving in larger habitats.

Like gene flow, transfer of memes between neighboring bird groups is dependent on the migration of individuals between populations (Briefer et al. 2010). Migration acts as an effective homogenizing factor on inter population song variation (Lynch 1996). However, natural isolating barriers (i.e. vast distances, inhabitable areas, or mountain ranges) or anthropogenic barriers can limit meme flow between groups, resulting in localized song learning and formation of dialects unique to individual populations (Lynch 1996, MacDougall-Shackleton 2001, Parker et al 2012). Habitat patchiness and landscape connectivity can therefore alter song sharing and reduce acoustic diversity (Laiola and Tella 2006). In white-crowned sparrows (*Zonotrichia leucophrys*) MacDougall-Shackleton (2001) revealed localized song dialects unique to individual populations that were associated with reductions in gene flow. Also, Robin et al. (2011) demonstrated marked song divergence among populations of white-bellied shortwing (*Brachypteryx major*) in high elevation sky-island and attributed song variation towards the vast isolation between groups. Divergence in song variation and dialect formation can also continue post-isolation through random drift as well as localized mutation of memes during song learning (Lynch 1996, Valderamma et al. 2012)

Transfer of memes within and between populations equates to information transmission and therefore possible transmission of bird ‘culture’ (Lynch 1996, Laiola & Tella 2005). Birdsong culture is defined as the transmission and transformation of socially adapted behaviors and knowledge, passed from generation to generation through the transmission and learning of song (Lynch 1996, Laiola 2008). Bird culture is hypothesized to evolve over time and is transmitted among individuals in continuous populations through vocal behaviors and locally prevalent song elements (Lynch 1996, Laiola 2008). The field of cultural evolution investigates how various Darwinian evolutionary models can be applied to cultural change within a species (Crozier 2010). Comparable forces of mutation, migration/flow, drift, and selection have been suggested to have influence on both genetic and meme evolution over time (Lynch et al. 1989). For example, like genetic evolution, culture passed down through song can vary geographically between different populations surviving in different acoustic and environmental conditions (Fitch 2009; Lynch & Baker 1994). As mentioned earlier, these variations are dialects, which are similar to the different gene pools among populations of the same species. Over time, meme flow, mutation, and localized variation of bird culture contributes to evolution. Unlike biological evolution, however, cultural evolution may be much more rapid and can happen over a few generations.

Applying biological evolution theories to the evolution of birdsong culture also allows researchers to investigate the erosion of meme variation and song degradation that may occur with decreasing population size over time. Reductions in population sizes are usually a result of an anthropogenic disturbance, but have historically and naturally occurred when individuals of a species colonize a new area (i.e. founder event). However, increasing habitat fragmentation as a result of anthropogenic causes, which

promotes population reduction, has received the most concern (Laiola & Tella 2007). Habitat fragmentation influenced by anthropogenic forces is recognized as one of the greatest threats to the survival of wildlife populations (Kruess & Tschamtkke 1994). Habitat fragmentation can reduce the size of already small populations, increasing their susceptibility to inbreeding depression, genetic drift, and Allee effects. (Laiola 2008). Fragmented populations also have less movement of individuals between patches, thus reducing called gene flow or the exchange of genes.

Laiola and Tella (2005, 2007) demonstrated song erosion in the Dupont's lark (*Chersophilus dupont*) by revealing decreased song complexity in smaller, distant populations. The reduction of population sizes and habitat fragmentation may affect the “memetics” of populations similar to the way they effect the genetics of populations. Small populations may display higher rates of song and cultural mutations, reduced social learning, and increased cultural drift. Reduced movement of individuals between fragmented populations dramatically reduces the amount of song and subsequent cultural information that is normally shared between them. A species with declining numbers can therefore hit a song culture bottleneck, similar to a genetic bottleneck, resulting in the loss of song variation and cultural information. In documenting the erosion of song and bird culture, researchers are able to demonstrate the need to conserve avian habitats to protect remnant songbird populations and their vocal complexity.

Population Dialect Formation: Habitat Heterogeneity and the Acoustic Adaptation

Hypothesis

Bird vocalizations, especially elaborate songs, may be energetically costly to produce (Boncoraglio and Saino 2007). Therefore, fitness advantages exist for efficient

communication from the signaler (i.e. male bird), through the habitat, and to the receiver (i.e. female birds). Efficient communication, however, can be difficult to achieve and can be highly dependent on different habitat types. Stemming from this concept came a highly investigated hypothesis that attempts to explain population song variability as a function of habitat variations. What is now termed the Acoustic Adaptation Hypothesis (AAH, also called “Sensory Drive Hypothesis, Endler 1992, Tobias et al. 2010) was first proposed by Morton (1975) and assumes that structural differences between habitats will influence sound transmission and signal propagation. Under the AAH, the habitat structure, its composition, and the environment will impose a selection force on the optimization of sound transmission and efficient signal broadcasting in birds. At the population level, selection will lead to the vocalization variant that is most efficiently transmitted through the local habitat to the intended receiver (i.e. a receptive female) with minimal sound degradation. Over time and across generations, these variants should persist in the population through the localized sharing involved in the song learning process. The results of such acoustic adaptations are song variants of different spectral and temporal structures that occur in different habitat types across populations.

A major prediction of the AAH is that high pitched (high frequency), faster paced signals are favored in open habitats and low pitched (low frequency), slower paced signals are favored in denser habitat This has been demonstrated by Boncoraglio & Saino (2007), Tobias et al. (2010), and Kirschel et al. (2011). Over time, there will be selection for the most efficient song type as a function of variation in habitat structure (e.g. density, vegetation type, etc) across different environments and populations. Even genetically related populations, homogenized by the influences of gene flow, have shown vocalization divergence among populations living in different environments,

demonstrating habitat-related selection and maintenance of vocal variation (Nicholls et al. 2006).

The main influences of habitat structure on signal transmission are degradation and attenuation of song components. Degradation is the sum of the structural changes that the signal accumulates at various distances from the source as it passes through the transmission habitat (Morton 1986). These structural transformations can occur from scattering or refraction through obstacles (i.e. trees, brush, leaves, etc.) or timing rearrangements by echoes and reverberation (Bradbury & Vehrencamp 1998). Generally, habitats with denser vegetation, foliage, and overall dense structure have been shown to increase rates of signal degradation. For example, Great Tit (*Parus major*) song has been shown to experience significant degradation during periods of accelerated foliation and leaf bursts of deciduous forests in the spring (Blumenrath and Dabelsteen 2004). Acoustic degradation attributable to habitat density and structure has been demonstrated in other species of birds as well, including five antbird species (Nemeth et al. 2001) and various Amazonian birds (Tobias et al. 2010).

The second major influence, attenuation, refers to the progressive weakening of signal force or intensity (i.e. volume, energy, or amplitude) through the acoustic habitat. Attenuation happens as the propagation distance increases (Forrest 1994). As sound is propagated away from the source and through the acoustic habitat over a distance, the energy or intensity (measured as acoustic power per unit area) of the sound is spread over a larger surface area and may become lost to atmospheric absorption and vegetation structure. Dense foliage and vegetation, along with cold temperatures and moist air, is known to increase attenuation (Aylor 1972). Conversely in open vegetation structures and warm, dry air climates the forces of attenuation are more relaxed (Harris 1996).

Over time, degradation and attenuation should drive the evolution of bird song transmission and sound propagation towards achieving the most effectively transmittable sound structure with minimal deterioration. Habitat heterogeneity may even affect the physical singing positions and location of the signaler in its habitat. Tobias et al. (2010) demonstrated that transmission height, along with signal frequency and habitat type significantly affects attenuation in an Amazon bird community. The singing of higher frequency, fast paced signals from a low perch suffered more distortion than did the singing of slow-paced, low frequency signals from a higher perch in denser habitats. Therefore, habitats of more open structure allow for low perch transmission of high frequency, fast-paced signals whereas dense vegetation requires high perch transmission of low frequency, slow-paced signals (Tobias et al. 2010). Habitat structure has also been shown to influence frequency modulation during song transmission. Nicholls & Goldizen (2006) revealed that satin bowerbirds use less frequency modulation in dense rainforest habitats compared to greater modulation in open, eucalypt-dominated habitats. These results have been moderately supported in Boncoraglio and Saino's (2007) first ever large-scale meta-analysis of current research testing the AAH.

There is also increasing evidence that varying ambient noise levels in avian habitats can influence divergence of bird song structures. Like attenuation and degradation in varying habitat structures, high levels of environmental noise are great challenges for singing birds that use acoustic communication in their survival. Ambient noise may come from sources such as the general urban environment (e.g. cars, factories, etc), other animal species (e.g. calling insects and other song birds), and abiotic features of the environment (e.g. wind and rain noise)(Slabbekoorn and den Boer-Visser 2006, Fernández-Juricic et al. 2005). Some species of birds use vocal plasticity as a strategy to

deal with complex environmental noises, such as those in urban settings, compared to neighboring population in natural environments. In the case of the European blackbird (*Turdus merula*), Mendes et al. (2011) documented vocal adjustment by birds in three sites along an urban to rural gradient in Spain. Compared to natural populations, blackbird populations in pure urban settings demonstrated shifts in their entire vocalization towards higher frequency songs to avoid masking by ambient noise that mainly occurs at lower frequencies. Similar evidence of acoustic plasticity and song adjustment has been demonstrated in many other songbird species across the globe (Slabbekoorn and den Boer-Visser 2006, Fernández-Juricic et al. 2005). Over time, song variability between natural and urban established populations can lead to distinct dialect formation and possibly genotypic changes, as suggested by various authors (Mendes et al. 2011, Slabbekoorn and Ripmeester 2008). Further research may be able to demonstrate that variable ambient noise levels, coupled with the variable habitat influences summarized in the AAH, drive song evolution between bird populations towards optimal song structuring in a competitive acoustic environment.

Acoustic Research in Hawaii

There exists a strong need for research that further examines variation in avian acoustics and dialect formations across geographic ranges in order to document the potential effects of habitat fragmentation, habitat destruction, and population declines. In Hawaii, for example, little to no research has been conducted on acoustic communication of any species of Hawaii's honeycreepers, a group of songbirds (Subfamily Drepanidinae) endemic to Hawaii and one of most spectacular displays of adaptive radiation in birds. Current populations, however, are well known for their dramatic declines and high rates of extinctions due in part to the interacting pressures of disease,

habitat destruction, introduced predators, and competition with alien species. Of the 41 historical species, 17 are thought to be extinct and 14 are federally listed as endangered, while only 3 species and subspecies are robust enough in terms of geographic range and population size to be of minimal concern (Atkinson & LaPointe 2009). Current populations exist in highly fragmented habitats across a wide range of sizes, elevations, structures, and environmental factors. Variability in population dynamics makes Hawaii's honeycreepers a model group for acoustic research. Although conservation efforts across the islands are striving to protect the remnant populations of Hawaii's precious honeycreepers, it is vital to preserve and document the acoustic variability of the remaining populations before Hawaii's forest go silent forever. Results of such investigations could influence the conservation efforts of dwindling native Hawaiian bird populations.

Objectives/Hypotheses

The goal of this project was to document acoustic variability and possible song degradation across five populations of Hawaii 'Amakihi (*Hemignathus virens virens*, or HAAM) on the windward side of Hawaii Island. Some populations exist in fairly intact habitats with low levels of disease, and predation, such as those at high elevation in the Hakalau Forest National Wildlife Refuge and Keauhou forest. Other populations however, such as those in low-elevation Puna forests, exist in more degraded habitats, with greater levels of disease and predation risk. These low-elevation populations may be increasing in population size relative to just a few decades ago (Spiegel et al 200x) despite high disease occurrence, possibly due in part to evolved disease tolerances (Woodworth et al. 2005). Other populations, such as those in mid-elevation Volcano and Saddle Road forests experience moderate disease stresses and habitat destruction. Also in

some areas where continuous populations exist, there are sharp differences in habitat structuring and vegetation compositions. Overall, variability in different biotic and abiotic factors exists among populations, which, along with geographic isolation, may have influenced localized song structures. By recording songs of male birds from different populations at different sites and using sound analysis software to measure and compare song components, I hypothesize that:

A) Variation in song structure exists between populations on the windward side of Hawaii Island. Population variation may be attributed primarily to localized song sharing due to population isolation. As previously discussed, this is credited as the major influence of song variation between populations of individual oscine species (Tobias et al. 2010).

B Expanding populations in the low-elevations will have less song variation. Conversely, larger and more stable populations at mid to high elevations, such as HFNWR, will exhibit higher song variation. Also, founder effects experienced in expanding, small populations may result in lower song variability and complexity compared to an established bird population (e.g. Parker et al. 2012, Baker et al. 2006).

C) Controlling for simple variation between populations, within population differences will exist between different habitat structures (e.g. open vs. closed forests) as predicted by the AAH. Open forests may allow for higher frequency measures compared to closed forests, which should select for lower frequency song parameters.

Methods

Study Species

HAAM is a generalist honeycreeper species considered the most adaptable of the Hawaiian native forest birds (Lindsay et al. 1998), often found feeding on nectar, fruits, and insects in vegetation ranging from the forest floor to the heights of the tallest trees. Amakihi are also a relatively sedentary species (Lindsey et al. 1998). Adult males are generally a bright yellow-green with black lores (Figure 1a) while females are a drab olive color (figure 1b). HAAM maintain slightly overlapping type A home ranges throughout the year for copulation, nesting, and foraging, with tightening of territories during the breeding season (Kamil & van Riper III 1982, van Riper III 1987). HAAM is one of the 3 Hawaiian honeycreeper species whose populations are thought to be fairly stable on Hawaii Island (Atkinson & LaPointe 2009). Along the windward or eastern slope of Hawaii Island, HAAM populations survive in forest fragments of various sizes, structure, and across different elevations.

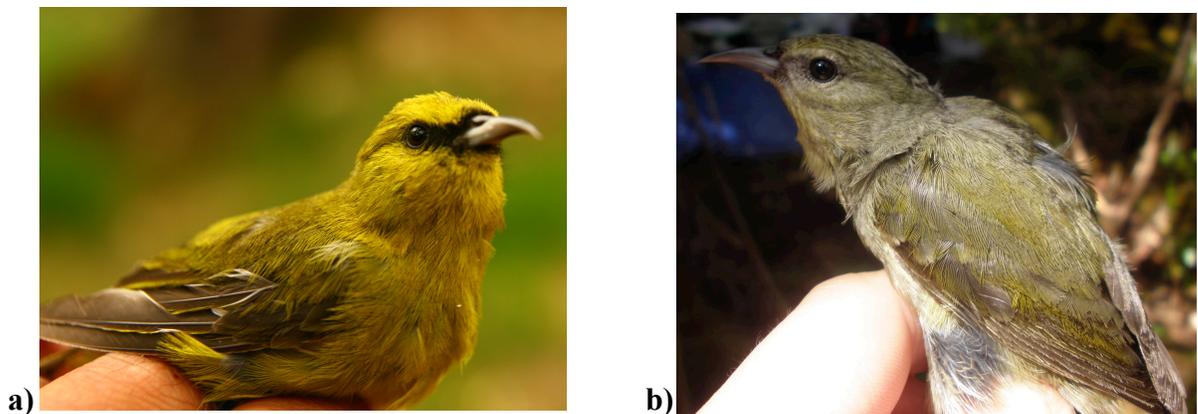


Figure 1. a) An adult ASY male Hawai'i `Amakihi characterized by its bright yellow plumage and fully developed, black streaked lores (*Hemignathus virens virens*), photograph taken at the Hakalau Forest National Wildlife Refuge and b) a drab olive-green adult female Hawai'i `Amakihi from the Kipuka area (Photo: A. Fournier)

Research Sites

Five recording areas were chosen across windward Hawai‘i Island at different elevations where known HAAM populations exist based on previous research (van Riper III 1987, Kilpatrick et al. 2006, Hart and Freed 2003, Flashpohler et al. 2010) and personal observation. Research sites include low elevation Kalapana/Puna/Kea‘au area (PUNA), Hawai‘i Volcano National Park (HAVO), Keauhou Ranch (KEAUH) and adjacent Kilauea Forests, the Kipuka (KIPU) areas south of saddle road on the flanks of Mauna Loa, and the Hakalau Forest National Wildlife Refuge (HFNWR)(Figure 2). Within each area, between 2-8 individual recording sites were chosen for sub-sampling. Each area was chosen such that connectivity and mixing between HAAM populations was minimal to absent. The five recording areas differ in elevation, degree of isolation and habitat disturbance, and climate (Table 1). All sites were dominated by ohia (*Metrosideros polymorpha*) forest, however there was some variability among sites in native/nonnative plant assemblages and habitat heterogeneity. Puna/Kea‘au areas are unique due to the recent (~10-15 years) recorded resurgence and expansion of HAAM populations into locations historically void of any HAAM individuals (Spiegel et al 200x), despite the high levels of infection with avian malaria (Woodworth et al. 2005).

To address the question of the effect of habitat structure on HAAM song, two different vegetation types were specifically chosen at both the HFNWR and Keauhou Ranch. Both vegetation sites differ greatly in their habitat structure and vegetation composition. The first type is labeled the open Koa plantation. These plantations are dominated by recent (~10-20 years) outplantings of the native Koa tree (*Acacia koa*), which are a dominant canopy tree in native Hawaiian montane forests and a fundamental tool in many reforestation efforts across the state. These plantations have a relatively

sparse understory that is dominated by introduced pasture grasses with low numbers of natural and outplanted native plant species. The second vegetation type is the native closed forest, characterized by old-growth Koa/Ohia (*Metrosideros polymorpha*) forest with established and intact under and mid-story vegetation structure. Both vegetation types at each site are continuously inhabited by 'Amakihi populations. HFNWR and Keauhou Ranch share similar elevation levels, annual rainfall, historical forest communities, recent (30-200 years) forest disturbances (e.g. cattle ranching, fire, logging, and harvesting), and current conservation efforts.

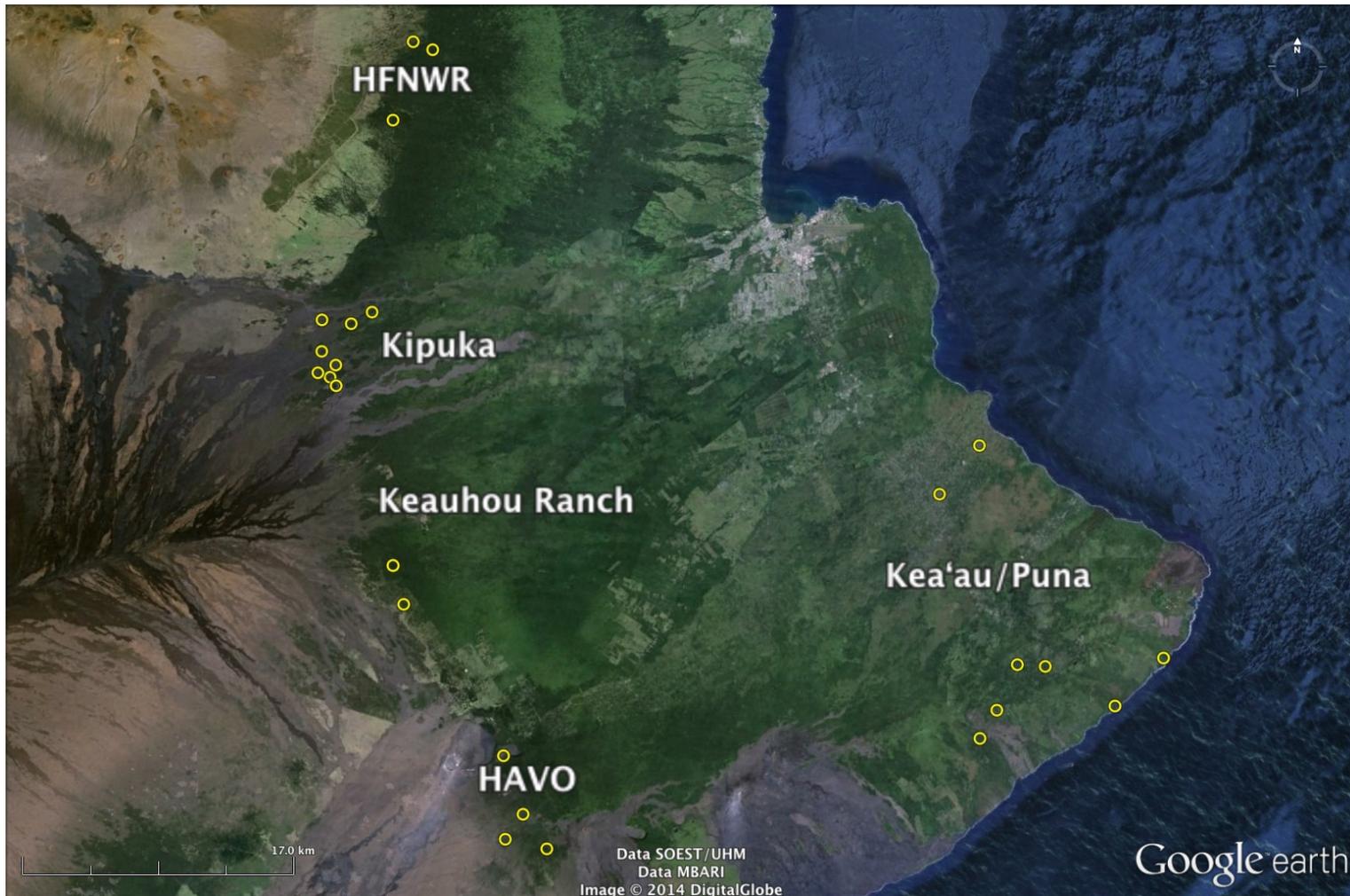


Figure 2. Five HAAM recording sites on the windward side of Hawai'i Island: lower Kea'au/Puna area, Hawai'i Volcano National Park (HAVO), Keauhou Ranch (KEAUH) and adjacent Kilauea Forests, the Kipuka (KIPU) areas south of saddle road on the flanks of Mauna Loa, and the Hakalau Forest National Wildlife Refuge (HFNWR).

Table 1: Description of each recording site

Site	# Recording Sites	Sample Size (# birds)	Elevation Range (m)	Distance to Nearest Site (km)	Annual Rainfall (mm)	Habitat Vegetation Description
PUNA	8	110	0-300	~17.0	2001-3550	Short, mid story scrubby mixed 'Ohia and nonnative trees. Tall nonnative canopy. Dense native and nonnative understory. Widespread urbanization
HAVO	4	53	900-1200	~6.0	2001-2750	Short scrubby 'Ohia/Mamane with varying density native and nonnative understory. Patchy vegetation in some areas interspersed by lava fields
KEAUH	2	62	1500-1850	~6.0	2001-2750	Dense forest comprised of old-growth, native vegetation with areas of actively reforested pasture comprised of younger, open Koa plantations
KIPU	8	53	1500-1800	~9.0	2001-2750	Densely vegetated patches of variably size comprised of old growth, native vegetation. Patches are intermixed with short, young vegetation on young lava substrate.
HFNWR	3	50	1600-1900	~9.0	2001-2750	Dense forest comprised of old-growth, native vegetation with areas of actively reforested pasture comprised of younger, open Koa plantations

Acoustic Recording

Male HAAM are known to sing two songs: a primary song given solely by adult males and an infrequent sub-song given by either sex (van Riper III 1987). The primary male song, which can be described as a high-pitched, undulating trill (Figure 3) is the focus of this research. Male HAAM increase the rate of singing during the morning hours, with song rates decreasing through the day until just before dusk, when they increase again (van Riper III 1987). Singing rates increase during the breeding season, peaking in late October then again in late February. Recording efforts in this study were concentrated around these heightened singing periods from 2011-2013

Male HAAM vocalizations were recorded using a Marantz PMD661 professional field recorder (Marantz America, LLC.) connected to a highly directional Sennheiser ME 67 shotgun microphone (Sennheiser Electronic Corporation) with a Rycote Softie Cover all mounted in a Rycote Pistol-grip (Rycote Microphone Windshields Ltd) on a photographer's tripod. The Sennheiser ME 67 microphone has a frequency response of 40 Hz-20 kHz (+/-2.5dB) and a maximum input sound level at 125 dB @ 1 kHz (THD = 1%). All tracks were recorded in 24bit WAV format at a 44.1 kHz sampling rate.

Upon entry to recording sites, adult male HAAM were visually located with binoculars or generally located by listening for songs or calls. Adult males older than their second year are identified by their bright yellow plumage and fully developed, black streaked lores. No vocalization recordings were obtained from drab-green singing males because these are likely either juveniles or young second-year birds that may still be learning or practicing their song. Once potential adult males were located, individuals were followed and monitored until the identified adult male began vocalizing, thus

initiating a recording attempt. Visual confirmation of each recorded singing adult male was maintained during all singing bouts. An entire singing bout was recorded until the male stopped singing or flew off. If a male continued to sing after he moved off, the bird was pursued and recorded for as long as possible. Once a male was deemed no longer recordable, recording efforts were moved a distance of at least 25m away from the direction of the recently recorded male in order to reduce pseudoreplication and the chance of re-recording an individual male. Depending on visibility and accessibility, recording was done at a distance no further than 10-15m from the singing male in order to capture the entirety of the male's song in its highest quality. Perch type and height, behavior, and any relevant information were noted before, during, and after bouts.

In the KIPU and HFNWR sites, past and current banding efforts have yielded hundreds of individual HAAM that have been sexed and color-banded. These known individuals were identified during recording trials by their unique color band combinations.

Song Analysis

The Raven 1.4 (Cornell University) sound analysis program was used to generate spectrograms and analyze HAAM recordings. All recordings were opened in Raven using the same window presets and size (Hann window, 600 samples, 3 dB Filter Bandwidth 106 Hz). Only male HAAM songs of the highest acoustic and visual quality with minimal background noise or sound disturbance were used for analysis. Using only the clearest and pure songs allowed for accurate and precise measurements. Songs of poor quality or songs that overlapped with other forest birdcalls or ambient background noises were not used for analyses.

Using the analysis tool in Raven, measurements of song parameters and complexity were made following Tubaro et al. (1993), Irwin (2000), Westscott and Kroon (2002), Robin et al. (2011), and Pieplow and Francis (2011). Parameters measured for each song included: song length (seconds), high frequency (Hz), low frequency (Hz), frequency bandwidth (Hz), peak frequency (Hz), number of syllables, average number of notes per syllable, and average number of frequency direction changes per syllable (Table 2). High and low frequency are the maximum and minimum frequencies reached by the entire song. Frequency bandwidth, also known as frequency range, is the difference between the high and low frequencies attained by the entire song. Peak frequency, also known as the dominant frequency, is the frequency level at which the most energy is expelled within the song. Notes are defined as any continuous vocal utterance and a syllable is two or more notes grouped to form a single coherent unit (Marler & Isaac 1961). A frequency direction change is noted when a change occurs in the direction, whether ascending or descending change, within a syllable. Using these measurements, I was able to quantify and compare the complexity and structure of songs across all five recording sites, as well as between the two vegetation types replicated at KEAUH and HFNWR.

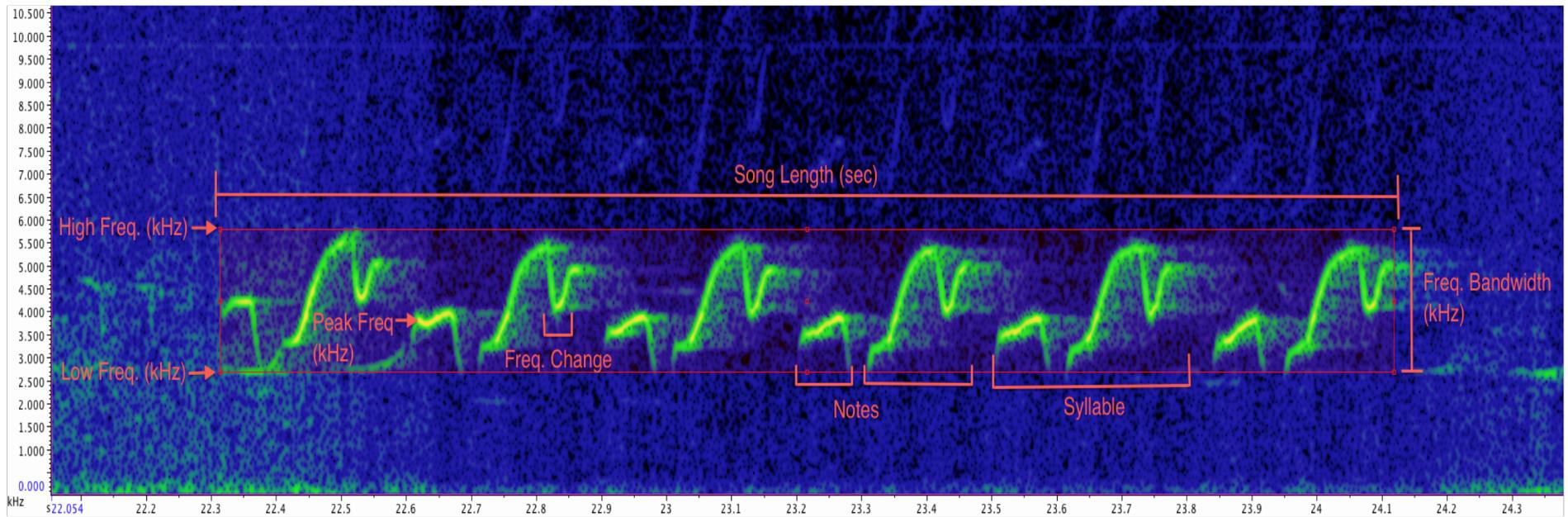


Figure 3. Representative HAAM song spectrogram with all 8 measured song parameters: song length (seconds), high frequency (Hz), low frequency (Hz), frequency bandwidth (Hz), peak frequency (Hz), number of syllables, average number of notes per syllable, and average number of frequency changes per syllable. All song parameters were measured using the Raven 1.4 sound analysis program.

Table 2: Description of 8 measured song parameters.

Song Parameter	Description
Song Length (sec)	Total length of song from beginning to end
High Frequency (Hz)	Maximum, highest frequency level attained over the entire song
Low Frequency (Hz)	Minimum, lowest frequency level attained over the entire song
Frequency Bandwidth (Hz)	Frequency range. Difference between the high and low frequencies
Peak Frequency (Hz)	Dominant frequency. Level at which the most energy is expended within the song
Notes per Syllable	A single, continuous vocal utterance
Syllable	Two or more notes grouped to form a single coherent unit
Frequency Direction Change	Change occurring in the direction, whether ascending or descending change, within a syllable

Classifying Song Types

Two separate visual song classification methods were used to measure the degree of variability in the visual song structure only across all 5 sites. Following the analysis of an individual HAAM song in RAVEN, a PNG image file was stored for each song. Each image retained a snapshot of the spectrogram in RAVEN with the highlighted HAAM song. Only images of analyzed songs were used for song categorization. An individual representative image of each recorded bird song was used for categorization. Sorting was not conducted for comparison between open and closed vegetation sites. Documenting and comparing visual song structuring within and between sites added to the measurements of complexity as well as comparisons of variability.

Classification 1

Using notable visual characteristics (e.g. unique shapes, note trajectories, undulations of notes, syllable complexity, etc.) song images were grouped into song types by first identifying general characteristics of the repeated syllables (e.g. presence or absence of notable large differences/similarities) that either separated or grouped song images.

From general characteristics, I continued to separate songs progressively with further detailed or specific similarity/differences. Separating song images into progressively detailed song types stopped when no notable, reliable differences in song images were observed. The result was a hierarchical breakdown of song images from all sites; similar to the way in which a phylogenetic tree is built using the genetic relatedness of groups (example in Figure 4). Site names or any part of image file names that connected it to a certain site were removed in order to decrease any site bias during separation and grouping.

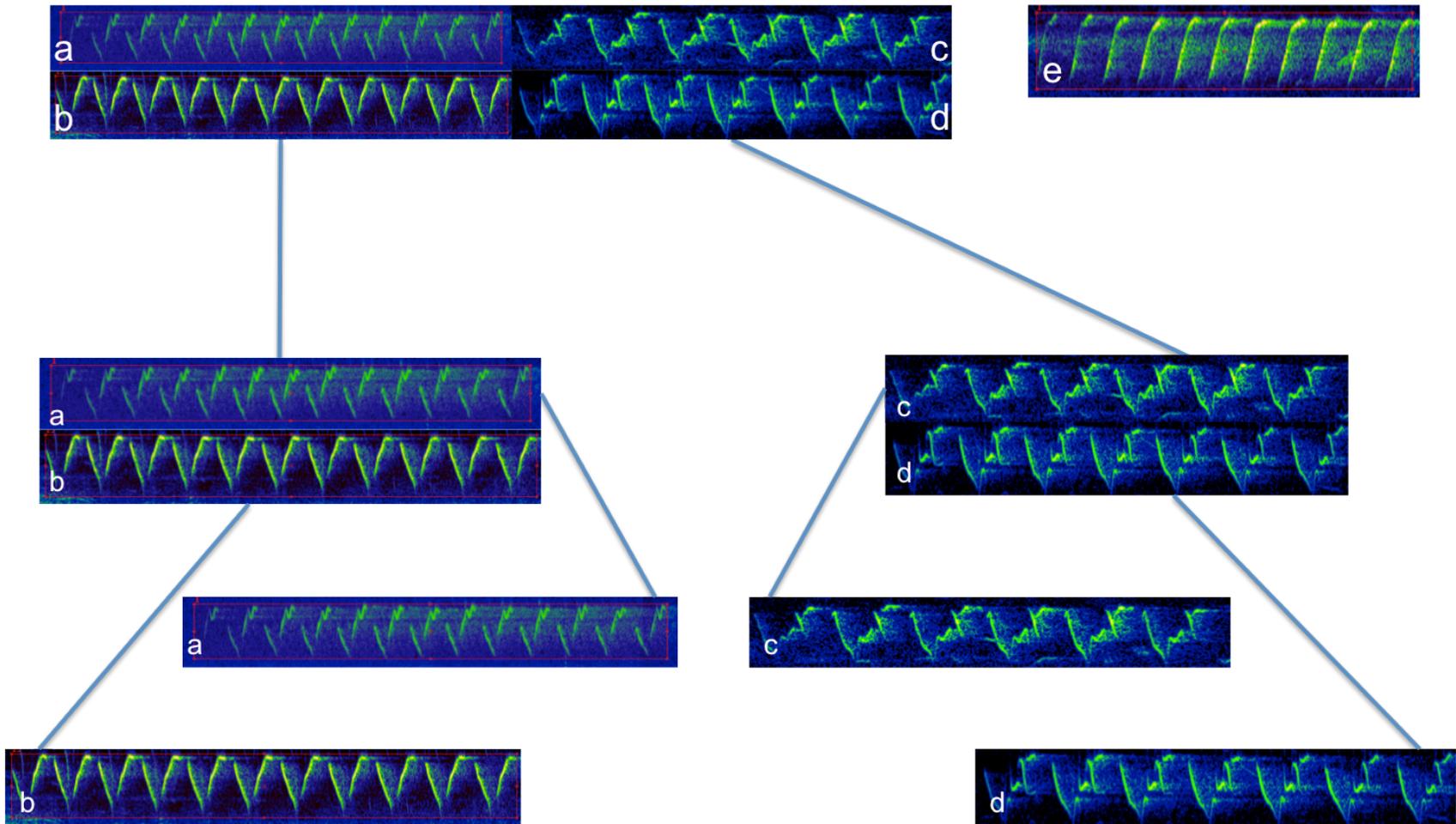


Figure 4: Example of song categorization 1. Songs a through d would be first grouped into one group due to the general check-mark shape they share, while image e would be placed in its own group. Then a+b and b+c would be grouped into two separate groups based on the shape of the upward ascending portion of the syllable. Finally, image b would be separated from image a based on the shape at the apex of the ascending note, and image c would be separated from image d based on the number of notes that make up the ascending portion of the syllable.

Classification 2

In order to further investigate variability in song structure and complexity, and to also complement the results of the first classification type, I conducted a second classification process of all saved song images from all sites. Song images were grouped by first identifying the common syllable unit that is repeated within the HAAM trill. Then the start and stop position of each individual note within the syllable was mapped from beginning to end using the abbreviated H for a note starting/stopping in a high position and L for a note starting/stopping in a low position. The result for each song image was a sequence of H and L (ex HLH or HLHLLH, example in Figure 5) mapping the pattern of high and low beginnings and ends for each note within the sequence of notes (or single note syllables) that comprise a single repeated syllable. Song images were then grouped together by the similar H and L sequences. A total of 41 H and L sequences ranging from simple LH or HL up to more complex sequences such as LHLLHLHLLH were identified. This method allowed for a non-subjective method visually classifying songs based on structure.

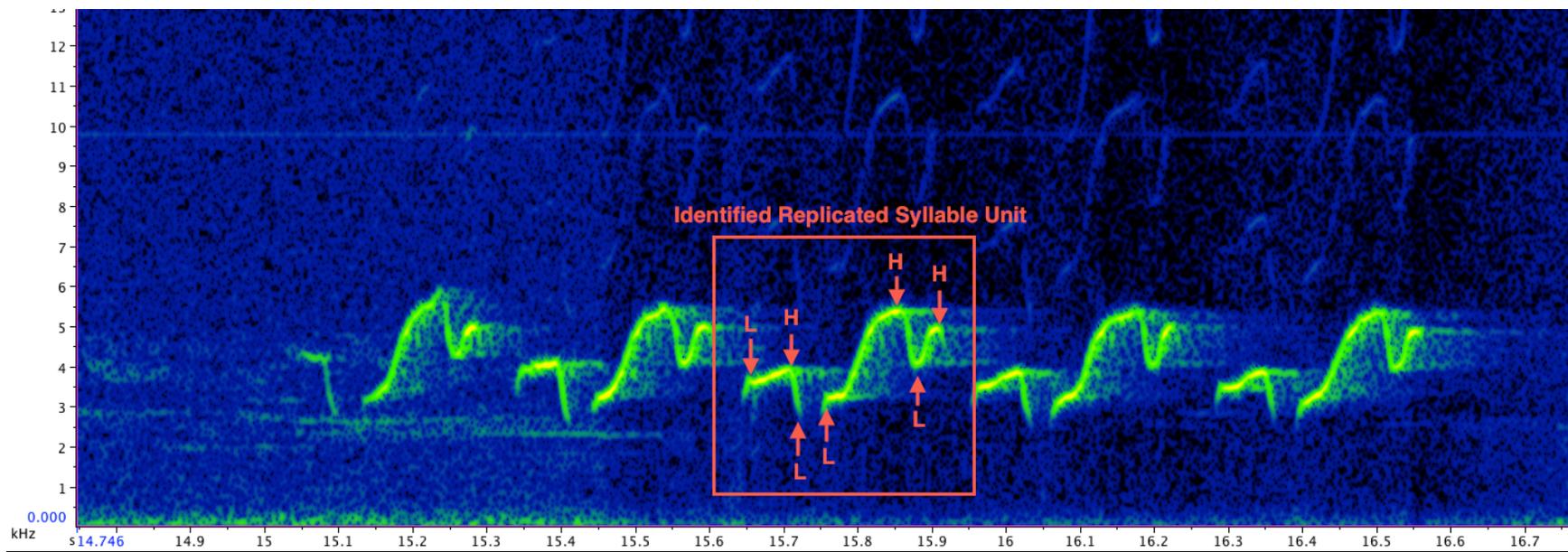


Figure 5. Example of song categorization 2 where first the replicated syllable unit is identified within a given HAAM song, and then the pattern of high and low start and stops are mapped. The above HAAM song would fall into a group labeled LLLH

Vegetation Measurements

In order to assess the effects of vegetation structure on HAAM song characteristics, I needed to quantify the differences in vegetation characteristics between both open Koa plantation and native closed forests at the HFNWR and KEAH sites. To demonstrate structural differences between vegetation sites, I conducted basic vegetation surveys using the point intercept (PO) crown method. PO methods allowed us to quickly and efficiently assess vegetation height structures as well as estimate ground cover in each vegetation site. The PO method is considered one of the most objective and efficient data collection methods to estimate cover values with minimal bias and error (Bonham 1989).

Twelve 50m transects were randomly placed in areas where recordings were obtained at each open and closed vegetation site. Transects were marked using a 50m long forester's open reel tape. Using a 5m long collapsible pole with marked intervals at .5m and 1-5m, a recorder started at 0m and at every 2m interval they would note how many plant crowns intercepted the pole and its trajectory in 8 categories: at 0-.5m, .5-1m, 1-2m, 2-3m, 3-4m, 4-5m, 5-10m, and at >10m. Plant crown intercepts were noted when the pole intercepted the outside edge or drip line of the plant crown being assessed with all of the spaces within the crown. Although the pole that was used was only 5m long, recorders were accurately able to assess the height of 10m by simply estimating double the poles height. To assure proper vertical placement of the pole at each 2m interval a plumb bob was used as an aid. A total of 24 transects were completed across the two open Koa plantation sites and two closed native forest sites totaling 600 individual sampling points.

Statistical Analyses

The statistical program R version 2.12.1 (R Development Core Team 2012) was used for all statistical analyses. Both R and the statistical program JMP were used to generate graphs. All data were initially saved in Excel and converted to the proper format to be imported into R. Excel was also used to generate any necessary data tables. All appropriate statistical tests used a set α of 0.05. I examined variance in song measures for individual HAAM within each site by examining means and 95% confidence intervals. Within site variances in song measures overlapped for >80% of all birds, demonstrating minimal within-site variance for unique song types.

Data selection and initial analysis: All 5 Sites

I recorded >1000 songs during the 2012 and 2013 HAAM breeding season at all five sample sites. Sample sizes at each site ranged from 50 – 110 birds. The number of songs analyzed from an individual bird ranged from a single song up to 15 usable songs. Song parameters were averaged for all birds with >1 analyzed song. Ultimately I used a data set containing song parameters from 329 total birds for analyses across all 5 sites.

I conducted a principal components analysis (PCA) on all 8 song parameters across all five sites and, following examination of PCA outputs, observed five components (PC) with Eigenvalues greater than 1 that accounted for 87.14% of variation within the data (Table 3). The variables that loaded maximally (loading >0.30) into the five PCs were song length, low and high freq, and freq bandwidth in PC1, # of syllables, notes per syllable, and # freq change into PC 2, song length, high freq, and notes per syllable into PC 3, low and high freq into PC 4, and peak frequency into PC5. Peak frequency was the only variable that did not load into PC1-PC4 (Table 4).

Table 3. Importance of each of the 5 retained principle components for the all five site PCA.

Importance of Principle Components: All Five Sites					
	PC1	PC2	PC3	PC4	PC5
Standard Deviation	1.4789	1.2285	1.1587	0.9985	0.9671
Proportion of Variance	0.2734	0.1887	0.1678	0.1246	0.1169
Cumulative Proportion	0.2734	0.4621	0.6299	0.7545	0.8714

Table 4. Loading value for each song parameter into each of the 5 retained principal components for all five sites PCA. Maximally loaded variables for PCA across all sites were those >0.3 absolute value highlighted in yellow.

Principal Component Loading: All Five Sites					
Song Parameter	PC1	PC2	PC3	PC4	PC5
Song Length	0.3186934	-0.285432857	0.5672891	-0.20359363	-0.02030642
Low Frequency	-0.513379	-0.052780474	0.0625347	-0.63643993	-0.017012
Highest Frequency	0.3389257	-0.058679146	-0.4500431	-0.66732182	-0.11323645
Frequency Bandwidth	0.6200008	0.004731216	-0.33341736	0.082512	-0.05798387
Peak Frequency	-0.1578642	-0.243968846	-0.08829383	0.17605474	-0.93026632
Number of Syllables	0.1890473	-0.65052123	0.29015315	-0.08618139	0.02803111
Notes per Syllable	0.2295967	0.367474755	0.44515242	-0.02402345	-0.14892492
Number of Frequency Changes	0.1490984	0.542715685	0.26536998	-0.24973501	-0.30782107

Data selection and initial analysis: Open vs. Closed Vegetation Sites

During the 2013 HAAM breeding season I recorded 1-15 usable songs from each of 42 birds from closed vegetation sites and 38 birds from open vegetation sites. Song parameters were averaged for all birds with >1 analyzed song. I conducted preliminary t-tests comparing both open sites at HFNWR and KEAH as well as both closed sites at HFNWR and KEAH and revealed no difference (p-value >0.05) in all 8 song parameters between our replicated sites.

I also performed a PCA on all 8 parameters measured from both vegetation sites. Following examination of PCA outputs, I found that five components (PC) with Eigenvalues greater than 1, accounted for 87.95% of variation within the dataset (Table

5). The variables that loaded maximally (>0.40) into the five PCs were low freq, high freq, and bandwidth into PC1, song length and # of syllables into PC2, peak freq, notes per syllable, freq change into PC3, high freq and notes per syllable into PC4, and peak freq, notes per syllable, and # of freq change into PC5 (Table 6)

Table 5. Importance of each of the 5 retained principle components for the open vs. closed PCA.

Importance of Principal Components Open vs. Closed					
	PC1	PC2	PC3	PC4	PC5
Standard Deviation	1.47230	1.30400	1.16140	0.99860	0.90670
Proportion of Variance	0.27100	0.21250	0.16860	0.12460	0.10280
Cumulative Proportion	0.27100	0.48350	0.65210	0.77670	0.87950

Table 6. Loading values for each song parameter into each of the 5 retained principal components for open vs. closed PCA. Maximally loaded variables for PCA across all sites were those >0.4 absolute value highlighted in yellow.

Principal Component Loadings Open vs. Closed					
Song Parameter	PC1	PC2	PC3	PC4	PC5
Song Length	-0.172886453	0.664363781	-0.091048808	0.177175902	-0.13964396
Low Frequency	0.50835277	0.073244059	-0.349279513	0.290657806	-0.353112173
Highest Frequency	-0.452872095	-0.180802781	-0.246222341	0.456097016	-0.27663889
Frequency Bandwidth	-0.653708814	-0.165290943	0.10619814	0.06618907	0.090270175
Peak Frequency	0.027187519	0.010501444	0.604536248	-0.395015955	-0.43407562
Number of Syllables	-0.118780476	0.639579155	0.293296067	0.179562166	-0.03618997
Notes per Syllable	-0.098872806	0.28784355	-0.418162181	-0.482192122	0.460240817
Number of Freq Changes	-0.233742578	0.034923445	-0.414082337	-0.500930973	-0.607931419

Final Statistical Analyses of Song Data

To test for differences in song between sites, I conducted a MANOVA (multivariate analysis of variance) with retained PC scores. However, I was only able to utilize the first two PC values from the PCA performed on all 5 sites. Multivariate tests require more degrees of freedom than response variables and therefore I was only able to

incorporate the two PC into a MANOVA in R. To test for differences between open and closed understory vegetation types, the first 5 PC values for each vegetation category were used in a MANOVA test.

Additionally, I conducted a discriminant function analysis (DFA, also known as linear discriminate function analysis (LDA) to examine and validate whether songs could be grouped according to site by the measured song parameters.

To examine which parameters led to differences in song between sites I conducted separate ANOVA for each of the PC scores used in the appropriate MANOVA. Tukey's honestly significant difference (HSD) post hoc tests were later used to examine the effect details within each ANOVA.

Vegetation Measures

To prepare vegetation data for statistical tests comparing differences in open and closed forest types at the HFNWR and Keauhou sites, I first computed the proportion (%) of vegetation 'hits' among each height category for each transect. This allowed for an investigation into the vertical distribution profile of vegetation for each transect and vegetation type. Proportions were then arcsine transformed in R. ANOVA was used to test for differences between overall open and closed vegetation values as well as differences between each category.

Results

Hawai'i 'Amakihi Song Structure and Variation Among Sites

The song of the Hawai'i 'Amakihi at all five of the study areas was comprised of a high pitched, undulating trill and was given solely by males, and was similar to that

described by vanRiper (1987). A multiple analysis of variance test demonstrated that male HAAM songs differed significantly across all five sampled sites ($F_{4,323}=23.5750$, Pillai=0.4520, $P<0.00001$). Post hoc ANOVA revealed that both PC1 ($F_{4,323}=34.112$, $P<0.00001$) and PC2 ($F_{4,323}=14.808$, $P<0.00001$) differed significantly among all five sites. Post hoc Tukeys HSD, which tests differences between each site against another, revealed varying degrees of differences between individual sites in both PC1 and PC2 (Table 7). PC1 (maximally loaded by song length, low and high freq, and freq bandwidth) differed significantly ($P<0.03$) between Puna-HFNWR, Puna-KEAUH, Puna-KIPU Puna-HAVO, HAVO-KEAUH, HAVO-KIPU, HAVO-HFNWR, KIPU-KEAUH, and KIPU-HFNWR. PC2 (maximally loaded by song length and # of syllables) differed significantly ($P<0.01$) between Puna-HFNWR, Puna-KEAUH, Puna-KIPU, HAVO-HFNWR, HAVO-KEAUH, HAVO-KIPU, and KIPU-KEAUH. Means and 95% confidence intervals for each song parameter from each site are reported in table 8.

Table 7. Results of a Tukeys HSD post-hoc test conducting site-by-site comparisons of each PC that was used in previous ANOVA and MANOVA tests. P-values were interpreted relative to an α set at 0.05.

All Sites TukeysHSD Post-Hoc		
Site vs. Site	PC1 P-Value	PC2 P-Value
KEAUH-HFNWR	0.26	0.97
KIPU-HFNWR	0.03	0.69
PUNA-HFNWR	0.00	0.00
HAVO-HFNWR	0.61	0.01
KIPU-KEAUH	0.00	0.26
PUNA-KEAUH	0.00	0.00
HAVO-KEAUH	0.00	0.02
PUNA-KIPU	0.00	0.00
HAVO-KIPU	0.52	0.00
HAVO-PUNA	0.00	0.92

Table 8. Means and 95% confidence intervals for each song parameter from each recording site

Song Length (Sec)			
Site	Mean	95% Confidence Interval	
HAVO	1.81	1.76 - 1.85	
HFNWR	1.75	1.71 - 1.78	
KEAU	1.81	1.76 - 1.79	
KIPU	1.81	1.76 - 1.85	
PUNA	1.66	1.62 - 1.69	
Low Frequency (Hz)			
Site	Mean	95% Confidence Interval	
HAVO	1826.09	1796.83 - 1855.35	
HFNWR	1856.87	1814.56 - 1899.17	
KEAU	1781.24	1738.65 - 1823.84	
KIPU	1919.53	1875.08 - 1963.98	
PUNA	2273.50	2228.36 - 2318.65	
High Frequency (Hz)			
Site	Mean	95% Confidence Interval	
HAVO	5769.24	5737.62 - 5800.85	
HFNWR	5917.19	5884.62 - 5949.75	
KEAU	5995.42	5956.91 - 6033.93	
KIPU	5745.48	5685.06 - 5805.90	
PUNA	5868.80	5833.84 - 5903.75	
Frequency Bandwidth (Hz)			
Site	Mean	95% Confidence Interval	
HAVO	3943.16	3900.30 - 3986.10	
HFNWR	4060.32	4010.90 - 4109.70	
KEAU	4214.18	4150.20 - 4278.15	
KIPU	3825.94	3772.23 - 3879.65	
PUNA	3595.29	3539.14 - 3651.43	
Peak Frequency (Hz)			
Site	Mean	95% Confidence Interval	
HAVO	4406.31	4315.81 - 4496.81	
HFNWR	4605.00	4488.83 - 4721.17	
KEAU	4310.76	4209.42 - 4412.10	
KIPU	4504.96	4416.91 - 4593.00	
PUNA	4577.34	4493.39 - 4661.29	
Number of Syllables			
Site	Mean	95% Confidence Interval	
HAVO	10.15	9.87 - 10.43	
HFNWR	8.39	8.16 - 8.61	
KEAU	9.14	8.84 - 9.44	
KIPU	7.33	7.13 - 7.53	
PUNA	9.46	9.16 - 9.76	
Number of Notes Per Syllable			
Site	Mean	95% Confidence Interval	
HAVO	2.46	2.34 - 2.56	
HFNWR	2.10	1.94 - 2.24	
KEAU	2.35	2.06 - 2.65	
KIPU	2.23	2.12 - 2.33	
PUNA	1.72	1.63 - 1.80	
Number of Frequency Changes			
Site	Mean	95% Confidence Interval	
HAVO	2.34	2.18 - 2.50	
HFNWR	3.38	3.24 - 3.51	
KEAU	2.59	2.45 - 2.74	
KIPU	3.07	2.83 - 3.31	
PUNA	2.04	1.87 - 2.22	

After an examination of mean song parameters, mean low frequency of PUNA HAAM song was nearly 400 Hz higher than any other site, whereas other sites only differed from each other by ~150 Hz (Figure 6).

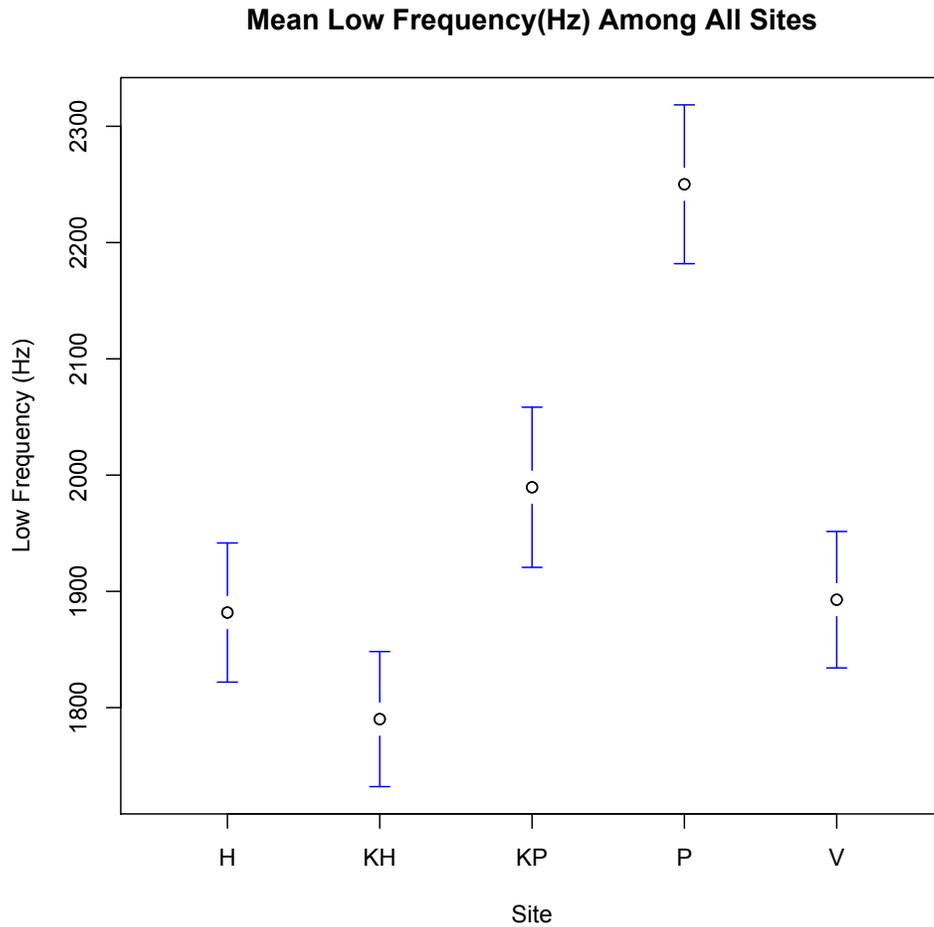


Figure 6: Plot of mean low frequency in Hz (open black circles), and 95% confidence intervals (blue lines) across all five sites: HFNWR=H, Keauhou=KH, Kipuka=KP, Puna=P, and HAVO=V.

Two linear discriminate functions successfully contributed to separating all 5 sites (Figure 7). LD1 demonstrated a proportion of trace of 0.68 (68% discrimination/separation) with song length, number of syllables, and # of frequency changes the most contributing song parameters (Figure 5). LD2 demonstrated a

proportion of trace of 0.22 (22% discrimination/separation) with song length, number of syllables, and # of notes per syllable the most contributing song parameters.

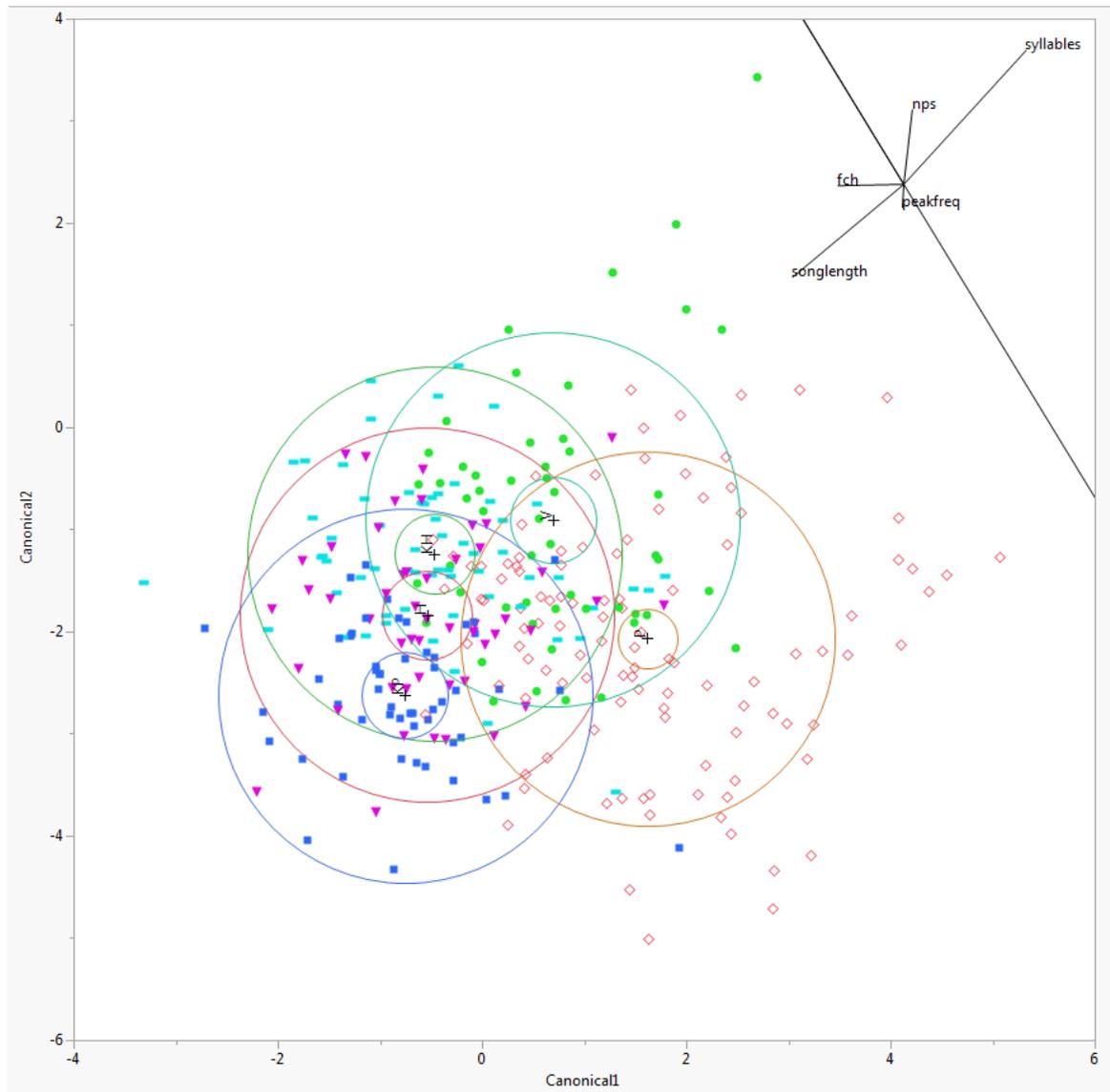


Figure 7 LDA canonical plot comparing song from 329 HAAM recorded during the 2011-2013 breeding season from 5 sites. Each dot represents a single HAAM bird. Circles represent 95% confidence intervals of the multivariate means of the 5 respective populations. Sites include the Kea'au/Puna area (P, open pink diamonds), Hawai'i Volcano National Park (V, green dots), Keauhou Ranch (KH, light blue rectangle) and adjacent Kilauea Forests, the Kipuka (KP, dark blue squares) areas south of saddle road on the flanks of Mauna Loa, and the Hakalau Forest National Wildlife Refuge (H, purple triangles).

Song Categorization

Categorization 1

Song categorization type 1 resulted in a total of 30 song types after a hierarchical breakdown of song structure (table 8). The number of song types varied across all five sites: Puna=14, HAVO=9, KEAUAH=11, KIPU 14, and HFNWR= 10 song types. To obtain a better understanding of how the number of song types reflects the variability or complexity of song structures within each population sample, I computed a simple ratio between number of song types and sample size of recorded individuals at each site (song type ratio). This resulted with KIPU demonstrating the highest ratio at 0.264 and the smallest ratio demonstrated by Puna at 0.127 (Table 9).

Table 9. Results of song categorization 1

Song Categorization 1 Results			
Site	Sample Size	# Song Types	Song Type Ratio
PUNA	110	14	0.127
HAVO	53	9	0.170
KEAH	62	11	0.177
KIPU	53	14	0.264
HFNWR	50	10	0.200

Categorization 2

Song categorization type 2 resulted in a total of 41 song types (table 9). The number of song types varied across all five sites: Puna=15, HAVO=12, KEAH=16, KIPU=21, and HFNWR=14 song types (Table 9). Song type ratio was greatest at KIPU and lowest at Puna (Table 10).

Table 10. Results of song categorization 2

Song Categorization 2 Results			
Site	Sample Size	# Song Types	Song Type Ratio
PUNA	110	15	0.136
HAVO	53	12	0.226
KEAH	62	16	0.258
KIPU	53	21	0.396
HFNWR	50	14	0.28

I also observed numerous song types from PUNA birds that were notably simple and basic in structure, compared to song structures from all other sites. Simple songs include those comprised of simple note shapes, such as the most basic song observed across all sites and found only in PUNA, described as a plain upslur note with no other offshoot structures (Figure 8). Unreported in the results, I also observed the lowest mean notes per syllable and lowest number of frequency changes from PUNA, both measures of complexity and variability in song. Although PUNA still shared various song types with other sites overall I noted the simplest types in numerous birds sampled and observed the lowest measures of complexity and variability from PUNA.

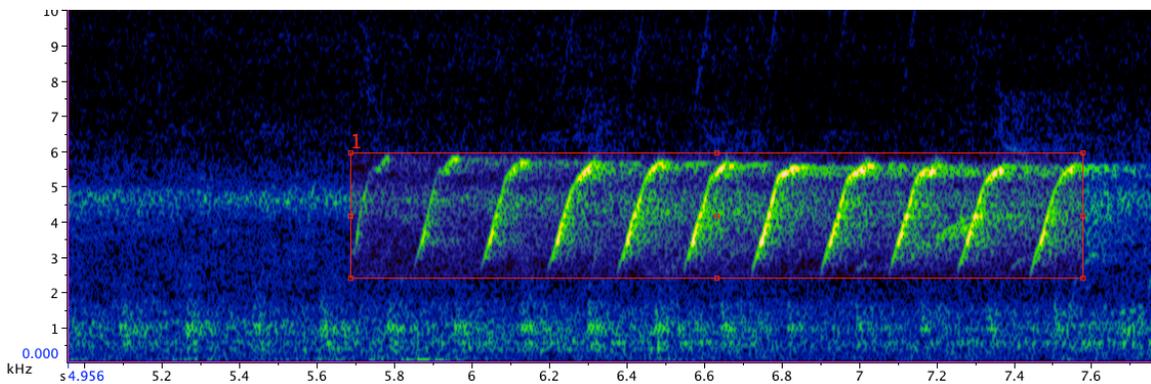


Figure 8. Example of the simplest HAAM song structure that I observed across all of our recording sites between all recording seasons. This song was found only in the Kea‘au/Puna area from more than one bird and from more than one recording site.

Song Differences Between Open & Closed Vegetation

Open vs. Closed: Vegetation Measurements

Analysis of vegetation structure measurements demonstrated highly significant differences between open and closed forest types at the HFNWR and Keauhou sites ($F_{1,46}$, $P < 0.00001$). Post-hoc analysis of variation demonstrated significant differences in all categories ($F_{1,46}$, $P < 0.0001$) except for the 0.5-1m category, where no difference was observed.

The open Koa plantation sites were comprised of close (~5m) to proximally spaced (20m) plantations of 15-25 year old Koa trees. The highest vegetation cover among these sites was observed in the 0m-0.5m category (69.65%) with the second most cover in the 0.5m-1.0m (9.08%) (Fig. 7). High vegetation values at the ground level below 1m were due primarily to the high abundance of extremely dense, tall, introduced pasture grasses at both open sites. In contrast to the structure observed in the open Koa plantation sites, closed vegetation values were much more evenly distributed across the vertical vegetation profile (Figure 9). Although the highest vegetation proportion value was in the 0-0.5m category (23.69%), both mid-story and canopy cover values were fairly uniform across the vertical profile (ranging from 8.05-15.82%). Unlike the 0-0.5m category in the open sites, which were dominated by dense pasture grasses, groundcover in native forests sites were primarily comprised of native ferns, tree and shrub saplings, and interspersed native and introduced grasses. Mid-story vegetation was made up of assemblages of various native tree ferns, dense shrubs, and tree species. Canopy trees (5-10m and >10m) were dominantly old-growth native Koa and 'Ohia (*Metrosideros polymorpha* spp) with some older trees normally found in the mid-story areas.

Open vs. Closed Proportion of Point Intercept Vegetation Cover Hits

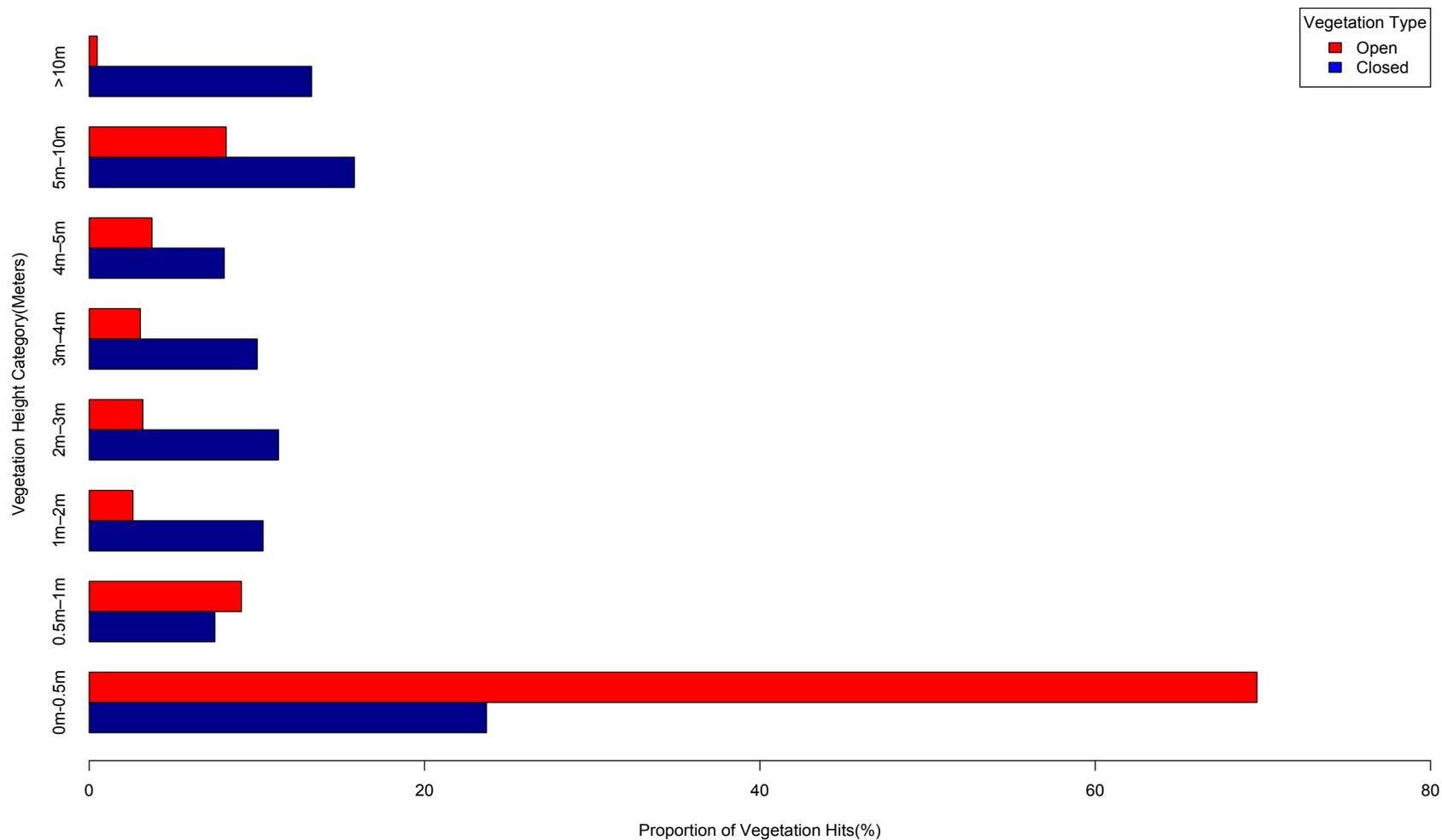


Figure 9. Horizontal histogram comparing the proportion of vegetation cover “hits” (x-axis) within each vegetation height category (y-axis) from the lowest, ground level (0-0.5m) to the highest canopy cover (>10m). Open sites were characterized by extremely dense ground cover comprised of pasture grasses, very few mid story structuring, with semi open canopy. Dense vegetation sights were characterized by more even structure with moderate vegetation at all height category

Open vs. Closed: Song Differences Between Open & Closed Vegetation

A multiple analysis of variance test demonstrated that male HAAM songs differed significantly between the open Koa forest plantation and dense native forests at the HFNWR and KEAH areas ($F_{1,78}=5.5923$, Pillai=0.27423, $P=0.0002$). Post hoc ANOVA tests demonstrated statistical difference only within PC3 (maximally loaded by peak frequency) between open and closed HAAM song ($F_{1,78}=26.548$, $P<0.00001$, Table 11). All other PC's were not significant in ANOVA tests. A linear discriminate analysis (LDA) was significant in separating the songs of individual HAAM by open and closed sites (Figure 10).

Table 11. Results of the post-hoc ANOVA I performed that compares each PC between open and closed vegetation sites. Significance was only observed in PC3, which was loaded maximally by peak frequency. P-values were compared to a α set at 0.05.

Open vs. Closed Post-hoc ANOVA Results		
PC	F-value	P-value
PC1	0.0003	0.9861
PC2	0.0976	0.7555
PC3	25.884	<0.0001
PC4	0.0187	0.8915
PC5	1.8836	0.1739

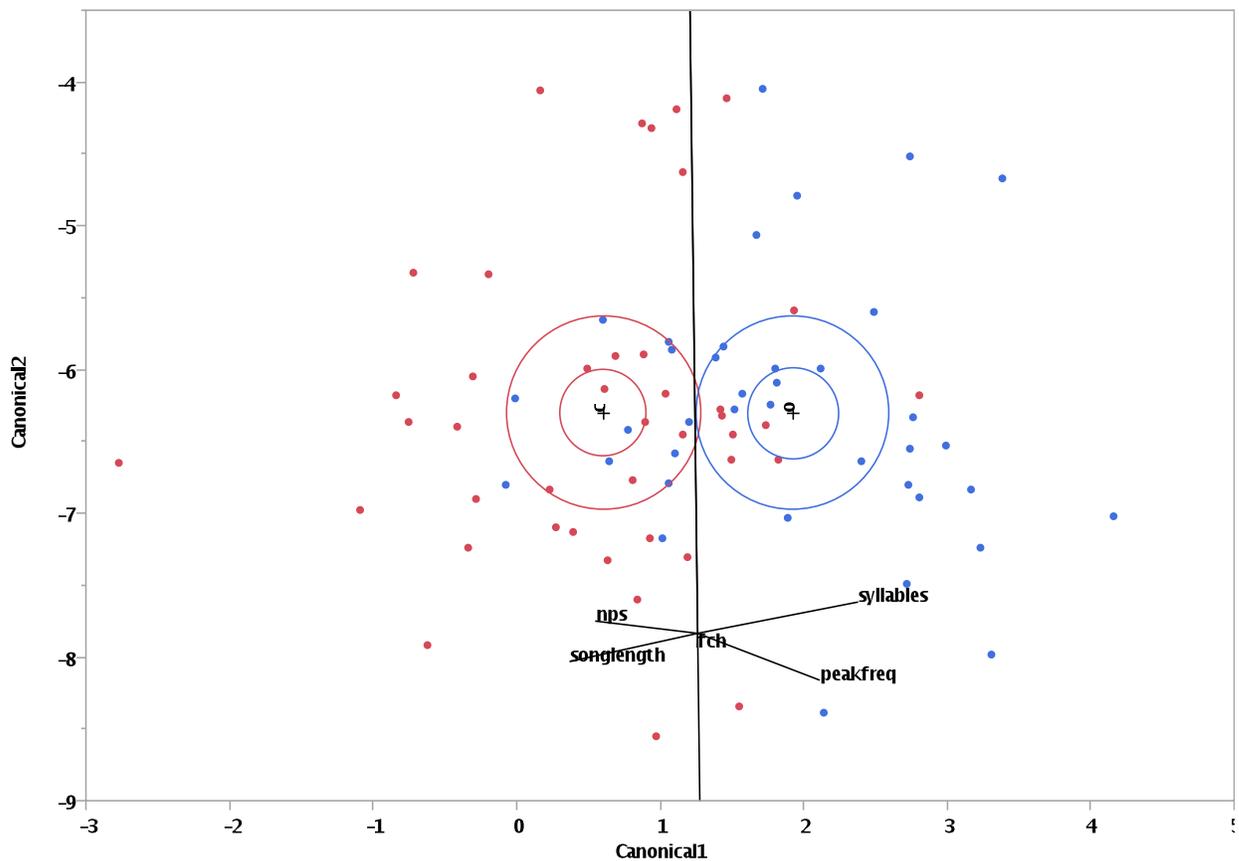


Figure 10. LDA canonical plot comparing song from 80 HAAM recorded during the 2013 breeding season from open (O, blue dots) and closed (C, red dots) vegetation sites at KEAUH and HFNWR. 42 birds were recorded from closed vegetation sites and 38 birds from open vegetation sites. Each dot represents a single HAAM bird. Circles represent 95% confidence intervals of the multivariate means of the 2 respective populations.

The results of our exploration into the effects of vegetation structure on song characteristics revealed strong differences in HAAM song between open Koa plantations and dense native forests. Of all the song parameters that were compared between sites, differences in peak frequency (frequency level with the most energy) were the most significant. Songs produced by birds from open vegetation sites had more energy at a higher frequencies compared to birds from closed vegetation sites, which produced songs with more energy at much lower frequencies (Figure 11).

Closed vs. Open Mean Peak Frequency(Hz)

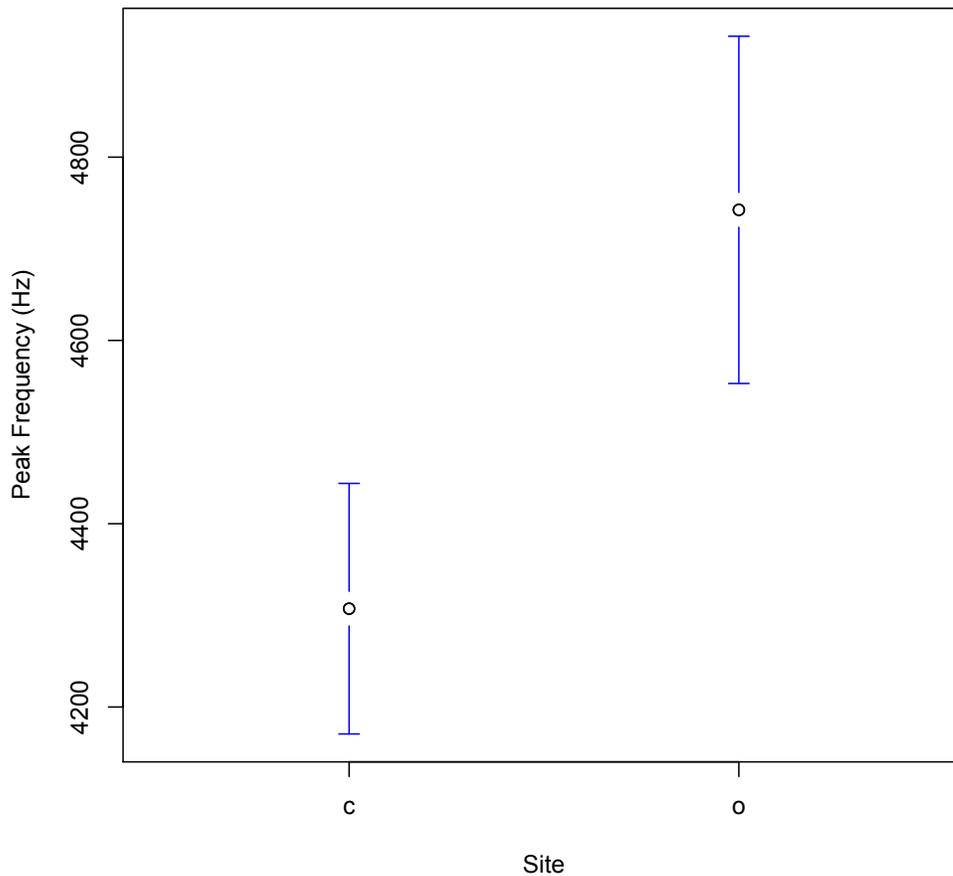


Figure 11: Plot of mean peak frequency in Hz (open black circles), and 95% confidence intervals (blue lines) for HAAM at closed and open vegetation sites.

Discussion

This study was the first to investigate acoustic variability in any native Hawaiian passerine species. In the past, researchers, birders, and conservationists have commented anecdotally on the audible variation and general differences among Hawaiian forest bird vocalizations across Hawai‘i Island. With this research, I was able to document differences in HAAM song parameters across all five sample sites on the windward side of the Big Island, and also to document differences in spectral characteristics of song between habitat with large differences in vegetation structure.

Song Variation Across All Sites: Isolation & Localized Song Learning

What may be driving the observed variation in song across our sample sites? The answer could come with a simple investigation into forces that drive genetic divergence. Population isolation and lack of connectivity between groups can dramatically limit mating opportunities between groups thus reducing gene flow (Robin et al. 2011). This effect may be analogous to song divergence in passerines. Isolation limits meme (i.e. song subunits) flow through reduced song sharing and singing exposure. Combined with the effects of drift and the dynamic interplay of localized song learning mechanisms (e.g. mistakes during song learning, this may result in local song dialect development over time. Song variation is known to exist microgeographically in White-crowned sparrows (*Zonotrichia leucophrys*, Nelson 1998), and exists at the continental scale for other species, such as the blue-tit (*Cyanistes caeruleus*, Doutrelant & Lambrechts 2001). Robin et al. (2011) demonstrated song divergence in 3 populations of white-bellied shortwing (*Brachypteryx major*) at high elevation, isolated sky islands in India, and accredited the observed differences to population isolation. Parker et al. 2012 also found evidence of song divergence in translocated, highly isolated populations of North Island saddleback birds (*Philesturnus rufusater*) in New Zealand.

The variation in song characteristics that I observed among our sampled HAAM populations likely owes to the combined effects of population isolation, drift, and localized song learning and sharing. Historically, populations of HAAM on Hawai'i Island presumably inhabited wider regions across the island and were more contiguous than today, as suggested by Foster et al. (2007) where HAAM populations around Hawai'i Island demonstrated shared, ancestral haplotypes. Prior to western contact, HAAM populations were likely isolated solely by natural phenomenon, such as vast lava

flows that cut through once continuous forest, natural breaks in forests, or high elevation mountain regions around the island. Currently however, our sampled populations, along with other unsampled populations surviving in different areas around Hawai‘i Island, exist in fragmented, isolated groups. Lava flows, large-scale anthropogenic habitat destruction, and avian disease have created isolating barriers that greatly reduce the mixing of individuals between populations. Subsequently, this has likely suppressed the dynamics of song sharing and meme flow on a large scale, contracting song-learning processes to localized scales. The result is most likely the variability demonstrated in this research: divergence in song parameters among populations and localized song variability unique to each group. These processes are highly analogous to the means in which isolating barriers lessen rates of gene flow, and coupled with drift, facilitate processes of genetic divergence.

Population song variation observed in this study parallels the findings of Foster et al. (2007), where significant population structuring in mitochondrial and nuclear DNA markers was observed in Hawai‘i ‘Amakihi, based on allele frequencies and on the abundance of private alleles. The observed population genetic variability occurred along an elevation gradient where sampling sites at low, mid, and high elevation demonstrated strong differentiation in DNA structuring (Foster et al. 2007). Multiple locations where DNA was sampled by Foster et al. (2007) at each elevation overlap with areas I sampled HAAM song, such as numerous areas across low elevation Kea‘au, Puna, and Kalapana, mid elevation sites within Hawaii Volcanoes Observatory, and within the Keauhou Ranch area. These areas at low, mid, and high elevation demonstrated notable song structuring and strong population variation, similar to the findings of Foster et al. (2007), suggesting possible analogous structuring in both song and genetics.

Song Variation Across All Sites: Why PUNA So Divergent?

Of the divergence observed among all HAAM populations, the unique separation of PUNA from the other 4 sites was the most notable and intriguing. HAAM populations in PUNA have become famous in recent years for their well-documented tolerance to avian malaria (*Plasmodium relictum*). For decades upon its arrival, widespread malaria decimated populations of native Hawaiian honeycreepers at low-elevation areas along the Puna and Kea'au coast. Extreme bottlenecking of HAAM populations occurred throughout low-elevation Puna, reducing populations to just few remnant groups. Yet despite high disease pressure and the prevalence of mosquitoes in low-elevation habitats, remnant HAAM populations developed a heritable tolerance to the disease, driven by constant selection pressures (Foster et al. 2007). Today, HAAM have since spread to areas once void of native birds, and are thriving in even denser populations elsewhere across Puna. These low elevation HAAM populations are currently isolated from their higher elevation neighbors (>1000 m). Foster et al. (2007) investigated whether these disease-tolerant populations of HAAM descended from migrants from higher elevation populations or were they simply descendents from a remnant population that survived the disease onslaught. Using DNA from current populations, as well as museum samples gathered from 1898 and 1948-49, they revealed that lowland HAAM are, and have historically been, differentiated from their higher elevation neighbors. Also, despite greater sampling at low elevation, genetic diversity was greater at high elevation areas. Mid elevation sites, however, were not as strongly different from high elevation sites.

The findings of Foster et al. (2007) provide evidence that the low-elevation HAAM populations contracted under disease pressures, yet eventually evolved survival mechanisms that allowed them to thrive under the same disease rich environment and

expand into areas void of native birds. This process may help clarify our observed separation of PUNA HAAM song from all other sites. When populations of HAAM became reduced during the years of avian disease epidemic, song variability and the pool of song types likely became reduced as well. Song variation experienced a bottleneck as populations shrank, parallel to the loss of genetic variation with reductions in population size. Over time, as disease tolerance developed allowing for HAAM range expansion across PUNA and Kea'au areas, founder effects (e.g. loss in variation or halting potential growth in song variation) may have occurred. For both types of song classification I conducted (1 and 2), PUNA was found to have the least amount of song variation, across all sites.

This process has been demonstrated by numerous investigations into similar instances of novel colonization events or contractions in population sizes and their effects on bird song. As mentioned earlier, Parker et al 2012 found evidence of song divergence in translocated-induced isolated populations of North Island saddleback birds (*Philesturnus rufusater*) in New Zealand. In this situation, extensive translocation of groups from a single threatened population onto protected islands of differing size and isolation, previously void of saddleback birds, allowed researches to track the induced bottleneck and founder events of North Island saddleback bird acoustics. Translocated birds demonstrated marked reduction in song variability, with two distinct song lineages developing in < 50 years (Parker et al. 2012). In another threatened New Zealand bird species, the North Island Kokako (*Callaeas wilsoni*), Valderrama et al. 2012 found that population growth rates and size were positively correlated with measures of song variability and diversity. In smaller populations, they witnessed smaller song repertoires and higher levels of song sharing (eg. lower song diversity/variability), owing to lower

availability and generation of novel song examples. Also, in fragmented populations, they observed evidence that suggest microevolution in song diversity that occurs in island colonization events (i.e. founder effects). These findings, along with many others, shed light and reasoning on possible processes that have driven the decline in song variability and complexity among PUNA HAAM.

On the other end, highest variability, measured as the number of song types relative to sample size, was observed across the KIPU area. I originally assumed that the HFNWR sites would exhibit the highest measures of variability and complexity of song types. HAAM populations at the HFNWR survive in the highest contiguous habitat and have the most stable population rates of all our sites (personal communication, unpublished data), which should allow for higher song variability and complexity (Valderamma et al. 2012). Conversely the KIPU area is a network of “forest islands” or patches of different sizes and isolation from each other, with an intermittent mix of short, rto inhibit bird movement between kipuka. The area is also adjacent to other areas of HAAM populations, such as the Hilo Watershed forests to the north, lower Waiakea Forests to the east, and Kulani forests in the distant south. This unique habitat configuration and arrangement could perhaps be facilitating higher rates of song complexity and variability. For instance, researchers from University of Michigan Tech (unpublished research) showed that HAAM in the KIPU area, along with other native forest birds, exhibit faithfulness to specific kipuka but move about the entire area quite frequently, visiting different kipuka and areas between throughout the year. Mixing about the area throughout the year equates to higher meme flow and sharing of song types, as a given learning bird is subjected to adult singing from numerous birds at any given time. Conversely, across HFNWR Paxton et al. (unpublished data) radio tracked I‘iwi

(*Vestiaria coccinea*) and ‘Apapane (*Himatione sanguinea*), two Hawaiian honeycreeper species thought to have dramatic movements throughout the year, and showed that they actually remain in an area for quite some time during the breeding season. Paxton et al. (unpublished data) also found similar behavior in other marked individuals from other native forest bird species, including HAAM. This suggests that HAAM populations at HFNWR are more sedentary in their movements during the breeding season, unlike the higher movement rates of their HAAM neighbors in the KIPU area.

Another possible influence on the notable divergence of PUNA populations from all other populations may be the effects of anthropogenic noise in suburban settings. Similar to the effects of vegetation density, songbirds have been shown to shift singing behaviors in urban settings by singing at higher frequencies to avoid sound masking by low frequency noise (Mendes et al. 2011, Slabbekoorn and den Boer-Visser 2006, Fernández-Juricic et al. 2005). Nearly all of our recording sites across the Kea‘au/Puna area were directly in or around suburban that contained automobile traffic, construction sites, and houses. All other sites were not in urban settings and are instead natural forested areas void of high urban noise influence.

Observed shifts in low frequency song parameters may represent a novel shift in Kea‘au/Puna HAAM song in response to increasing urbanization and the subsequent increase in low frequency noise. However, Nemeth and Brumm (2010) demonstrated with great tits (*Parus major*) and European blackbirds (*Turdus merula*) that adjustments in song frequency and pitch only had a marginal effect on increased communication distance in urban settings, whereas amplitude adjustments had a larger effect. Future research could further target high urban areas in the Kea‘au/Puna where HAAM exists

and compare song amplitude measures of HAAM in Kea‘au/Puna areas that are distant from sources of urban noise.

Song Variation Across All Sites: Other Possible Factors

Various factors that have the potential to influence bird song variability may be ruled out in our study of HAAM song. For instance climatic factors, such as precipitation, wind, temperature, and humidity are known to dramatically influence the absorption of sound in space (Kirschel et al. 2009). In American wood warblers (*Parulidae*), variability in climatic conditions and the subsequent absorption levels was shown to dramatically influence signal structures (Snell-Rood 2012). However, differences in climatic conditions are unlikely to have influenced our observed differences in HAAM song because all sites were in humid wet forests. Songbird populations that occur in different elevations have also been shown to differ in their song structure. For example, Green hylia (*Hylia prasina*) in sub-Saharan Africa sang at lower frequencies at higher elevations (Kirschel et al. 2009). An effect of elevation is unlikely in this study due to the strong song similarities between sites of different elevation ranges (e.g. HAVO-KIPU, HAVO-HFNWR, and HAVO-PUNA) as well as strong song differences between sites of similar elevation ranges (e.g. HFNWR-KEAUH, KIPU-HFNWR, and KIPU-KEAUH).

Other documented habitat and environmental parameters are perhaps accountable for the observed HAAM song variation across our sample sites. As mentioned in detail, habitat vegetation heterogeneity, such as vegetation densities, structure, and height, is a documented influence in shaping the acoustic structures of songbirds, and its effects are well summarized within the Acoustic Adaptation Hypothesis (Boncoraglio and Saino

2007). The overall driving force is a bird's evolutionary need to be able to optimally transmit its vocalizations through its given habitat.

While all study sites generally occurred within *Metrosideros polymorpha* forest, there was some dissimilarity in vegetation among sites. For example, the overall forest structure observed at the HFNWR, KEAUH, and KIPU sites were comprised of similar tall, old growth Koa and Ohia canopy with moderate to densely structured mid and understory native specie assemblages. The KIPU area, however, doesn't contain the same contiguous forests as KEAUH and KIPU. Instead it contains a unique mix of "forest islands" of different size and isolation surrounded by a matrix of native shrubs and short trees on young lava flows. Sampled sites across the HAVO area on the other hand, are generally a much shorter forest structure with openly spaced vegetation, comprised of mixed native/nonnative species assemblages. Lastly, the PUNA sites vary greatly among themselves from areas with tall nonnative trees such as albizia (*Falcataria moluccana*), ironwood (*Casuarina equisetifolia*), and cecropia (*Cecropia obtusifolia*), with short understory vegetation comprised of all nonnative species, to tall, densely structured forests throughout the vertical profile comprised of both native/nonnative species. All sites contain varying amounts of habitat disturbances that resulted in open corridors throughout the research sites.

Open vs. Closed Vegetation & HAAM Song

This observation supports the predictions of the acoustic adaptation hypothesis (AAH) as well as findings of other studies that examine the effects of vegetation on song structure. Because avian vocalizations are a central aspect of their social behavior, there should be a selective advantage to maximizing sound transmission fidelity within a given

habitat. Under the AAH, open vegetation allows for higher frequency song components and complexity, while dense vegetation selects for lower frequency components and less complex song. In a comprehensive meta-analysis of the evidence for the acoustic adaptation hypothesis, Boncoraglio & Saino (2007) demonstrated that of all song variables across the numerous studies they analyzed, peak frequency was the only variable that differed between habitats in the direction predicted by the AAH when their analysis was restricted to the Oscines. This is consistent with the general idea that vegetation structure selects for a sound that can achieve maximum broadcast. Although a given bird sings a song that spans a characteristic bandwidth, which may be structured spectrally (e.g. high and low frequencies) in a way that follows the AAH, song at peak frequency will be audible at larger distance from the singer than song structured at other frequencies (Boncoraglio and Saino 2007). In other words, adjusting peak frequency is more efficient in maximizing broadcast efficacy compared to adjusting overall song frequency.

In their meta-analysis, Boncoraglio & Saino (2007) also suggested that, excluding heritable shifts in peak frequency, some species might lack the acoustic phenotypic plasticity that allows for the adjustment of peak frequency that would result in notable differences between populations from different habitats. Learning plasticity in song denotes the ability for a learning bird to adjust peak frequency over time. Nemeth et al. (2013), however, provided strong evidence otherwise. In the common blackbird (*Turdus merula*), a species of songbird that thrives in both natural forests and noisy urban areas, they found that city birds adjusted their peak frequencies above the 2.2 kHz level to prevent masking by the low frequency noise of the city environment. Forest birds, in their natural setting without the disruption of urban noise, sang at a lower peak frequency level

1.8 kHz. Peak frequencies were suggested to change by either singing different element types that are naturally stronger at different frequencies or simply by shifting the spectral energy within their song (Nemeth et al. 2013). Slabbekoorn and Peet (2003) demonstrated very similar shifts in frequency by great tits (*Parus major*) to avoid masking in urban conditions, and suggested that learning plasticity coupled with broadcast maximization selection allow for frequency shifts. Species lacking plasticity may lose on breeding opportunities or other social benefits. Song plasticity may represent a general behavioral mechanism allowing bird species to maximize their reproductive and social success in heterogeneous sound environments, such as urban vs. forest settings or in our study, HAAM populations in open vs. closed vegetation habitats.

An interesting aspect of our findings is the short period since our open habitat sites were transformed. Although ancient Hawaiian people frequented the HFNWR and the KEAUAH area, no large settlements or agriculture were established and thus their impact on habitat structure was likely minimal (Tomonari-Tuggle 1996, personal communication). Historical logging of large native hardwoods is the oldest significant disturbance at both of our sites, dating back to some of the earliest foreigners requiring wood for ship maintenance as well as early wood trades. It was, however, selective and likely didn't result in wide scale clearing. The onslaught of intense land clearing for ranching and subsequent browsing and trampling by ungulates had the greatest impact that led to the widespread, open understory vegetation that exists today. Ranching at both sites date back from the mid to late 1800's and continued on till the mid '90s at HFNWR and 2003 at KEAUAH. This puts our open sites at a 120-160 year old age. In that relatively short time scale, our findings suggest that HAAM residents shifted their peak frequency to maximize the efficacy of their broadcast signals.

Other components of HAAM song, such as high/low frequency, may not have had ample time to shift towards an optimal transmission levels. Otherwise, perhaps the selective pressures imposed by vegetation aren't strong enough to transform the entire song structure. Rather, shifts in peak frequency may represent a sufficient modification in achieving maximal sound transmission within each habitat. Peak frequency could possibly be a flexible characteristic of HAAM song, similar to previously presented examples, enabling it to shift relatively quickly. In all likelihood, dramatic clearing of forest habitats relaxed the signal attenuating pressures faced by birds in densely forested habitats. It is quite possible that song be flexible enough that HAAM are able to shift peak frequency levels when moving between open and closed habitats.

It should be noted that unlike in our song comparison between vegetation, peak frequency was a weak contributor to the variation observed across all vegetation sites. In our principal component analysis that involved all measured parameters across all sites, peak frequency did not load maximally until PC5, which contributed the least to our observed variability. This may denote that either vegetation may not be a proper explanation for the observed song differences across all sites, or other frequency or temporal song parameters fit the vegetation structure at each given site. Proper vegetation measurements at each site and in-depth analysis of song parameters could perhaps shed light on this subject.

Future Research

The pioneering nature of this research project, coupled with the lack of acoustic research in Hawai'i, and the fragility of Hawai'i forest birds invoke numerous questions about the acoustic environment of Hawaii's songbird species and populations. For

instance little is known if individual HAAM maintain the same song type or song parameters from year to year. In this research, I was unable to record song from known, banded individuals in consecutive years to compare if individual song differs over time. However, I was able to visit the exact same recording sites in HAVO in our second year of recording, and no new song types or song structures were observed in the second year. Proper procedures would involve recording known, banded birds throughout different times of the year and also over consecutive years to see if song shifts over time.

In order to completely understand the effects of vegetation on HAAM song and to determine whether each site has distinct acoustic environments, controlled playback experiments could be performed following Tobias et al (2009) and Nicholls & Goldizen (2006). These experiments would aim to quantify the transmission properties of each vegetation type. This would involve a standard approach of broadcasting and rerecording previously recorded and filtered HAAM sound stimuli in the different habitats and quantifying the extent to which the sounds are degraded.

Another interesting area of study could investigate song recognition by HAAM populations. Although I demonstrated song differences by populations, are songs actually an honest signal indicative of a bird belonging to a certain population? Are individuals from a population able to distinguish between songs from their own population versus a song from another? Methods of song playback would be appropriate to answer such questions. By playing high quality local song (e.g. songs belonging to a population) and foreign male song to focal male and female HAAM in the breeding season, researchers could test whether geographic song variation contains identifiable traits that are functional to a certain population. Westcott and Kroon (2002) followed the same protocol in the Golden Bowerbirds (*Prionodura newtonia*). In comparing song from 5 isolated

populations they demonstrated marked geographic variation in multiple song traits. They then conducted playback experiments that involved broadcasting local and foreign advertisement song to a focal breeding pair and measuring their response. In these experiments, males responded more strongly to song from local dialects than from foreign dialects (Westcott and Kroon 2002).

With song variation demonstrated across our windward Hawai'i Island HAAM populations it would be interesting to extend our scope and incorporate populations from elsewhere on the island. Numerous other HAAM populations exist all around Hawai'i Island from Manuka and Ka'u populations to the south, Pu'uwa'awa'a and Honaunau populations to the east and southeast, and perhaps the most northern Hawai'i island populations that occur in the Kohala mountain range. HAAM populations also occur at high elevation Mauna Kea and Mauna Loa mountain areas. Researchers could visit these sites, record and analyze HAAM song, and perform the same statistical tests to build an island wide perspective of song variation about Hawai'i Island.

Larger scale investigations may also compare song differences among the different species and subspecies of 'Amakihi that survive on the other Hawaiian Islands. Prior to 1995, 'Amakihi on Kaua'i, Oahu, Maui, and Hawai'i were considered a single species: the common 'Amakihi (*Hemignathus virens*). Work with mitochondrial DNA by Tarr & Fleischer (1993) was later used to separate groups resulting in the Kaua'i 'Amakihi (*Hemignathus kauaiensis*), 'Oahu 'Amakihi, and two subspecies of the Hawai'i 'Amakihi occurring on Maui (*Hemignathus virens wilsoni*) and our sampled bird on Hawai'i Island (*Hemignathus virens virens*). How are songs within the subspecies of the Hawai'i 'Amakihi on Maui and Hawai'i Island? Do differences and similarities

correspond with patterns of genetic differences across all species of ‘Amakihi across their statewide range?

Summary

This acoustic study was the first to ever embark on an investigation into the acoustic realm of any native Hawaiian passerine species. I was able to demonstrate notable variation in Hawai‘i ‘Amakihi song across 5 research sites on the windward side of Hawai‘i Island, likely due to population isolation and localized song learning are likely the initial driving force. This research project also provided strong evidence of song divergence in ‘Amakihi song between open and closed vegetation areas, supporting well-established hypotheses underlying the Acoustic Adaptation Hypothesis. We can only hope that our research will invoke active inquisition into areas of acoustic research with Hawaii’s native forest birds, thus continuing to forge novel areas of research in the islands. Hawaii’s native honeycreepers are a precious resource that is unfortunately famous for dramatic population declines and fragility, especially in the face of climate change. Acoustic research, along with all areas of scientific study, must continue in Hawai‘i before its forests go silent forever.

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