AEDES MOSQUITOES IN HAWAII

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By

Jonathan C. Winchester

Thesis Committee:

Daniel Rubinoff, Chairperson

Durrell Kapan

Shannon Bennett

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CHAPTER 1. HISTORY OF MOSQUITOES IN HAWAII

1.1. Introduction

The presence in Hawaii of insect disease vectors presents risks to public health, economic well-being, and conservation of rare native species. Mosquito-borne dengue and avian malaria are just two important examples impacting Hawaii in the last century. While geographic isolation limits the arrival of alien species, the rarity of these immigration events also makes the islands uniquely vulnerable in several ways. Island ecosystems are susceptible to invasion (Elton 1958; Denslow 2003), and this includes invasion by vectors, pathogens and parasites (Riper III et al. 1986). Further, human populations in islands with low endemicity of disease tend to be immunologically naive (Cliff and Haggett 1984), and political support and institutional memory for epidemic disease prevention may be poor when the interval between epidemics is long (Slosek 1986; Chadee and Rahaman 2000).

Mosquitoes are a family of flies (Diptera: Culicidae) in which the adult females generally feed on the blood of vertebrates. The blood feeding adaptation exposes mosquitoes to a wide variety of pathogens, and they often vector disease from one host to the next, both within and between species (Marquardt 2005). Although Hawaii has no native mosquitoes, six species of biting mosquitoes have been introduced. Two of these mosquitoes, *Aedes aegypti*, commonly known as the yellow fever mosquito, and *Aedes albopictus*, the Asian tiger mosquito, are efficient *Flavivirus* vectors and have been responsible for epidemics of dengue in Hawaii and elsewhere (Effler et al. 2005; Gubler 1989). *Ae. aegypti*'s arrival over a century ago was followed a few years later by *Ae. albopictus*, and both species dispersed throughout the islands. *Ae. aegypti* disappeared from the two northernmost major high islands, Kauai and Oahu, near the end of World War II, and declined further with the *Aedes aegypti* Eradication Program (AaEP) in the 1960s.

Although the general outline of these events is well known, a thorough understanding of the historic record of introduction, population growth and interaction between these mosquito species is important to understand how these disease vectors came to be where they are today. In particular, *Ae. aegypti* is more anthropophilic and accounts for higher levels of human disease transmission worldwide (Lambrechts et al. 2010): its displacement by *Ae. albopictus* has potential disease impacts. Hawaii is a model for examining the mechanisms behind this displacement. Here we analyze the historical records of mosquito introductions in order to understand the factors that have led to the current distribution of Hawaii's mosquitoes. These factors may include some or all of the following: changes in climatic conditions; changes in the landscape; changes in dispersal rates. Importantly, all of these factors might be influenced, intentionally or otherwise, by human activities such as mosquito control.

1.2. Methods

Literature search was conducted utilizing Pubmed (http://pubmed.org), the Armed Forces Pest Management Board's Defense Pest Management Information Analysis Center Literature Retrieval System (AFPMB LRS; http://afpmb.org/lrs), Google Scholar (http://scholar.google.com), and Google Books (http://books.google.com). All literature was reviewed which contained the keyword "Hawaii" in combination with "mosquito." Sources containing the words "mosquito eradication" or "*Aedes* eradication" were reviewed for mention of Hawaii. The literature describing Hawaii's dengue fever epidemic of 1943-1944 referred to older accounts of the insects' introduction to Hawaii. In many cases these references cited even earlier sources written by early visitors to Hawaii. I reviewed these primary sources for information regarding mosquito introductions and their presence in particular times and places. I obtained some documents from the Hawaii Department of Health (HI DOH) and the U.S. Department of Health, which were available from state archives and archives at the Vector Control offices in Aiea and Hilo. I also interviewed entomologists at HI DOH for information on the most recent *Aedes* mosquito surveys, but none of the state employees who conducted the *Ae. aegypti* eradication work in the 1940s - 1960s are still alive (C. Jacobsen, pers. comm.). I reviewed records and documents from several other sources: Centers for Disease Control and Prevention (CDC), the Army Office of Medical History, and the library of the Florida Medical Entomology Laboratory in Vero Beach. Medican entomologist Dr. Duane Gubler, Sc.D., formerly of the University of Hawaii, provided a number of out-of-print records on Hawaii mosquitoes. Gordon Patterson (author of *The Mosquito Wars* and *The Mosquito Crusades*) provided helpful advice on locating people and records connected to mid-twentieth century mosquito control. Unfortunately just as in Hawaii, I was not able to find anyone left alive on the U.S. mainland who was involved with the Hawaii portion of the *Aedes aegypti* Eradication Program (AaEP).

Significant events related to mosquito history in Hawaii are summarized in a timeline (Figure 1.1) and presented in two sections: first, significant events in the introduction and spread of mosquitoes; then, records related to mosquito-borne disease transmission and consequent public health human activities. Results of statewide surveys for *Ae. aegypti* presence locations are mapped in Figure 1.2. I follow by discussing the current state of vector control in the state and implications for public health.

1.3. Results

1.3.1. Introductions

The Hawaiian Islands were free of mosquitoes before Captain Cook's first landing in the islands in 1778 and for several decades thereafter, a fact confirmed by native and European writers (Malo 1903). The insects' inability to complete their life cycle without fresh water prevented them from reaching the islands during the days of canoe travel, but it seems they traveled readily in the freshwater stores of European sailing ships. Differing accounts of the first encounter with biting flies in Hawaii—which must have been noteworthy to those who had never known this annoyance—indicate there may have been multiple introductions, but every account is within a year or two of 1826 (Figure 1.1). The creation of the new Hawaiian language word "*makika*" is further evidence that in spite of the "hordes of flies" reported by Captain Cook's assistant, mosquitoes were not then present (Brigham 1911; Bryan 1915, 388). This first mosquito to arrive in Hawaii was *Culex quinquefasciatus*, a nocturnally feeding species whose affinity for foul water with high organic content made it well suited to Hawaii's flooded wetland taro farms. Although it is now present on all the islands, the dispersal took many years, and the mosquito, Hawaii's only vector for avian malaria, is not a serious pest or disease vector to humans.

Aedes aegypti, Hawaii's second mosquito, was noticed several years before the arrival of *Aedes albopictus*, the third introduction. The time frame can be inferred from the observations of two British entomologists, Percy H. Grimshaw and R. C. L. Perkins, who worked in Hawaii in the 1890s, recorded in *Fauna Hawaiiensis*, the first attempt to catalog all of the Hawaiian Islands' land animals. *Ae. aegypti* was not noticed by either author until after 1892, and *Ae. albopictus* had become common by 1907. Grimshaw, in his *Diptera* volume, notes the presence of *Cx. quinquefasciatus* mosquitoes in 1892, but does not mention *Aedes* (Grimshaw 1901, 6). However, Perkins, in his introduction (sometime between 1901 and 1910) writes:

> "[*Stegomyia*] *scutellaris* [i.e., *Aedes albopictus*] is of comparatively recent importation, as it did not come under my notice during the earlier days of my collecting, though now very numerous and conspicuous. Both it and *Stegomyia fasciata* [i.e., *Aedes aegypti*] are very troublesome not only on the lowlands but also in parts of the lower forests" (Perkins 1913, clxxxi).

Allowing that *Ae. aegypti* may have been present but not yet widespread in 1892, it is likely that it was present five to fifteen years before *Ae. albopictus.* Although it was common on most of the main islands, *Ae. aegypti* is only recorded once on Kauai, by entomologist D.D. Bonnett (Hess 1957). Perkins may have overlooked its absence there during his surveys, or it may have been common and then declined in a trend similar to that in Honolulu.

Although *Ae. aegypti* had the advantage of arriving first, it did not long remain the dominant mosquito in Honolulu. The ratios of *Ae. aegypti* to *Ae. albopictus* in city mosquito surveys from that period (Anon. 1949), show a trend of *Ae. albopictus* displacing *Ae. aegypti* during the period from 1910-1920 (data in Table 1.1; timeline inset on Figure 1.1). This pattern of decline in *Ae. aegypti* is typical after the introduction of *Ae. albopictus* and is currently being observed on the island of Bermuda (Kaplan et al. 2010). In Honolulu the ratios and abundances of mosquitoes were quite variable depending on location, as are rainfall, temperatures and wind across the city, but *Ae. albopictus* was consistently present throughout the lowland forests and upper valley residential areas, to the extent that it was known as the "forest mosquito," in contrast with *Ae. aegypti,* the "town mosquito" (van Dine 1904). It is interesting to note that while very low numbers of *Ae. aegypti* were found in 1948, just before its disappearance from Honolulu, the mosquito had recovered from a similar low in 1926. Usinger (1944) attributed *Ae. albopictus'* abundance in Honolulu to the forested ridges that extend down into the city, and suggested that eradication of this species would never be possible.

Similarly, Bonnett (Anon. 1949) reported that the *Ae. aegypti* individuals found in 1948 were all from the flat coastal areas of Honolulu (between Beretania Street and the ocean).

Other mosquitoes introduced to Hawaii are not linked to transmission of any human or animal disease there. Briefly, they are: *Ae. vexans nocturnus*, first noticed in 1962 (Joyce and Nakagawa 1963); *Wyeomyia mitchellii*, arriving around 1980 (Shroyer 1981); *Ae. japonicus japonicus*, detected (on the Big Island only) in 2004 (Larish and Savage 2005). Mosquitoes in the genus *Anopheles*, many of which are notable for their ability to vector human malaria, occasionally arrive on Oahu[1], but they have never established a permanent population (Furumizo et al. 2005). Finally, four species of non-biting *Toxorhynchites* mosquito were introduced in hopes that their predatory larvae would reduce populations of the pest mosquitoes[2]; at least two of these are still common on one or more islands (pers. obs.).

1.3.2. Early *Aedes* control efforts

The present-day distribution of *Ae. aegypti* and *Ae. albopictus* populations in Hawaii is influenced not only by their order of arrival, but also by human efforts at vector control and eradication. Soon after the *Aedes* mosquitoes arrived in Hawaii, public perception of mosquitoes began to develop from simple annoyance into a realization of the hazard they posed for vector borne epidemics. During this period it was demonstrated that mosquitoes vector malaria (Ross 1898) and yellow fever (Reed et al. 1900). In 1900, Hawaii experienced a traumatic epidemic of Bubonic plague (Ikeda 1985) with two important outcomes: the burning of the city's Chinatown district required the payment of over two million dollars compensation, and a sanitary inspector was appointed to initiate a vector control program (Morris 1995). In 1903, Hawaii's first major dengue epidemic infected an estimated 30,000 people. By 1907 dengue had been shown to be transmitted by mosquitoes (Cleland et al. 1916, Cleland et al. 1919). While dengue ("boo-hoo fever")

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1 *Anopheles* was first trapped on Oahu about 1920 (van Zwaluwenburg 1927), followed by one in 2003 (Furumizo 2005) and most recently in 2010 (I. Sutherland, pers. comm.). Joyce (1961) records 17 dead specimens from fogging (disinsection) of arriving aircraft (Joyce 1961).

2 These were *Toxorhynchites inornatus*, in 1929 (Williams 1931); *Tx. brevipalpis* in 1950 (Bonnett and Hu 1951); *Tx. amboinensis* in 1951 (Nakagawa 1963); and an unsuccessful attempt to introduce what is probably now known as *Tx. hypoptes* in 1953 (Hu 1955).

was believed to be less dangerous to white people than to native Hawaiians (Morris 1995), yellow fever was much more feared. The 1878 Mississippi Valley epidemic had killed thousands of Americans (e.g., 5000 in Memphis, Tenn.) and cost an estimated $100 million. During the 1898 Spanish-American War, more soldiers lost their lives to yellow fever than to combat (Army Medical Department 1976). However, the feasibility of defeating the disease via mosquito control was proven repeatedly, first in Havana, then in the construction of the Panama Canal, as well as the quelling of a 1905 New Orleans outbreak (where 4046 had died in 1878), and the elimination of yellow fever from Rio de Janeiro in 1908 (Pan American Health Organization 1992). With these events in recent memory, health officials in America's newly annexed tropical territory had good reason to conduct an aggressive mosquito control campaign.

The first recorded effort toward mosquito control in the territory is also one of the first examples of successful biological control of mosquitoes. At the suggestion of the USDA medical entomologist L. O. Howard, in 1904 the territorial legislature funded a biologist to travel to Texas to acquire live specimens of the top-minnow *Gambusia affinis* and return them to Honolulu for breeding and distribution throughout the state (van Dine 1907, Pemberton 1963). While this effort succeeded in reducing numbers of *Cx. quinquefasciatus*, the minnow was not effective in controlling *Aedes* mosquitoes because the small temporary containers used by this species are inaccessible to water-borne predators like fish.

In 1910 the Territorial Board of Health sent an employee to spend six months studying yellow fever elimination methods in South and Central America (Morris 1995). A case of shipboard yellow fever in 1910, followed by a single case of yellow fever transmission in Honolulu in 1911 (the only one to ever occur in the state) underscored the danger, and a permanent force of forty mosquito inspectors was established in 1912 (Morris 1995). Over the following years, the hundreds of thousands of inspections, thousands of legal citations, and miles of ditches dug coincided with the changing mosquito ratios described already (Figure 1.1 inset). Hawaii continued to have dengue cases until 1915 (Usinger 1944), after which none were reported for almost 30 years.

Like many American cities, Honolulu nearly eliminated *Ae. aegypti* from the city and with the disappearance of dengue after 1915, mosquitoes faded from public concern. A new danger was worldwide influenza epidemics from 1918-1919, which included 12,000 cases in Hawaii (Barry 2004). In the following decades Hawaii mosquito control was inconsistent at best, and was described by a CDC official and later Mosquito Control Bureau chief as "rather hit-or-miss" (Hess 1957, Nakagawa and Mikuni 1958). Hawaii's mosquito struggles during the 1920s - 1930s are described in local newspapers, which noted when mosquito complaints rose to newsworthy levels. Health officials would issue reminders about larval habitat reduction, sometimes accompanied by increased home inspections. Inspectors looked for mosquito breeding habitat, sealed the openings of cesspools, applied oil to cesspools and swamps, and collected refuse such as cans and tires. For example, a 1921 article announces the success of the Board of Health "war on mosquitoes," which included a district-by-district sweep of the city's problem areas (Anon. 1921), but by 1927, a headline announced "mosquitoes pestiferous again," with an official blaming "carelessness of public" (Anon. 1927). Territorial government attention to the issue was poor enough that the Chamber of Commerce formed its own mosquito and rat control program in 1930. During the Great Depression, the Works Projects Administration (WPA) supported some large scale labor intensive projects for malaria control on the mainland (Medical Department 1976), but in general, attention to mosquito control probably declined in Hawaii as it did elsewhere (Lipman 1935).

1.3.3. Great Depression

During the 1930s, on the mainland, mosquito scientists were publishing enthusiastic recommendations for the eradication of mosquitoes by such methods as filling or draining wetlands and importing predators. The leading scientists in this campaign were on the U.S. east coast, initially in New Jersey but increasingly from Florida as well. They developed formulations and procedures for controlling mosquitoes with poisons like Paris Green (copper(II) acetoarsenite) and mechanical means like spreading diesel oil on water surfaces to prevent larvae from breathing. In 1939, an amazing new weapon for agricultural insect control was discovered, dichlorodiphenyltrichloroethane (DDT). This event coincided with the nation's preparations for war, and DDT quickly was adopted as a major tool for the new organization called Malaria Control in War Areas (MCWA, precursor to the CDC), created with the mission to create mosquito-free zones around every area supporting the war effort. Their history is described by Gordon Patterson in *The Mosquito Crusades* (Patterson 2009). Experts who for years had spent as much effort fighting for funding as they had fighting mosquitoes were suddenly given virtually unlimited resources, and when they had succeeded to the point that no more mosquitoes could be found in military areas, they didn't stop. "Mobile units consisting of an entomologist and an engineer with a truck full of supplies and equipment…roamed the countryside looking for danger [i.e., from mosquitoes]" (Etheridge 1992, 16). In 1942, this organization had 4000 staff members.

1.3.4. World War II

Meanwhile, Hawaii's population nearly doubled from its pre-war level, with roughly half a million military and construction workers (Allen 1950, 219), and the combination of troops living in field camps, some fresh from training on the mainland, and others refitting from fighting in the Pacific Theater, made ideal conditions for mosquito-borne disease. In July 1943, the first cases of dengue fever in over 30 years were reported in Hawaii, beginning with commercial airline pilots who had recently returned from Fiji to a boarding house in Waikiki (Usinger 1944). Although the epidemic would eventually be contained to around 1600 cases, at the beginning there was no way of knowing whether it would be comparable to the 1903 outbreak or even larger. Mosquito borne epidemics have historically had major impacts on military campaigns (Medical Department 1976), and dengue in Hawaii, the United States' major Pacific stronghold and hub, was regarded as a very serious threat. An all-out effort was begun to control the outbreak. Mosquito control personnel were quickly shifted from the mainland to Hawaii, and local hires and detailed military personnel added to their ranks, until about 1500 people were assigned to mosquito control at the peak of the epidemic (Wilbar 1947). The intensity of this effort (1500 in Hawaii compared with 4000 for the entire mainland) was very high. The Army and Navy implemented or increased their programs of aircraft and ship spraying and inspection (Wilbar 1947). Every house and property in Honolulu was inspected (Figure 1.3), sometimes as often as every ten days (Medical Department 1976); rubbish items capable of holding water were collected for trash or recycling (Figure 1.4). Each sick person was quarantined under netting during the daytime, and houses "in and around a focus of cases" received repeated chemical spraying treatments: pyrethrum and oil on the vegetation, and "commercial fly spray" inside the houses (Usinger 1944). The epidemic mainly affected the flat coastal areas of Waikiki and Kakaako.

Just as they had on the mainland, MCWA workers broadened their scope after the initial job was done, first to rural areas of Oahu, then to the neighbor islands, but they did not completely eliminate the yellow fever mosquito (Nakagawa and Hirst 1959). In 1946 federal assistance ceased, and the Territorial board of health resumed total control, maintaining a minimal program with about 30 permanent mosquito control personnel assigned to Honolulu and Hilo (Nakagawa and Hirst 1959). These men continued the job that the MCWA had nearly finished, and after about 1949 *Ae aegypti* was never again seen on Oahu (Nakagawa and Hirst 1959).

1.3.5. Post-war

The same characteristic that makes *Ae. aegypti* dangerous as a disease vector is also its weakness; eradication was possible because of this species' affinity for humans. *Ae. aegypti* are anthropophilic (meaning they feed on humans) and endophilic (meaning they rest indoors), characteristics that allow them access to water, food and shelter in urban landscapes where these resources are otherwise rare (Christophers 1960). However, these same characteristics make them easy to control. In contrast, *Ae. albopictus*, preferring to breed and rest in vegetation or forests (Usinger 2007), could never be permanently eradicated in Honolulu because no part of the city is far from forested ridges. It is interesting to note that Waikiki and Kakaako, the main foci of epidemic transmission in 1943-1944, are the flat parts of the city furthest from the mountains, and therefore may have had relatively higher ratios of *Ae. aegypti* to *Ae. albopictus.*

Elsewhere in the world, after the combat ended and focus shifted to occupation and rebuilding, there was a military-like campaign to eradicate *Ae. aegypti*, the yellow fever mosquito, beginning mainly with South American governments (Slosek 1986; Soper 1963; Schliessmann 1967). A new enthusiasm for top-down disease control became evident at this time, capitalizing on public health and epidemiological lessons learned during the war. The US Public Health Service was created in 1944, the MCWA was expanded to become the CDC (initially Communicable Disease Center, today the Centers for Disease Control and Prevention) in 1946, and the World Health Organization was created in 1948. Having seen the complete eradication of *Anopheles gambiae* in Brazil (from 1930-1940) followed by early successes with *Ae. aegypti* in South America (in the 1950s), and perhaps looking for a way to build Cold War allies in the hemisphere, the United States CDC initiated its own campaign against the yellow fever mosquito with the launch of its *Ae. aegypti* Eradication Program (Schliessmann 1967).

Initially the AaEP focused on the continental USA and its southern neighbors. Hawaii received attention later on for two reasons. First, to be successful in the long term, the AaEP would have to achieve worldwide eradication of the domestic variety *Ae. aegypti* (Soper 1963; Gubler 2004); even though remote, Hawaii's mosquitoes still posed a risk not only to people in Hawaii but also to any other place Hawaii's ships or planes might arrive with mosquito eggs. Second, in 1956 state health officials were alarmed to discover *Ae. aegypti* had spread to Hilo on the Big Island, and requested help from the CDC in assessing and controlling the spread (DOH records, Hilo, accessed October 2010). This resulted in a 1957 statewide survey organized by the CDC (Figure 1.2a), which found the mosquito widespread on the Big Island, and also present on Molokai (but not nearby Maui and Lanai - together these three islands are called the Maui Nui complex), nor on the more northern islands of Oahu and Kauai (Hess 1957). While state personnel had made their own control efforts by focusing on eliminating larval habitat (source reduction) in Hilo and other areas, they did not have the resources or experience to organize an island-wide eradication effort. However, less than a decade later, the mainland AaEP was successful enough that its leaders could devote some personnel to focus fully on Hawaii, launching a promising eradication effort that began with another statewide survey in 1966 (Figure 1.2b). This program used the methods described above for Honolulu in 1943, with an organized schedule that divided the larger islands into sub-districts, in which each community was to receive inspections and treatments, repeated as many times as necessary. It was acknowledged that eradication would be difficult in the large areas inhabited by *Ae. aegypti* in Kona (Hess 1957, Wells 1968). After a promising beginning in Hawaii, in 1968, US involvement in the AaEP ended, influenced by concerns about DDT and by budget cuts. This left Hawaii with *Ae. aegypti* definitely eradicated from Kauai and Maui, but with their status unclear in the other islands of the

Maui Nui complex (although they were never again reported in these three islands) and the Big Island.

The next picture we have of *Ae. aegypti* in Hawaii is in 2002. A dengue fever epidemic that infected 122 people in Maui and Oahu was the first instance of dengue transmission where *Ae. albopictus* was thought to be the only possible vector (Effler et al. 2005). To confirm this, HI DOH conducted a thorough survey of all the main islands in 2002 (Figure 1.2c), in which they failed to find *Ae. aegypti* anywhere except dry, mainly leeward, areas of the Big Island (DOH 2002).

In early 2011, four confirmed dengue cases were reported from Honolulu and outlying communities. The only vector known on the island (Oahu) is *Ae. albopictus*. At the time of this writing (June 2011), the state has no mosquito control program due to funding cuts (Nakaso 2011), and new cases are still being reported.

1.4. Discussion

While Honolulu records in the first few decades of the 20th century show a decline in the proportion of *Ae. aegypti* resembling the pattern seen in similar areas when *Ae. albopictus* invades (Table 1.2). *Ae. aegypti* did not disappear from the islands during the 1890s-1940s, even after dropping to less than 1% frequency at the time of the 1926 Honolulu survey. This is an indication of the species' resilience and ability to persist as relict populations that re-invade suitable habitat. However, today there is no DDT use, and mosquito control receives relatively little attention. Air and boat traffic between the islands must provide ample immigration opportunity, so it is not clear why *Ae. aegypti* has not reinvaded its former habitat. Honolulu still has mosquito habitat—the bromeliads, lilies and pots in gardens and balconies still catch water and breed mosquitoes; the human refuse and natural containers that hold rainwater still exist—but something must be different, and understanding this is a key motivation behind my research. There are three related hypotheses for the lack of reinvasion to former *Ae. aegypti* areas: a change in niche, dispersal limitation, and competition. In Chapter 2 I will use a species distribution modeling tool to describe differences between the areas where *Ae. aegypti* persists and the areas where it has disappeared. In Chapter 3 I will look for patterns of co-existence or exclusion over time at specific Big Island sites where *Ae. aegypti* exists today.

These questions are highly relevant to public health. *Ae. aegypti* is the more dangerous of the two vectors because of its preference for biting humans. Since most dengue cases are mild, infected people may easily be well enough to travel and the virus is introduced to Hawaii fairly frequently (6–10 travel-related cases per year that do not result in on-island transmission, S. Bennett, pers. comm.). With only *Ae. albopictus* on Oahu (the most densely-populated island with over a million people), recent dengue outbreaks have not reached high numbers or long duration. If *Ae. aegypti* were to return to its former range on all the islands, both the likelihood and the cost of a dengue epidemic would increase substantially.

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Tables

Table 1.1. Changes in the relative abundance of *Ae. aegypti* and *Ae. albopictus* (Bonnet 1949).

Year	Percent *Ae. albopictus*	Percent *Ae. aegypti*
1911	50	50
1912	80	20
1913	33	67
1914	91.6	8.4
1915	91.6	8.4
1926	99.2	0.8
1943	85	15
1944	93.5	6.5
1948	99.3	0.7
1949	100	0

Table 1.2. Examples of outcomes from *Aedes aegypti* and *Ae. albopictus* encounters worldwide. Generally, *Ae. aegypti* is likely to become or remain dominant in hot tropical urban centers, while *Ae. albopictus* is likely to become or remain dominant in cooler areas with high forest cover.

Area	**Established Species**	**Invading Species**	**Outcome**	**Reference**
Cities in SE Asia (Bangkok, Calcutta, Kuala Lumpur, Manila)	*Ae. albopictus*	*Ae. aegypti*	*Ae. albopictus* decreased or disappeared in urban areas	Rudnick 1965 (Bangkok), Senior-White 1934 (Calcutta), Chan et al. 1971 (Singapore), Stanton 1920 (Kuala Lumpur).
Rural areas in SE Asia	*Ae. albopictus*	*Ae. aegypti*	*Ae. albopictus* remained common or no change noted	same as above
Continental US (most areas)	*Ae. aegypti*	*Ae. albopictus*	*Ae. aegypti* decreased or disappeared	Hobbs et al. 1991, Hornby et al. 1994, O'Meara et al. 1995
Urban areas in Southern US	*Ae. aegypti*	*Ae. albopictus*	*Ae. aegypti* broad decline, but some sites unaffected, some sites lost *Ae. aegypti*.	Hornby et al. 1994, O'Meara et al. 1995
Guam and Manila	*Ae. aegypti* (suppressed population)	*Ae. albopictus*	*Ae. albopictus* replaced *Ae. aegypti* after mosquito control	Gilotra et al. 1967
Honolulu	*Ae. aegypti*	*Ae. albopictus*	*Ae. aegyp*ti decreased	Chapter 2
Bermuda	*Ae. aegypti*	*Ae. albopictus*	*Ae. aegypti* populations crashed	Kaplan et al. 2010

Table 1.3 Locations positive for Ae. aegypti in the 1957, 1966, and 2002 surveys (Figure 1.2). Place names were georeferenced by searching the location in Google Earth, or by adjusting if the reference provided more specific information.

Island	Location	Reference
Maui	South edge Kihei	Hess 1957, Appendix C
Maui	North edge Kihei (2 miles from prev.)	″
Molokai	West of Pukoo	″
Molokai	West of Ualapue	″
Molokai	2.5 mi west of Kaunakakai	″
Molokai	Kaunakakai	″
Hawaii	Kailua	″
Hawaii	Keauhou	″
Hawaii	Honaunau	″
Hawaii	Kawaihae	″
Hawaii	Kawaihae Park	″
Molokai	Kaunakakai	Wells 1968, page 2
Hawaii	Honokaa	″
Hawaii	Kawaihae	″
Hawaii	Puako	″
Hawaii	Mahukona	″
Hawaii	Milolii	″
Molokai	Kaunakakai	Wells 1968, Table 7
Molokai	Kaulamaula	″
Maui	Kihei	Wells 1968, Table 10
Lanai	Waikapuaa	Wells 1968, Table 14
Lanai	Maunalei	″
Hawaii	Milolii	Wells 1968, Table 19
Hawaii	Opihali	″
Hawaii	Alae	″
Hawaii	Kawaihae	″
Hawaii	Mauna Loa (Manuka)	Wells 1968, Table 20
Hawaii	Hawi	″
Hawaii	Hilo (Banyan Drive)	Wells 1968, page 33
Hawaii	near Hilo	″
Hawaii	Hilo	″
Hawaii	Mauna Kea Resort	VCB 2002, Table 3
Hawaii	Puako Village	″
Hawaii	Holualoa Cemetery	″
Hawaii	Kealakekua - Upper	″
Hawaii	Captain Cook - Lower	″
Hawaii	Pebble Beach	″
Hawaii	Papa Bay Estates	″
Hawaii	Milolii Fishing Valley	″
Hawaii	Oceanview Estates	″
Hawaii	Kehena Estates	″
Hawaii	Pahala	VCB 2002, Table 2
Hawaii	Punaluu (Sea Mountain)	″
Hawaii	Naalehu	″

Figures

Figure 1.1. Timeline of mosquito introductions (below the line) and related public health events (above the line). The inset chart shows the number of Ae. aegypti as a percent of total Aedes mosquitoes, during the period the species was in Honolulu.

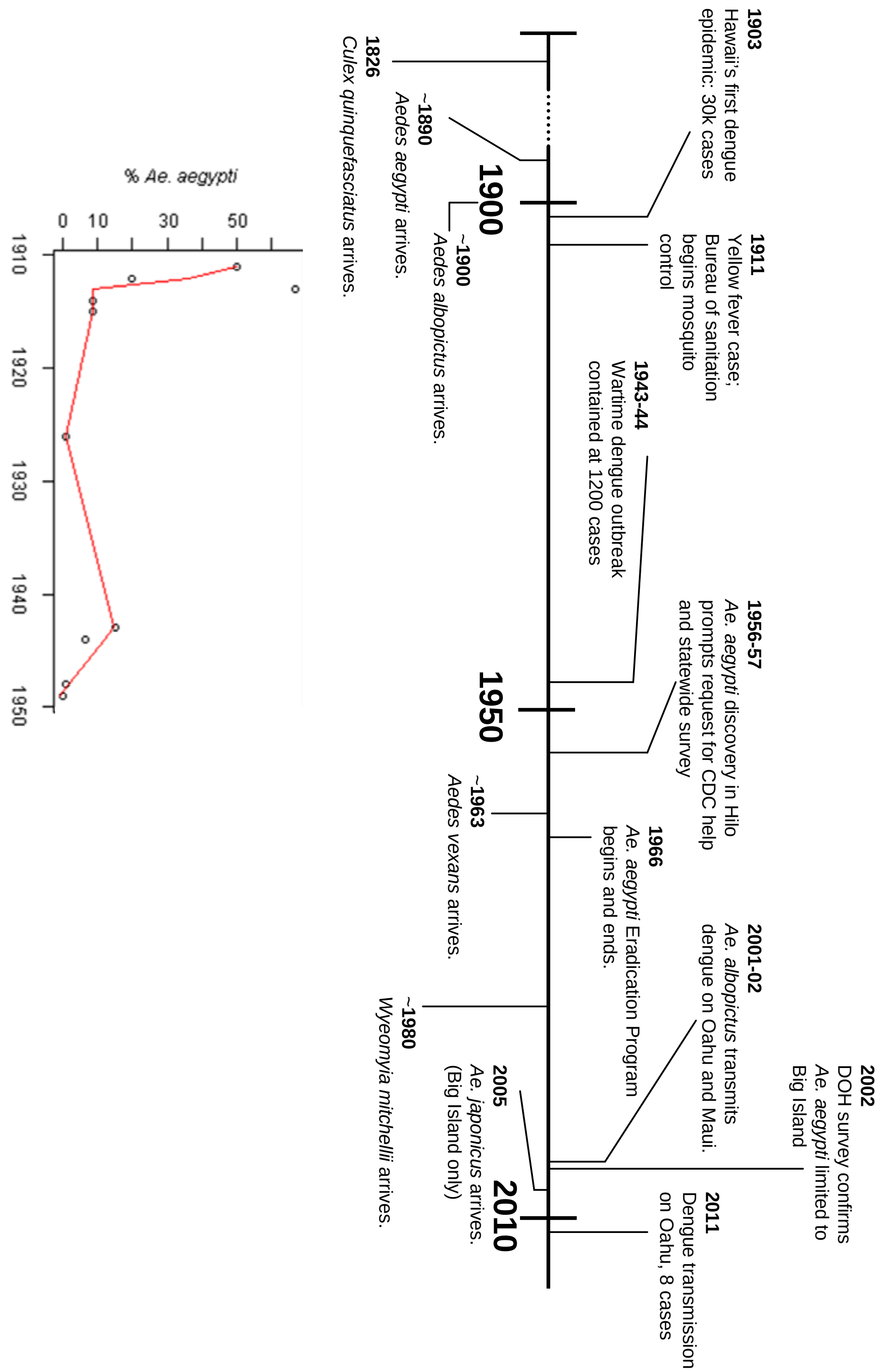

Figure 1.2. Ae. aegypti positive locations in surveys before (1957 & 1966) and after (2002 & 2010) the Ae. aegypti eradication campaign (see Table 1.3).

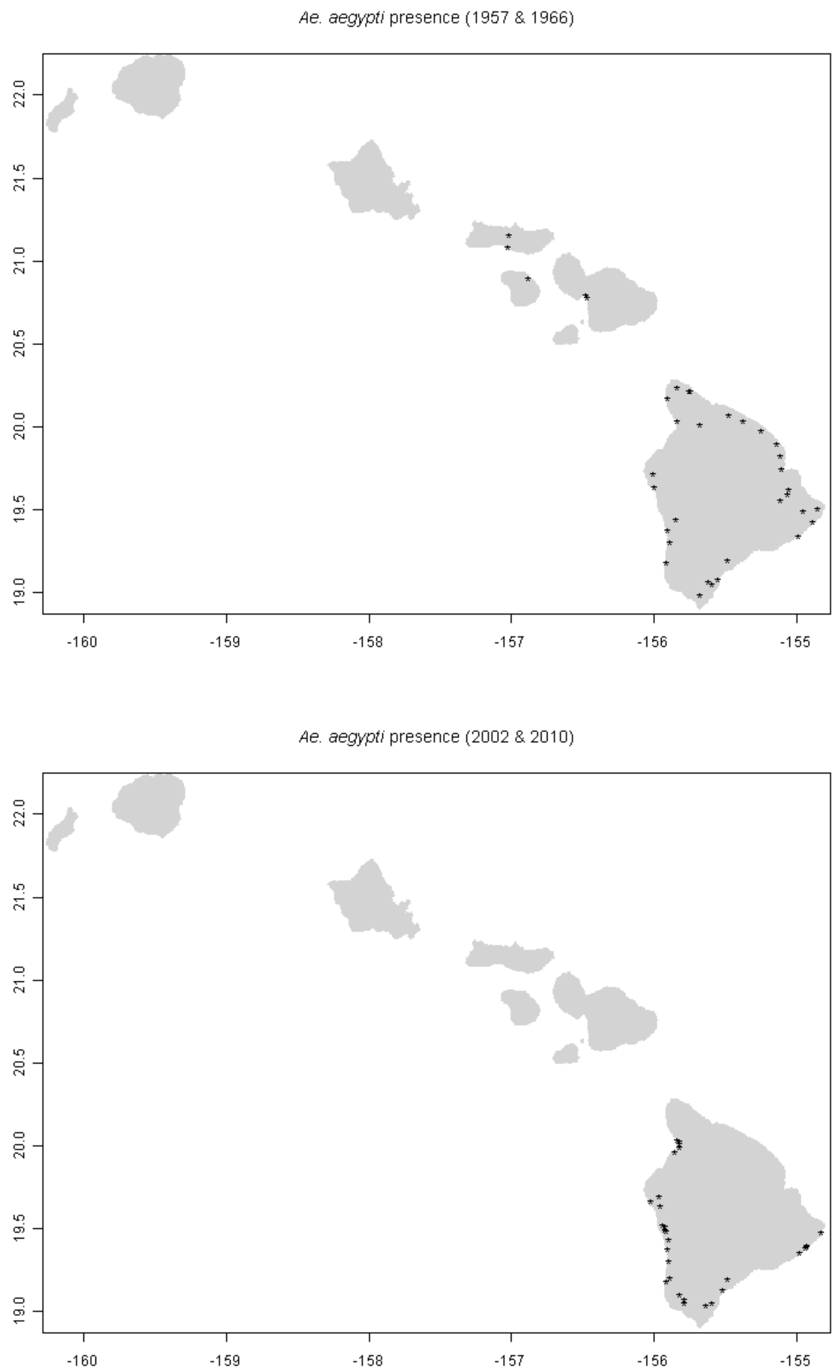

Figure 1.3 Spraying house by house in 1943. Trucks like this one on loan from the Army chemical corps truck carried a water/diesel/pyrethrum emulsion for vegetation spraying. Buildings in the areas of dengue cases received repeated treatments with DDT inside and out.

Figure 1.4. "Piles like these are springing up all over the city." (Honolulu Advertiser) Recycling drives for war materials like tin and rubber also had the benefit of removing mosquito habitat.

CHAPTER 2. SPECIES DISTRIBUTION MODELS

2.1. Introduction

Aedes aegypti and *Ae. albopictus* are two closely related mosquito species (members of the subgenus *Stegomyia*) that have spread from their respective origins in North Africa and SE Asia to new ranges worldwide. As treehole-breeding mosquitoes, they share two important adaptations – a tendency to oviposit in small containers, and the ability for eggs to remain viable and hatch after weeks (*Ae. albopictus*) or months (*Ae. aegypti*) of desiccation. These traits have made them especially suited to using human transportation and urbanization to invade new habitats around the world. Since both are vectors of human disease, their populations and movements are important to public health and epidemiology, and the study of their ecology is crucial in understanding and predicting changing disease risk, as these and other container-breeding *Aedes* species invade new areas worldwide. An example is the recent arrival of *Ae. japonicus* in N. America (Peyton et al. 1999).

Aedes aegypti is native to North Africa, where it adapted to breeding in the containers around human villages and cities in an environment that is largely arid and inhospitable to mosquitoes (Tabachnick 1991). It was introduced from Africa to the Caribbean by shipping in the 1500's and eventually spread throughout tropical and temperate cities in the New World (Tabachnick 1991). It reached Australia in the mid-19th century (O'Gower 1956), and over the next century appeared in most Asian cities. *Ae. albopictus* is native to Southeast Asia and spread during the 19th century to many Pacific Islands, but it was not known in any continent outside Asia until relatively recently. A new period of accelerated expansion for *Ae. albopictus* began with its arrival in North America in 1985, followed by Europe, Brazil, and Africa. Recent invasions of both species are linked to global trade in used tires (Reiter and Sprenger 1987; Benedict et al. 2007; Enserink 2010). As I reviewed in Chapter 1, both species arrived in Hawaii around 1890 (with *Ae. aegypti* 5-15 years before *Ae. albopictus*), making this one of the earliest locations to be invaded by both species.

2.1.1. *Aedes* interactions

The similar larval habitat utilized by *Ae. aegypti* and *Ae. albopictus* means that they are subject to exploitative competition when both species occupy the same area. Food is frequently a limiting resource for container breeding mosquito larvae, as hundreds or thousands of eggs may hatch together when a small container is inundated (pers. obs.). Ecological theory predicts that a species which is superior in exploiting a resource may exclude another species for whom that resource is limiting, but the outcome of competition is also influenced by the priority effect (Alford and Wilbur 1985), immigration (Horn and MacArthur 1972), and, in a metapopulation, recolonization rates (Levins 1969; Nee and May 1992; Nee et al. 1997). Spatial or temporal heterogeneity can alternately favor one species over the other, thereby preventing either species from excluding its competitor. Although field experiments in the United States (Juliano 1998) and Brazil (Braks et al. 2004) have found competitive advantage for *Ae. albopictus* larvae in containers, environmental factors determine if one or neither species will be dominant at the landscape scale, and a variety of competitive outcomes have been documented. These are summarized in Chapter 1, with the general trend being that urban development, especially with accessible indoor habitat, favors *Ae. aegypti*, while rural or forested areas favor *Ae. albopictus*. Some intermediate landscapes, such as suburbs and mixed residential/rural parts of subtropical Brazil and Florida, may support coexistence (Braks et al. 2003; Lourenço-de-Oliveira et al. 2004; Maciel-de-Freitas et al. 2006; Rey et al. 2006).

A long-standing explanation for the geographic patterns of segregation is that *Ae. aegypti*'s preference for urban or peridomestic habitat means that it always has shelter (in buildings) and water sources, even in locations and during periods that are dry (not unusual in North Africa, its native range). *Ae. albopictus*, breeding in more remote tree holes or tire dumps that are not artificially irrigated, is more dependent on precipitation but is common in areas that are not too dry. This pattern of urban and forest habitat preference is useful as a general rule, but not sufficient to explain some observed patterns of habitat partitioning, especially where both mosquitoes are found together at the same sites, year after year (Leisnham and Juliano 2009).

In the twenty-five years since *Ae. albopictus* invaded North America, scientists have had the opportunity to closely observe it in the wild with *Ae. aegypti* (which was already common), resulting in a deeper understanding of their interactions. An important difference between the mosquitoes may allow *Ae. aegypti* to be first to colonize a new container when it is flooded. If suitable water containers are not available, females of both species will oviposit in dry containers where they detect evidence of past flooding. If there is no water, embryos complete development to larvae in the eggs, then enter a suspended state called diapause, until environmental cues prompt them to hatch and emerge. *Ae. albopictus* eggs can normally hatch up to 30 days after oviposition, while *Ae. aegypti* eggs remain viable several times longer, with high hatch rates possible even eight months after oviposition (Christophers 1960). Thus a container that, when wet, is suitable for dominance by *Ae. albopictus* larvae, may be dominated by *Ae. aegypti* in the days or weeks that follow the first rain after a long dry period (Leisnham and Juliano 2009). Even if *Ae. albopictus* females find the newly flooded container and oviposit, food may already be scarce and newly hatched larvae (of either species) are often eaten by the later instar larvae that are already present (Edgerly et al. 1999). In colony and wild habitats, extended experiments with container drying, hatching and adult emergence coupled with microclimate observation have revealed that competitive dominance can depend on the length and frequency of container drying (Juliano et al. 2002; Aspbury and Juliano 1998), and therefore on geographic and yearly variation in dry season length. In containers where long dry periods occur, moisture may be a limiting factor for *Ae. albopictus*: since they are unlikely to have viable eggs remaining after a long dry period, their larvae will be not be present immediately after flooding, and *Ae. aegypti* can remain the predominant species in that container due to a container priority effect. Areas with pronounced wet and dry seasons may thus support a bimodal temporal pattern of dominance, with both species persisting year after year (Leisnham and Juliano 2009).

2.1.2. Interactions in Hawaii

Since the introduction of *Ae. aegypti* and *Ae. albopictus* to Hawaii in the 1890s, there have been two distinct competitive outcomes. For the first 50-60 years after these mosquitoes arrived there was broad coexistence, with both species found across a wide geographic and environmental range (Chapter 1). The survey records that are available for Honolulu show a decline in *Ae. aegypti* numbers as a percentage of total mosquitoes caught, during the period 1910-1926. This pattern has been seen elsewhere due to competition as *Ae. albopictus* completed its post-invasion expansion (Chapter 1), but it could also be an effect of mosquito control, especially in the years immediately after 1910 when the fear of yellow fever introduction was high (Chapter 1).

During and after the 1943-1944 dengue epidemic in Honolulu, a major mosquito control campaign caused a dramatic range reduction for *Ae. aegypti* (Chapter 1). However, even after DDT use ceased and mosquito control declined, *Ae. aegypti* remained absent from most of the island chain, a situation which has persisted for the past several decades, suggesting a shift in the balance between the two mosquitoes (Chapter 1). In Chapter 1 I discussed how a mosquito control campaign had a larger impact on *Ae. aegypti* than on *Ae.* albopi*ctus* and reviewed the records that show *Ae. aegypti* failed to reappear almost everywhere it was eradicated, even long after mosquito control stopped.

Similar to seed banks in plants (Harper 1977), egg dormancy in treehole mosquitoes via desiccation resistance may be an evolved response to environmental uncertainty. Delayed hatching can decrease density-dependent competition by reducing the number of larvae present immediately after flooding, and also increasing persistence of the population even when adverse environmental conditions have killed all adults. *Ae. aegypti*'s use of this temporal dispersal strategy may be the reason that it is commonly found in dry areas even after competitive displacement has reduced or eliminated its populations in the majority of its range. If *Ae. albopictus* imposes exploitative competition on *Ae. aegypti*, the existence of dry containers at certain times or places can provide the latter with a refuge from competition by removing *Ae. albopictus* eggs from the habitat while *Ae. aegypti* eggs persist through the dry period.

The hypothesis that *Ae. albopictus* excludes *Ae. aegypti* from returning to its former range generates three predictions whose effects should be clear when comparing the distribution of historic and modern *Ae. aegypti* with the range of *Ae. albopictus*.

1) *Ae. aegypti* today will be rare where *Ae. albopictus* is most common.

2) Compared with other islands, the Big Island, where *Ae. aegypti* still persists, will have the highest amount of land area as refuge from competition, i.e., areas that are suitable for *Ae. aegypti* but not for *Ae. albopictus*, as predicated by dry season length.

3) Areas with densest *Ae. albopictus* distribution will coincide with the areas where *Ae. aegypti* distribution declined over the 1966 - 2010 interval.

I test these hypotheses using species distribution modeling to compare *Ae. aegypti*'s modern and historic ranges with respect to inter-island variation in the amount of land favorable to *Ae. albopictus*. The SDMs also allow us to project the areas in the state most suitable for *Ae. aegypti* reinvasion, suggesting strategies for vector control officials to consider when planning prevention, monitoring and control.

2.2. Methods

2.2.1. Study Area

The Hawaiian Island chain includes areas of dense human habitation on the four main "high" islands, starting from the southeast with the largest and highest "Big Island" (Hawaii), the Maui Nui group (consisting of Maui and two smaller populated islands: Molokai and Lanai), Oahu, and Kauai at the northwest (Figure 2.1). The Big Island is the newest and still growing, with active volcanic activity, and age increases with distance from the southeastern area of island formation. The degree of erosion is related to age of the islands, although Oahu is an exception, having 99.5% of its land area within 600 m of sea level, compared with only 75% for its older neighbor, Kauai.

Temperature is an important factor in mosquito habitat suitability because of its effects on desiccation, metabolism, and (at low values) survivability. Temperature in Hawaii generally varies inversely with elevation (Figure 2.2). A consistent precipitation gradient is repeated on each island and group. Due to a high pressure cell northeast of the Hawaiian Islands, a consistent northeasterly trade wind blows across the island chain, creating a sharp weather contrast between windward to leeward areas (Giambelluca 1998). This pattern exists on each of the islands to some degree, with Hawaii the most extreme due to the highest mountains, and Lanai lying in the rain shadow of Maui's 3,000-meter Haleakala peak. Moisture is highest on the windward facing mountains and their drainage basins, making the east and north sides of islands most favorable for mosquitoes because they depend on water for larval development. The length and frequency of dry periods, an important variable for container-breeding mosquitoes, are impacted by Hawaii's seasonal regime, where the period from November to March is relatively wetter and cooler than the summer months (Figure 2.3).

Because human settlements provide an important source of food and larval habitat for mosquitoes, patterns of development are important to consider for their potential influence on *Aedes* populations. Human density on the Kona coast of the Big Island is relatively modest, although growth has increased sharply since 1970 (Anon. 2002). Coastal areas tend to be dry and warm, with isolated beach resorts and fishing villages separated by arid stretches of scrub and lava. Outside of developed areas with landscaping and irrigation, the age of most recent lava flow is the major influence on presence and type of vegetation. Buildings in remote coastal areas may not be connected to any city infrastructure and therefore have water catchment basins or tanks. Large areas consist of recent lava flows that have scrubby vegetation and little topsoil; small rock basins (often underground) and tree crotch holes in the alien species kiawe (*Prosopis pallida*) or haole koa (*Leucaena leucocephala*) can create localized and ephemeral habitat patches that may be important in sustaining *Ae. aegypti* populations.

2.2.2. Ecological Niche Modeling

A variety of statistical tools, often called ecological niche models (ENM) or species distribution models (SDM), can be used to predict the potential range of a species based on the characteristics of sites where the species has been found. Since absence of a species is difficult to document, there are a number of ENM techniques that work based on limited knowledge of where a species is present and require no knowledge of where it is absent (Pearce and Boyce 2006). Based on the principle that a species exists in a multidimensional range of conditions that constitute its ecological niche, these tools model the habitat suitability across a geographic extent, using as input raster maps of variables that either represent these ecological and environmental parameters (e.g. temperature, forest cover), or serve as proxy for them (e.g., altitude, remote sensing radiance). These models are often used in conservation to predict possible presence areas of a rare species, but are also useful in identifying potential locations for unwanted or invasive species such as mosquitoes (Peterson and Vieglais 2001).

Maximum Entropy Species Distribution Modeling (Maxent version 3.3.3e: Phillips et al. 2006; Phillips and Dudík 2008) is one of the most frequently used SDMs and has performed favorably in a recent test against 16 other methods (Elith et al. 2006). Maxent works by choosing a probability distribution that maximizes the explanatory value of the known species presence records, essentially assuming that they represent the absolute best habitat on the map. To compare these presence points with the background variation of the landscape, Maxent also randomly chooses pseudo-absence points from within the map extent. The algorithm then chooses a set of functions on the environmental variables that gives highest values at points on the map with known presence, and lowest values at the pseudo-absence points, while avoiding overfitting by penalizing complexity and rewarding smoothness (Elith et al. 2011). Like any prediction, the quality of a Maxent model is judged by its error rate, and models may be compared using a metric called AUC (area under the receiver operating characteristic), which assesses the ability to minimize Type I and Type II errors. Models with AUC > 0.75 may be considered potentially useful (Phillips and Dudík 2008). I used Maxent with selected geographic rasters and mosquito presence records to develop species distribution models.

2.2.3. Data

I compiled three sets of mosquito presence records (Chapter 1). Results for the 2002 Department of Health survey (Hawaii DOH, unpublished data) and my own surveys in 2009-2010 were combined for a total of 216 *Ae. albopictus* records and 26 modern *Ae. aegypti* records. The 33 historic *Ae. aegypti* mosquito records were combined from the 1957 CDC survey and the 1966 *Aedes aegypti* Eradication Program survey. There is no evidence of a change in *Ae. albopictus* range, so the historic and modern distributions are assumed to be the same.

I considered 27 environmental variables as potential predictors for mosquito habitat distribution (Table 2.1). These were chosen based on my judgment of their biological relevance to mosquito populations, and other habitat modeling studies (e.g., Moffett et al. 2007; Masuoka et al. 2008; Larson et al. 2010). These serve two purposes: first, to provide Maxent with information on the conditions at mosquito presence sites; and second, after model development, to allow prediction of the probability of presence at all sites on the map. The environmental datasets were used as raster map layers, all of which were coregistered to the same projection and extent, then resampled to 30 arc-second resolution. Climate rasters for monthly total precipitation, monthly maximum temperature and monthly minimum temperature (Daly and Halbleib 2006a, Daly and Halbleib 2006b), were processed using package the R package "dismo" (Hijmans et al. 2011b) to extract 19 "Bioclim" variables representing various aspects of temperature, precipitation and seasonality (Houlder et al. 2001; Beaumont et al. 2005). I downloaded DEMs for the Hawaiian Islands at 10 m resolution (NOAA 2007), derived slope and aspect rasters, then used bilinear resampling to upscale to 30 arc-second resolution using ArcGIS software (ESRI 2009). I obtained modified LANDSAT images at 30m resolution (Coastal Geology Group 2011), separated the three color bands and used cubic resampling in ArcGIS to upscale each to 30 arc-second resolution. From each of several subsets of highly correlated variables ($r > 0.9$), I selected the one variable that was most useful in jackknife trials of model performance (e.g., I kept annual mean temperature while dropping six other measures of temperature extremes). A summary of considered and selected layers is in Table 2.1.

2.2.4. Software settings / model parameters

Maxent models were developed using the default software settings recommended by the developers, which were calibrated based on trials of 226 plant and animal species so as to minimize the combined probability of Type I and Type II error (Phillips and Dudík 2008). Deviations from the default can produce a more conservative model (e.g., to clearly identify the best possible habitat for an endangered species) or less conservative (e.g., to find every possible area at risk of invasion). The output format was set to logistic, which scales the estimated probability of presence at each grid cell to values between zero and one. Eighty percent of the presence records were used to train the model, with the remaining records used as test points to validate the model. The process was repeated for 500 iterations. While *Ae. albopictus* was present on all the islands, the available collection records were biased towards Oahu and Kauai, so a bias raster was calculated as the total number of records per island, divided by the island's area below 1500 m elevation (Table 2.2). This reflected similar effort per land area on Hawaii, Kauai and Lanai, with the lowest intensity on Maui and Molokai (about a quarter of that on

Hawaii), and Oahu sampling about four times higher than Hawaii. For each model, the extent of the input rasters was limited to the islands or group where the species was present, (e.g., Hawaii for modern *Ae. aegypti*, and Hawaii and Maui Nui for historic *Ae. aegypti*). This means that Maxent was only allowed to choose pseudo-absence points from that extent, in order to avoid biasing the model against habitat that is suitable for *Ae. aegypti* on other islands. The results from each model were projected to cover the entire state.

2.2.5. Analysis methods

Maxent models were developed for all three sets of presence records (historic and modern *Ae. aegypti*, and *Ae. albopictus*) using the selected environmental datasets in Table 2.1. Each Maxent model was assessed by its performance in predicting the 20% of points that were set aside from the training points for validation. A random prediction has an expected AUC value of 0.5, and models with AUC above 0.75 are considered potentially useful (Phillips and Dudík 2008).

For each model, raster input layer importance was assessed one at a time using Maxent's jackknife option. This compares the basic model with and without the selected variable to assess how much it contributes; it also fits a model using only the selected variable to assess how much information that variable contains in isolation.

Niche overlap among the three models was compared using *D* (Schoener's *D*) and *I* (Hellinger distance / 2) statistics, calculated with the software package ENMTools (Warren et al. 2008). These metrics assess the similarity of two ENMs on a scale from zero (completely discordant) to one (identical). While these do not indicate whether the niches are more similar to each other than would be expected by chance, they were used to rank the relative values of the three pairwise comparisons.

With each of the three selected Maxent models, cumulative amounts of land area were plotted against the predicted occurrence probability to identify where the three models were most divergent, then a cutoff value was chosen to classify each grid cell into high or low quality habitat. The degree of spatial intersection among high probability habitats was compared to assess the hypotheses of competitive exclusion and refuge from competition. To assess the extent that the change between historic and modern *Ae.*

aegypti ranges was impacted by *Ae. albopictus*, a resampling trial was performed by generating 35 presence records, 1000 times, from the *Ae. aegypti* historic Maxent distribution and creating new “pseudo-modern” models based on these presence records. These models were analyzed for any tendency for segregation between *Ae. aegypti* and *Ae. albopictus* that was equally or more extreme than the segregation we observed.

2.3. Results

All three Maxent models have AUC’s well above 0.75, indicating a high ability to rank presence sites above non-presence sites. AUC’s for *Ae. aegypti* (historic), *Ae. aegypti* (modern), and *Ae. albopictus* are 0.911, 0.957, and 0.944, respectively (Figure 2.4).

Jackknife scores for each variable and each model are shown in figures. For historic *Ae. aegypti* (Figure 2.5) and *Ae. albopictus* (Figure 2.6) annual mean temperature was the variable with highest utility (that is, it made the largest contribution to Maxent’s ability to predict presence, both in isolation and in interaction with other variables). For modern *Ae. aegypti* (Figure 2.7), standard deviation of temperature was the variable with highest utility in isolation, and LANDSAT band 1 was highest in utility when combined with other variables.

Use of the Maxent-generated *Ae. albopictus* raster as an input layer for a modern *Ae. aegypti* Maxent model decreased the performance (AUC) from 0.957 to 0.923, so only the basic model was used for further analysis.

2.3.1. Model comparison

Niche overlap (Table 2.3) is assessed using both the *I* and *D* statistics in accordance with Warren et al. (2008). Although *I* is higher than *D* in all three pairings, both metrics are consistent in their rankings and show the *Ae. aegypti* (historic) and *Ae. albopictus* models to be more similar to each other than either is to modern *Ae. aegypti*.

Species distribution probabilities, predicted across the study area, are mapped in figures. At the broad scale, the three distributions share some common features. High altitude cold areas are poor habitat in all three cases. While there is no immediately clear visual difference between the historic *Ae. aegypti* (Figure 2.8) and *Ae. albopictus* (Figure

2.9) maps (both are broadly predicted in low elevation areas of all islands), the colored area that represents predictions of high probability for modern *Ae. aegypti* (Figure 2.10) is clearly smaller and concentrated in limited coastal ranges, especially on the south and west sides of islands. A plot of each model's cumulative distribution on the Big Island indicates how much land area is modeled to be available to each mosquito group at varying levels of probability. *A*e. *aegypti* (historic) is modeled with the most high probability land area, followed by *Ae. albopictus*, with *Ae. aegypti* (modern) having the lowest curve at all probability levels (Figure 2.11).

2.3.2. Statewide projection

Venn diagrams (Figure 2.12) are used to show the spatial intersection of land area that is classified as "high probability" (predicted occurrence probability > 0.25) for one, two or all three models. The areas which are high-HSI for historic *Ae. aegypti* but low-HSI for modern *Ae. aegypti* (areas of the green circles that do not intersect with the blue circles) indicate lost habitat, and the areas which are high-HSI for modern *Ae. aegypti* but low-HSI for *Ae. albopictus* (areas of the blue circles that do not intersect with the red circles) represent refugia where *Ae. aegypti* faces little competition from *Ae. albopictus*. The amount of land area represented in these diagrams varies according to the total land area on each island, and the fraction that is not too cold for mosquitoes; these are summarized in Table 2.4.

2.3.3. Resampling trial

On the Big Island, 21% of the map cells that are high-HSI for *Ae. albopictus* are also high-HSI for *Ae. aegypti* (modern). In 1000 repetitions of the resampling trial, the amount of overlap generated in Maxent models ranged from 68% to 100% and segregation was always much less extreme than the observed value ($p < 0.001$).

2.4. Discussion

2.4.1. Resampling analysis

Many map cells that have HSI > 0.25 under the historic *Ae. aegypti* model have HSI < 0.25 under the modern *Ae. aegypti* model. These are areas where the modeled likelihood of finding *Ae. aegypti* transitioned from high to low (an arbitrary threshold) after the eradication campaign and remained low to the present day. The fact that competition from *Ae. albopictus* is frequently cited as a limitation on *Ae. aegypti* populations suggests a question. In these Maxent models, is an area is more likely to change from high-HSI to low-HSI for *Ae. aegypti* if it is high-HSI for *Ae. albopictus* than if it is low-HSI for *Ae. albopictus*? Under the null hypothesis of no competitive effect, we would expect a negative answer, that is, an area's chance of losing *Ae. aegypti* is unrelated to the HSI for *Ae. albopictus*.

In fact, the relationship is highly significant, and it is very unlikely that the high segregation of modern *Ae. aegypti* high-HSI habitat from *Ae. albopictus* high-HSI habitat is due to chance. The resampling trial showed no tendency for randomly sampled presence points from historic *Ae. aegypti* to generate a Maxent model biased toward marginal *Ae. albopictus* habitat. In 1000 resampling trials, we habitat overlap values ranged from 68% to 100%, and never approached the observed degree of segregation (21% overlap).

As stated in the introduction, many instances are known where either of these two *Aedes* species has invaded and replaced the other, as well as examples where an invader reaches some level of coexistence with the original species. Mosquito control campaigns that are focused in human residence areas can result in localized eradication of *Ae. aegypti* (Chapter 1), but permanent eradication is unlikely unless immigration is impossible. In Central and South America and some Caribbean islands, even after the regional near-eradication of *Ae. aegypti*, the success was followed by cessation of mosquito control, and nearly every country saw reinvasion of the vector (Gubler 1989). Madagascar and Reunion may be the cases that are most similar to Hawaii; on both islands the two mosquitoes have been present together for about a century. In Madagascar the two species are largely segregated by climate zones, with little sympatry (Fontenille and Rodhain 1989). In Reunion, *Ae. aegypti* has decreased from high prevalence in 1907 to relict populations in remote valleys today (Bagny et al. 2009), possibly due in part to mosquito control in the 1950s. The benefit of the current study lies in using a new approach to re-examine an interaction that has been observed many times.

2.4.2. Pairwise comparisons

The niche comparison statistics *D* and *I* indicate that the models for *Ae. albopictus* and historic *Ae. aegypti* had more overlap with each other than either had with modern *Ae. aegypti*. While the majority of their similarity comes from their geographic co-occurrence in coastal areas of Hawaii, the pairwise comparison supports the idea that overlap between the two species was larger in the past than it is now. The dissimilarity between the two *Ae. aegypti* models indicates the shift to a realized niche that is clearly different (and smaller) than the one occupied 40 years ago.

The presence records are biased toward populated areas because the goal of the mosquito surveys in 1957, 1966 and 2002 was to detect *Ae. aegypti*, an anthropophilic mosquito. It is possible that the resulting models under-predict *Ae. albopictus* occurrence in forested and remote areas. While *Ae. aegypti* is not usually associated with remote areas, relict populations in Madagascar and Reunion islands have been observed persisting many years, breeding in treeholes and shaded rock pools (Fontenille and Rodhain 1989; Bagny et al. 2009). Rock basins and underground voids are common in the Kona district of the Big Island, whose many recent lava flows are sparsely vegetated and present a poor habitat for *Ae. albopictus*. We noticed that mosquitoes of both species are often abundant near the rock walls that are commonly used as livestock fences or boundary markers in Kona.

2.4.3. Best predictors

The Maxent software estimates the importance of each variable, according to how much it contributes to training the model, in two ways. The total amount of predictive information contained in an input layer is assessed by the quality of a model that is built using only that single layer as input. The amount of unique predictive information contained in an input layer is measured by its importance in interaction with all other variables. Since the three models co-vary so much, the relative ranking of predictors (Figures 2.5 - 2.7) is dominated by their utility in discriminating mosquito habitat from the background landscape, rather than by how well they delineate one type of mosquito habitat from another.

In the models presented here, annual mean temperature was consistently important in predicting distribution, and was the highest-ranked input variable for historic *Ae. aegypti* and *Ae. albopictus*. This is not surprising, as cold temperatures limit mosquitoes from most of the high islands' montane land area. Standard deviation of temperature was the highest-utility variable for the modern *Ae. aegypti* model, and its utility was highest at low values of temperature standard deviation. While there is no clear reason in mosquito biology why this was useful in helping Maxent to refine predictions, its variation across the landscape provides a likely explanation: on the Big Island (where the modern *Ae. aegypti* model was trained) temperature variability broadly co-varies with elevation, with the lowest temperature variation below 1000 m, and highest variation at the mountain peaks.

The three LANDSAT bands were also ranked high-utility in modeling all three sets of presence records, both in isolation and in combination with other variables. It is important to remember that utility does not imply a causal or linear relationship between the variable and the habitat quality, but only that changes in the raster at all or part of its range were correlated with change in the likelihood of presence. For example, LANDSAT band 1, the highest-utility variable for modeling modern *Ae. aegypti*, has its highest (i.e., most reflective) values in ranchland areas whose cover is grazed grass and rocks, and its lowest values on dark lava areas, such as those near the top of Mauna Loa. Both the highest and lowest values of this variable are largely in areas too cold for mosquitoes, and it may be simply coincidence that Maxent found the layer to be useful. This illustrates the potential danger of projecting the model to other islands.

While the Hawaii government has land use and land cover maps created from 1970s data (Hawaii Statewide GIS Program 2010), I found these to be inaccurate in 2010. Since remote sensing is commonly used in the creation of land cover and vegetation maps. I used the satellite imagery instead because it captures the same types of variation. Surprisingly, given the biology of these two species, precipitation in the driest quarter, a potential factor in container drying times, was not a strong predictor in any model.

I tested the effectiveness of using the distribution of the hypothesized competitor as a predictor of the modern *Ae. aegypti* distribution. The slight decrease in performance when *Ae. albopictus* was used as an input raster for modern *Ae. aegypti* (modern) is difficult to interpret. While a failure to improve performance would indicate that Maxent simply got no added predictive power from the input, a decrease in performance means that Maxent found the layer to increase training gain, but relied on the input too heavily and thus suffered from overfitting. While the use of a model based on limited point information is not unusual for Maxent (e.g., all the climatic variables in this study are modeled by interpolation from weather stations), descriptions of “nesting” models like this are unusual in published literature, and it would take further investigation to examine the reliability of this technique. It may be that because the niches are similar, Maxent found *Ae. albopictus* occurrence to be highly predictive on a broad scale (across all temperature ranges), so that it overestimated the positive correlation between the two, and thus performance was decreased at the fine scale where it really matters - where correlation is negative - in the narrow range of parameters that represents viable mosquito habitat.

As stated earlier, the environmental input variables listed in Table 2.1were chosen in part because of their potential to impact container breeding mosquitoes. Several other variables that are often used in ENMs were available, but not used. Land use and land cover are closely related to human development and settlement. Besides the quality issues discussed above, using these would have biased the results toward certain categories, since CDC and DOH surveys for mosquitoes were concerned with the risk posed by human disease vectors and therefore concentrated on the subset of potential mosquito habitats that are occupied by humans. Similarly, slope, a possible predictor of water accumulation basins, was excluded because mosquito sampling tended to focus on settled areas which do not reflect the range of very steep to very flat terrain that is found in Hawaii. During creation of the models, a variety of Maxent settings were tested, as well as different combinations of environmental input rasters. In various input parameters used for Maxent, the gradient and pattern seen across islands has been consistent (i.e., Kauai poorest for *Ae. aegypti*, Oahu with a larger suitable area, and Maui some good habitat but small area), so the analysis conclusions are not sensitive to the exact set of input parameters used.

2.4.4. Neighbor island presence and invasibility

Ae. aegypti local populations were present on Maui and Molokai but not Lanai in 1957 (Hess 1957), then in Lanai and Molokai but not Maui in 1966 (Wells 1968), but on none of the three in 2002 (Vector Control Branch 2002). A single female *Ae. aegypti* was trapped and confirmed in Molokai in 2009 by a member of our lab (P. Thongsripong, specimen). This intermittent presence and absence may indicate the Maui Nui group was marginal habitat even before the AaEP; it also demonstrates at least a moderate frequency of inter-island re-introductions. In spite of the unintentional introductions that must result from heavy inter-island air and sea traffic, *Ae. aegypti* has not reinvaded other islands in any permanent way, and has not even returned to much of its former habitat on the Big Island, though it is found regularly in certain parts of the windward side, within 40 miles of Hilo (pers. obs.; C. Jacobsen, pers. comm.).

Extrapolating modern *Ae. aegypti* predictions from the Big Island Maxent model to other islands is easily done with the software options, but the results should be treated with caution, because Maxent performs best at predicting habitat in the same area where it was trained (Edith et al. 2011). Because other regions or conditions are likely to have parameters and combinations of parameters that were not available in training the model, a projection of the models to a new space can be less reliable.

Given this caveat, it is interesting to note how the pattern of modeled habitat overlap varies across the islands (Figures 2.11 & 2.12). The areas that are modeled as high HSI for modern *Ae. aegypti*, but low HSI for *Ae. albopictus*, may broadly indicate refugia, where *Ae. aegypti* larvae can develop in relative freedom from the exploitative competition imposed by *Ae. albopictus* larv*Ae.* The Big Island has the most modeled refuge habitat in absolute terms, Maui Nui a smaller amount, Oahu very little, and Kauai none. Maui as well as Molokai and Lanai (not pictured) have high *proportions* of *Ae. aegypti* habitat modeled as refuge, but the *absolute amount* of *Ae. aegypti* habitat is small. Due to its higher amount of low-elevation land, Oahu has a higher amount of *Ae. aegypti* habitat in total area, but very little of it is predicted as refuge. Finally, Kauai has very little land area classified as modern *Ae. aegypti* habitat, and none of it is outside of *Ae. albopictus* habitat. (While these figures are based on a cutoff probability of 0.25, a similar pattern of overlap is seen at higher and lower cutoff values.) The presence on other islands of habitat that Maxent judges to be similar to *Ae. aegypti* habitat in Kona indicates that *Ae. aegypti* might readily invade the other islands if *Ae. albopictus* populations were low enough. This was, of course, the case when *Ae. aegypti* arrived around 1890 before *Ae. albopictus* was present in Hawaii (Chapter 1). An *Ae. aegypti* reinvasion could happen again if something were to reduce *Ae. albopictus* prevalence in urban sites like Honolulu. Several possible scenarios are: an extended dry period; reduction of tree cover in and around the city; or an insect control program with a non-target impact on *Ae. albopictus*.

Analysis of the statewide projection of modern *Ae. aegypti* indicates that, outside of the Big Island, Oahu has the most area at risk, with the southern and eastern coastal plains predicted as high probability habitat for *Ae. aegypti* (these are also the areas with highest human density and therefore most likely to sustain disease transmission). Maui has a much smaller area of high-probability *Ae. aegypti* area, mainly around its main city of Kahului (although this area was negative in 1957 and 1966), and Molokai has very limited high-probability areas on the south and western shores. As mentioned above, Kauai is modeled as largely unfavorable for *Ae. aegypti*, which is consistent with historical records.

2.5. Conclusion

These map results support the gross pattern that was already known from surveys: *Ae. aegypti* range today is smaller than it used to be, both in geographic area and in parameter space, while enhancing the ability to interpret and visualize their distribution. This analysis allows us to better predict the relative suitability of parts of the Big Island that are unsurveyed for mosquitoes, and, somewhat less reliably, to judge what parts of other islands may be most at risk of reinvasion, given no change in the competitive balance that exists today. These areas should be the focus of future monitoring efforts since they may be the areas most likely to support reinvasion. The modern *Ae. aegypti* model indicates that, aside from the Big Island, Oahu has more viable habitat for *Ae. aegypti* than any other island, with the low elevation areas of Oahu’s southeast and southern coastal plains being the most at risk for reinvasion. The difference between islands in the availability of habitat that is a refuge from competition for *Ae. aegypti* may offer an explanation for its failure to re-invade the other islands despite what must be a high rate of inter-island human-mediated introduction.

Because the current distribution of *Ae. aegypti*, within suitable mosquito habitat, is directly correlated with low levels of *Ae. albopictus* and not simply a function of environmental parameters, this study suggests that the presence of *Ae. albopictus* can block *Ae. aegypti* from an area. As suggested by Gubler (2003), the presence of the former species, a minor disease vector, may be a net benefit to public health, if it reduces the number of the latter species, a major disease vector.

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Tables

Table 2.1. Environmental datasets. *From each subset of highly correlated variables (0.95), only one was used. Land use / land cover, aspect and slope were rejected for bias.*

used	(BIOCLIM1) Annual Mean Temperature
used	(BIOCLIM2) Mean Diurnal Range (Mean of monthly (max temp - min temp))
used	(BIOCLIM3) Isothermality (BIOCLIM2/BIOCLIM7) (* 100)
used	(BIOCLIM4) Temperature Seasonality (standard deviation *100)
not used	(BIOCLIM5) Max Temperature of Warmest Month
not used	(BIOCLIM6) Min Temperature of Coldest Month
used	(BIOCLIM7) Temperature Annual Range (BIOCLIM5-BIOCLIM6)
not used	(BIOCLIM8) Mean Temperature of Wettest Quarter
not used	(BIOCLIM9) Mean Temperature of Driest Quarter
not used	(BIOCLIM10) Mean Temperature of Warmest Quarter
not used	(BIOCLIM11) Mean Temperature of Coldest Quarter
not used	(BIOCLIM12) Annual Precipitation
not used	(BIOCLIM13) Precipitation of Wettest Month
not used	(BIOCLIM14) Precipitation of Driest Month
used	(BIOCLIM15) Precipitation Seasonality (Coefficient of Variation)
used	(BIOCLIM16) Precipitation of Wettest Quarter
used	(BIOCLIM17) Precipitation of Driest Quarter
not used	(BIOCLIM18) Precipitation of Warmest Quarter
not used	(BIOCLIM19) Precipitation of Coldest Quarter
not used	Elevation
not used	Slope
not used	Aspect
not used	Land use / Land cover
used	LANDSAT band 1
used	LANDSAT band 2
used	LANDSAT band 3

Table 2.2. Bias categories for *Ae. albopictus*.

Island	Estimated survey intensity
Hawaii	2
Maui	1
Molokai	1
Lanai	3
Oahu	18
Kauai	4

Table 2.3. Niche comparisons

Pair	Niche overlap statistic (D/I)
Ae. aegypti (historic) vs. *Ae. albopictus*	0.7223 / 0.9292
Ae. aegypti (historic) vs. *Ae. aegypti* (modern)	0.4890 / 0.7618
Ae. aegypti (modern) vs. *Ae. albopictus*	0.4567 / 0.7408

Table 2.4. Area (km^2) with HSI > 0.25, by island

	Ae. aegypti (historic)	*Ae. aegypti* (modern)	*Ae. albopictus*	Exclusion	Refuge land area (% of Column 2)
Hawaii	2193	508	1455	1774	297 (58%)
Maui Nui	472	44	547	444	16 (36%)
Maui	378	36	319	355	12 (34%)
Molokai	56	6	216	5	2 (39%)
Lanai	38	3	12	37	1 (54%)
Oahu	366	91	320	304	7 (8%)
Kauai	49	1	252	48	0 (0%)
Total	3080	645	2573	2571	320 (50%)

Figures

Figure 2.1. The main Hawaiian islands.

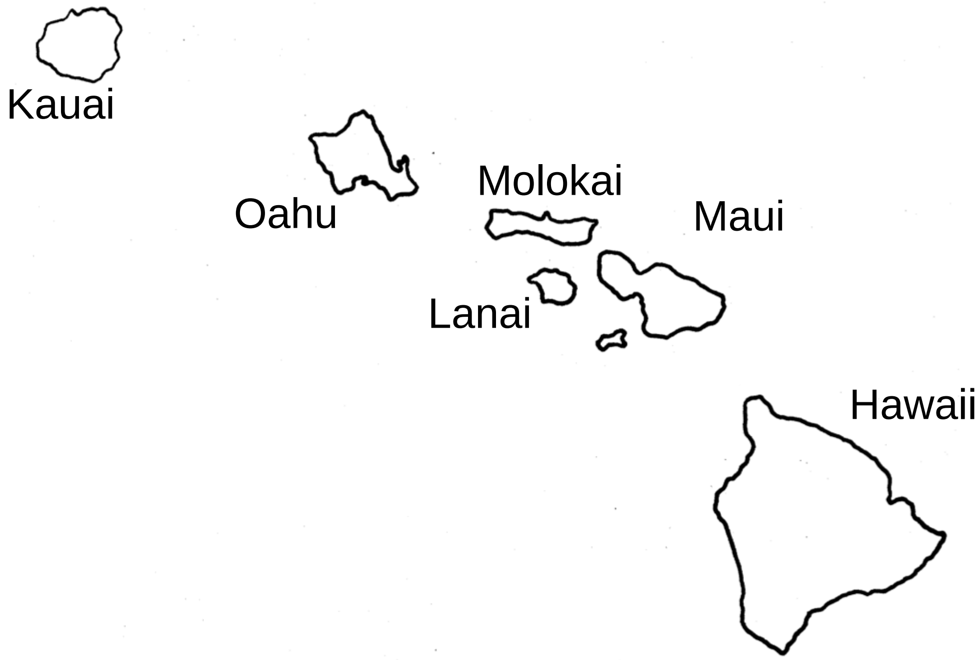


Figure 2.2. Annual Mean Temperature. Temperatures are tropical at sea level and decrease with elevation.

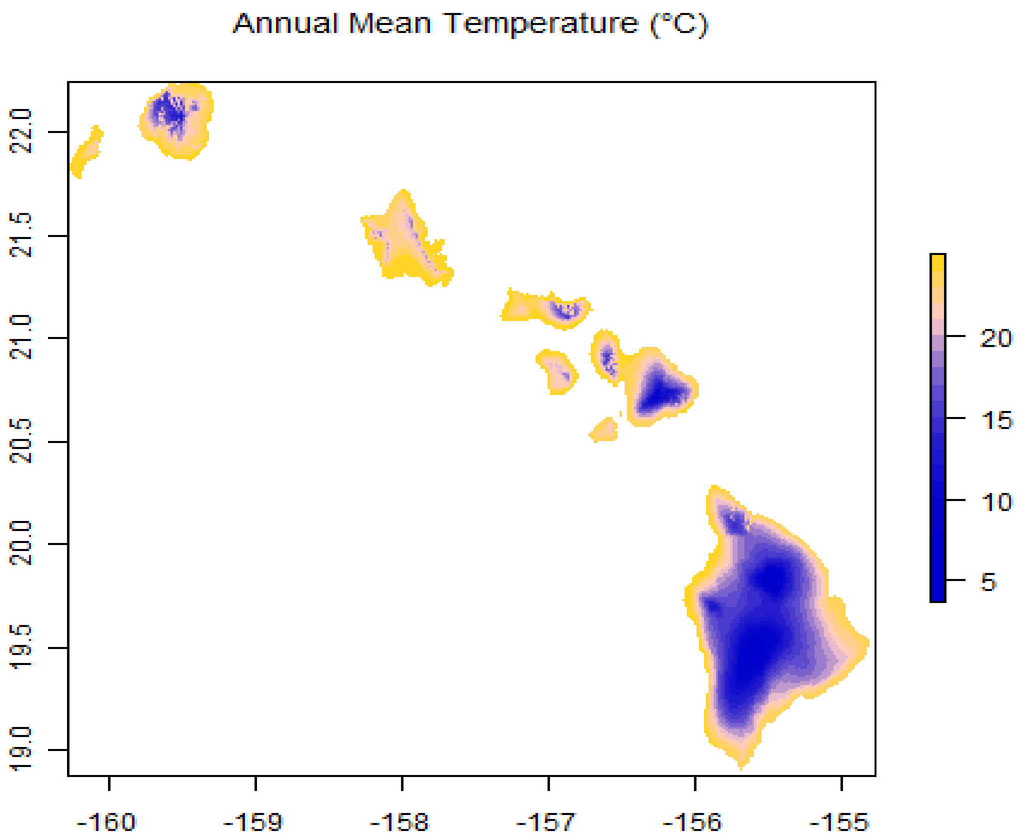


Figure 2.3. Annual Total Precipitation. The prevailing northeasterly trade wind creates a consistent moisture gradient across the islands. High rainfall on the windward mountains creates ideal mosquito habitat in the downslope drainage (i.e. where warmer and flatter). The wetter "cloud band" can be seen on the Big Island's west coast.

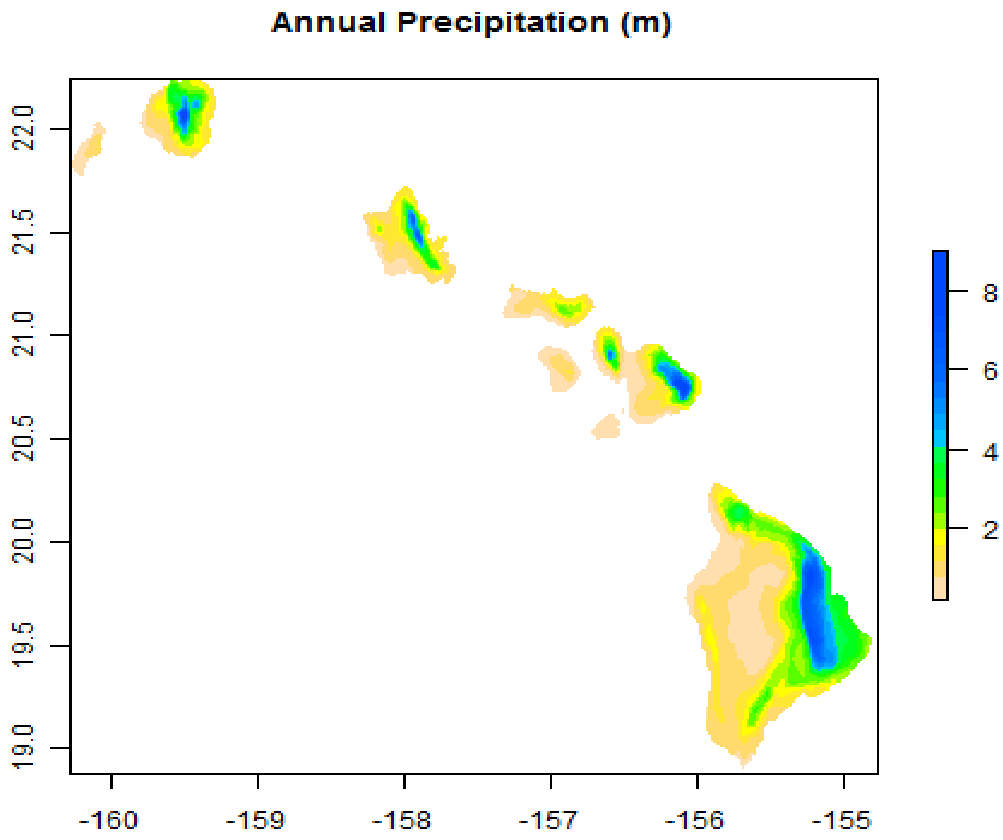


Figure 2.4. ROC (receiver operating characteristic) curves. *The curve displays the tradeoff between prediction sensitivity and specificity. Area under the curve (AUC) is expected to be 0.5 for a random prediction, and values greater than 0.75 indicate a potentially useful model. All three Maxent models created in this study have AUC above 0.9.*

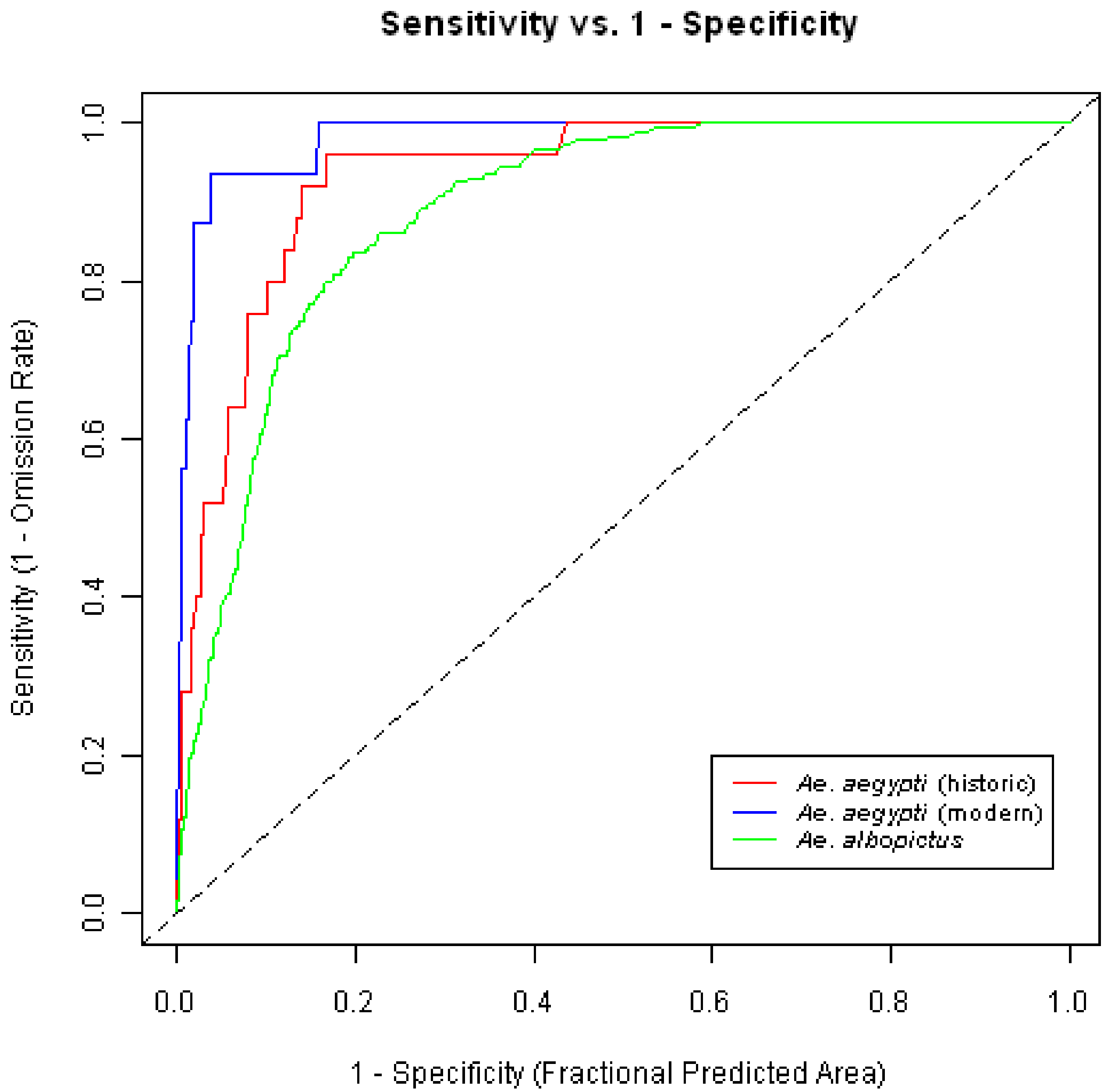


Figure 2.5. Jackknife for Ae. aegypti (historic). Regularized training gain for Ae. aegypti (historic) using selected variables. When used alone, annual mean temperature increased gain more than any other variable (dark blue); omitting annual mean temperature decreased gain more than omitting any other variable (light blue).

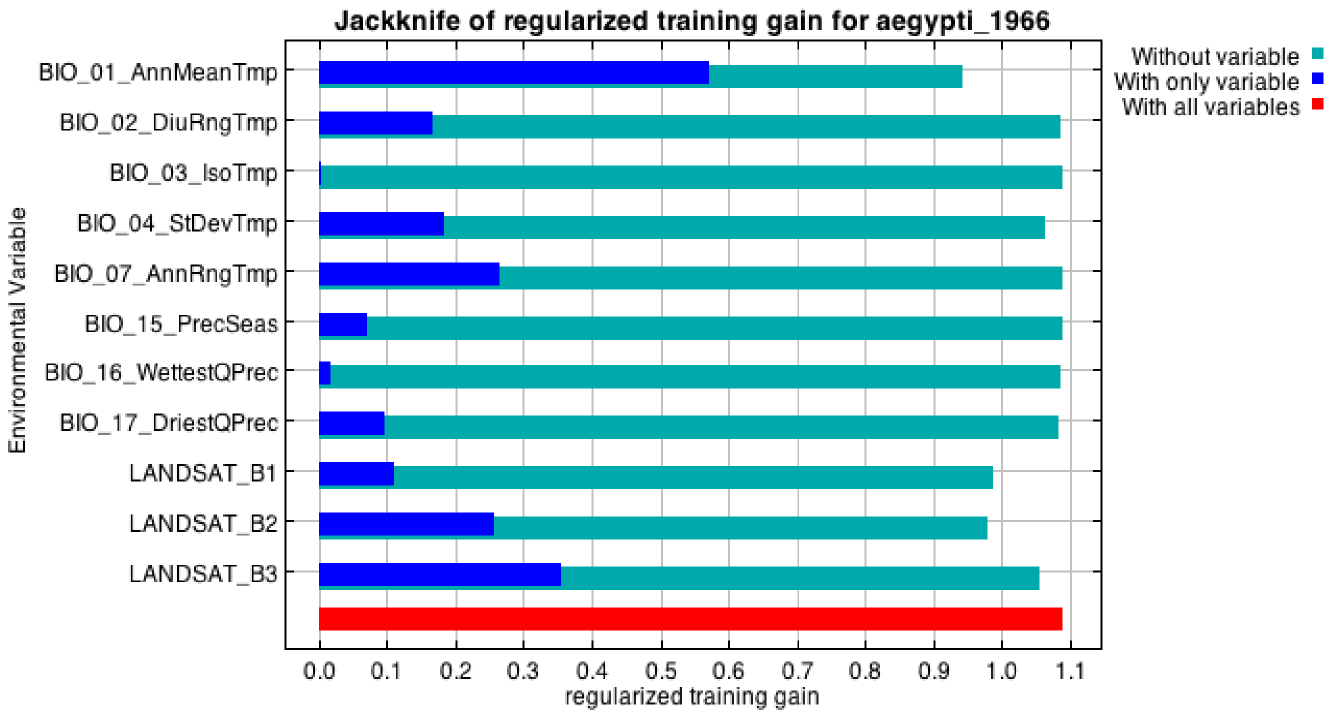


Figure 2.6. Jackknife for Ae. aegypti (modern). Regularized training gain for Ae. aegypti (modern) using selected variables. When used alone, standard deviation of temperature increased gain more than any other variable (dark blue); omitting LANDSAT band 1 decreased gain more than omitting any other variable (lght blue).

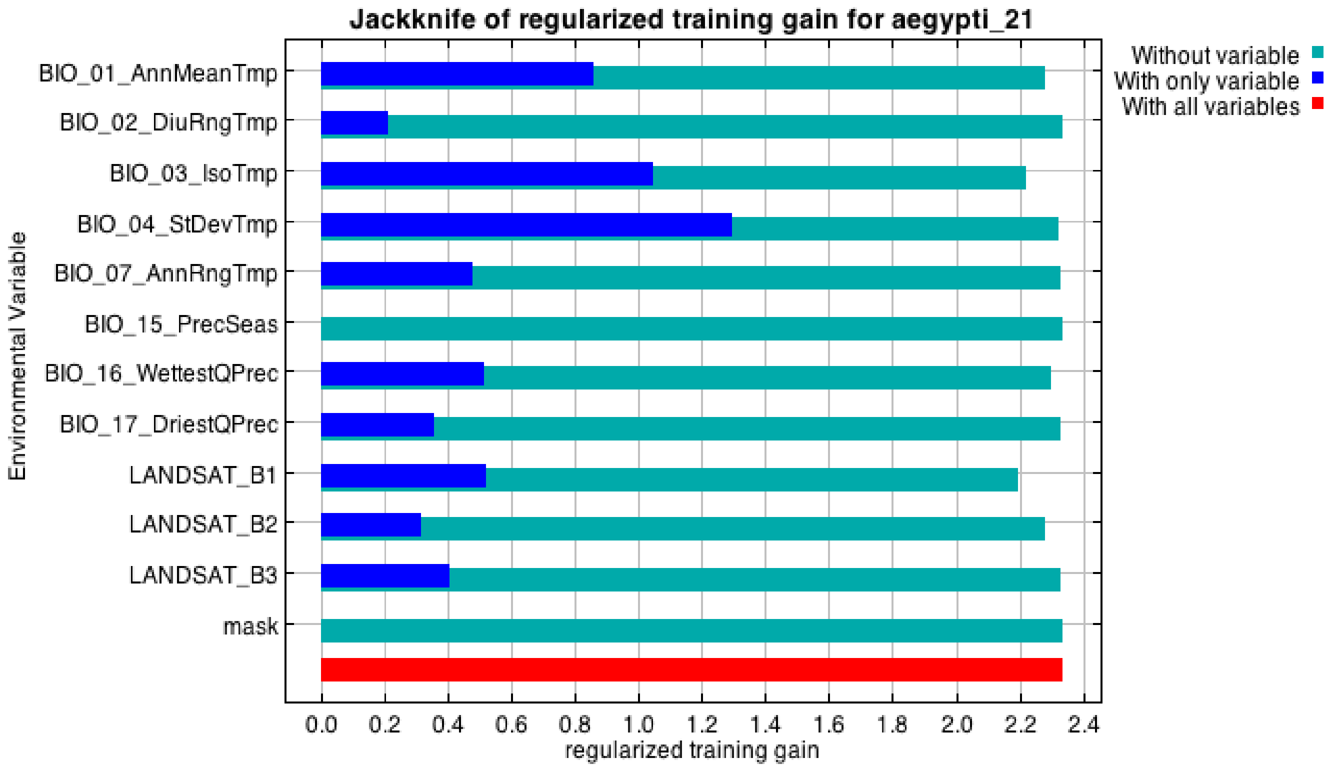


Figure 2.7. Jackknife for Ae. albopictus. Regularized training gain for Ae. albopictus using selected variables. When used alone, annual mean temperature increased gain more than any other variable (dark blue); omitting annual mean temperature decreased gain more than omitting any other variable (light blue).

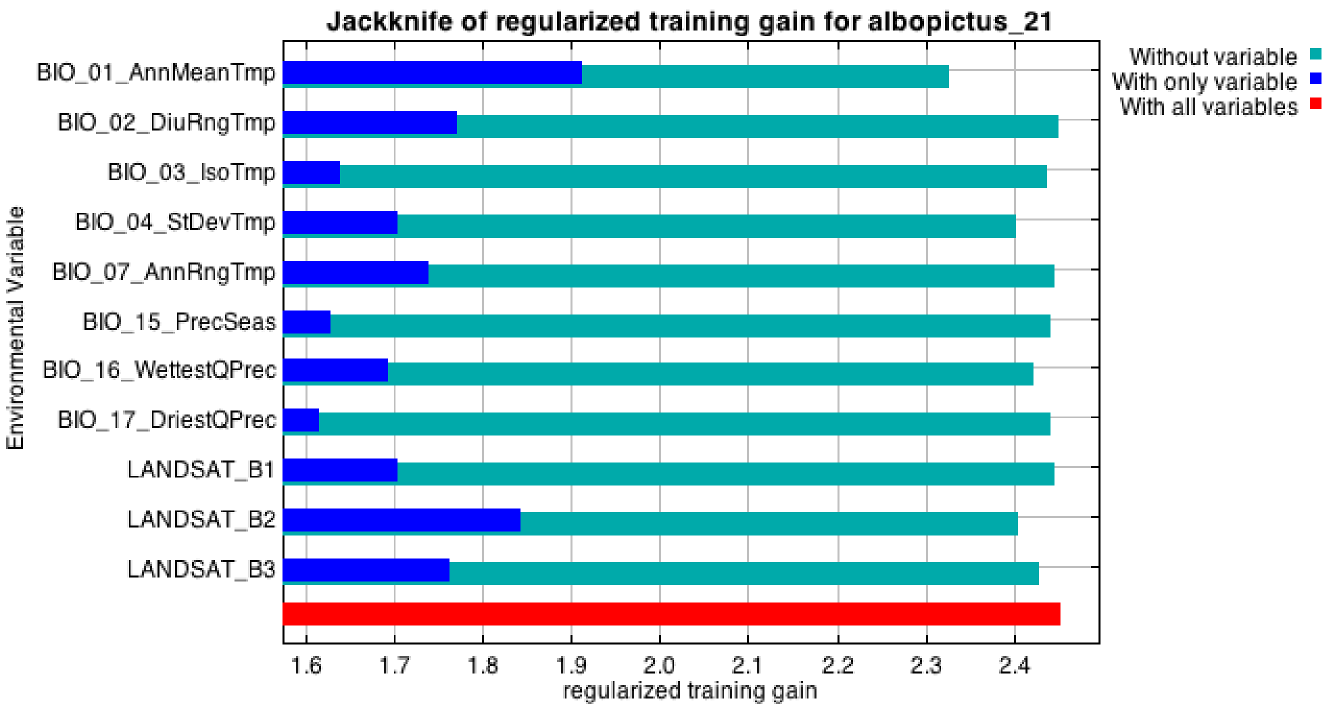


Figure 2.8. Historic Ae. aegypti HSI map (overlaid with presence records). Historic species distribution model for Ae. aegypti indicates widespread habitat at low elevations. The mosquito was not found on Oahu or Kauai during the 1957 and 1966 surveys.

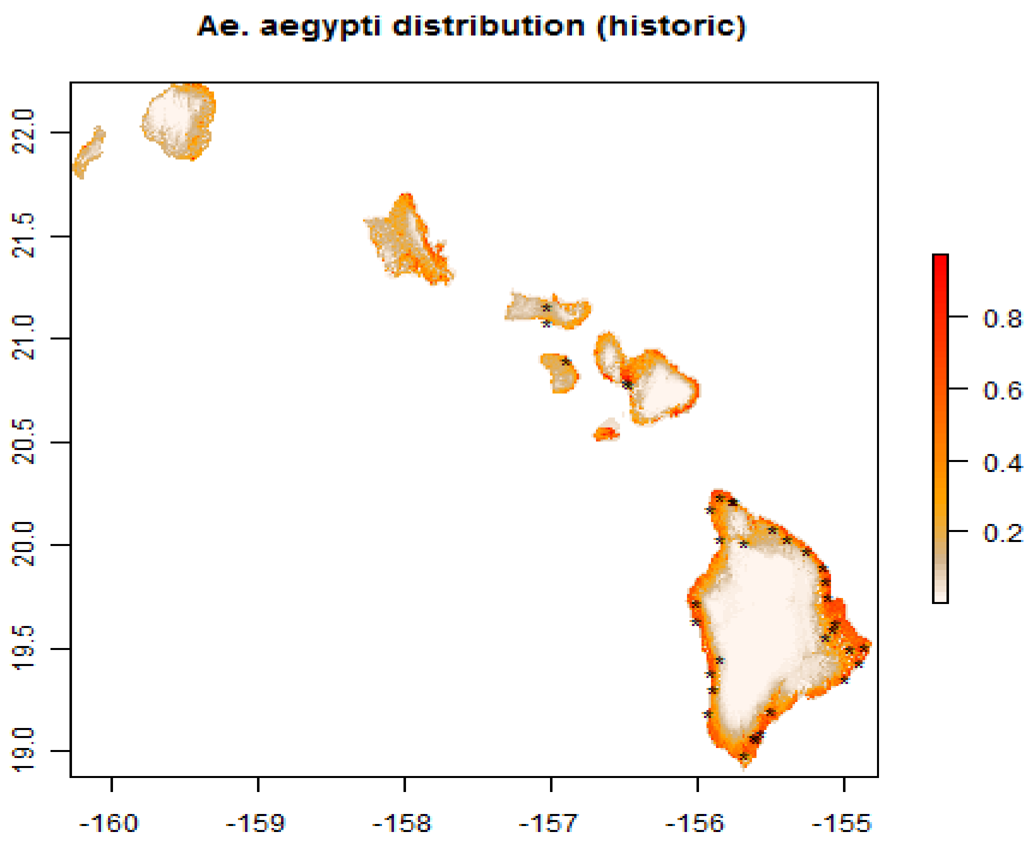


Figure 2.9. Modern Ae. aegypti HSI map (overlaid with presence records). Modern species distribution model for Ae. aegypti shows concentrations on west and south coasts of the Big Island, with colored patches of suitability on all islands except Kauai. The species is only found on the Big Island.

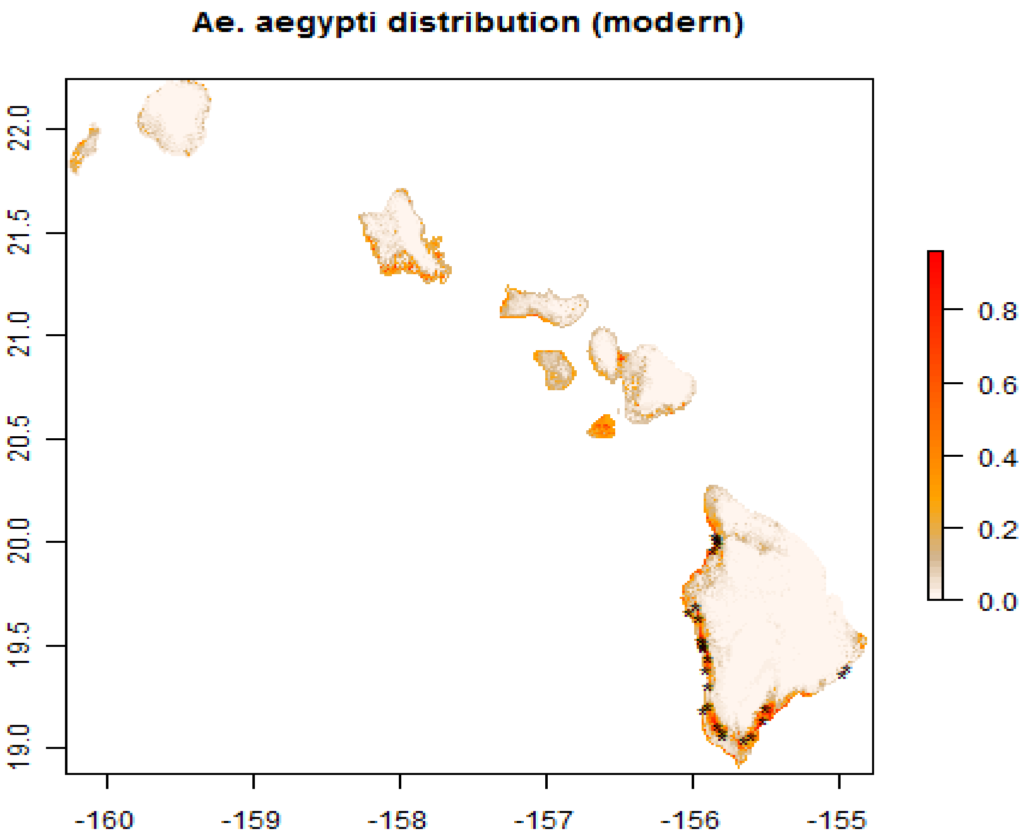


Figure 2.10. Ae. albopictus HSI map (overlaid with presence records). Species distribution model for Ae. albopictus predicts widespread distribution in the warm zones of all islands, especially windward sides. Survey intensity is biased by human population density, with Oahu (Honolulu County) the most heavily surveyed.

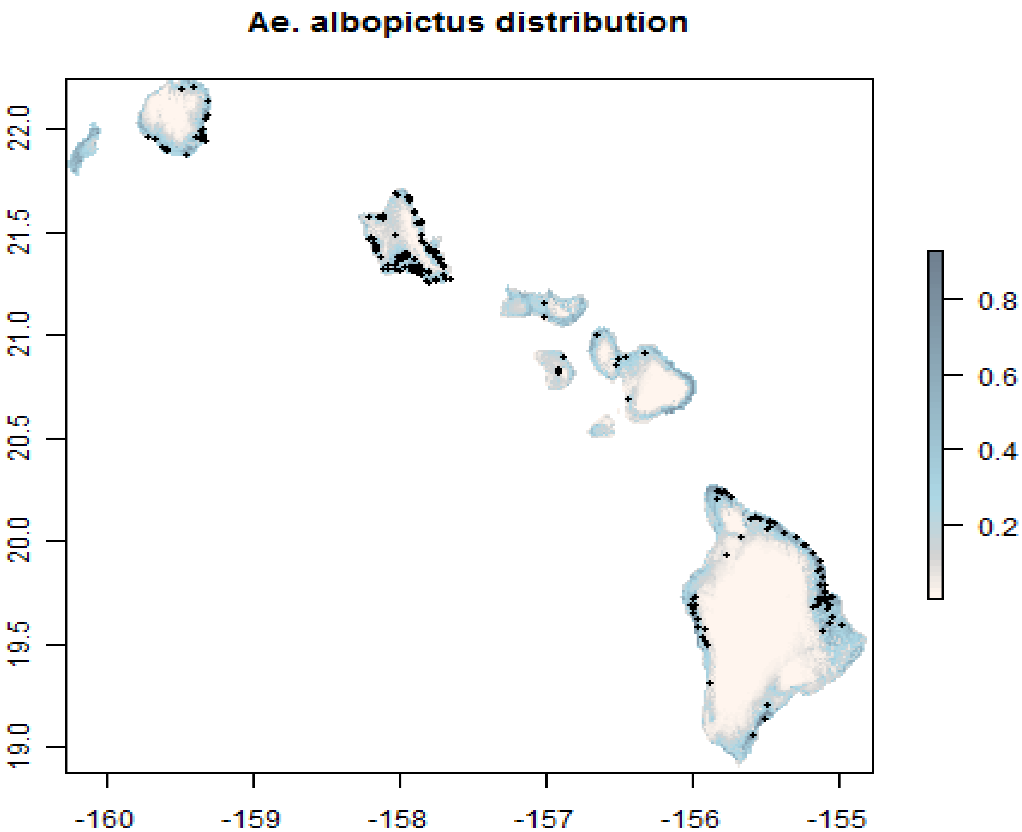


Figure 2.11. Cumulative land area by probability for all three models. Classifying total suitable habitat for each model at varying levels of probability reveals that Ae. aegypti (historic) is modeled with the most high quality land area, followed by Ae. albopictus, with Ae. aegypti (modern) having the lowest curve at all but very poor habitat levels. A cutoff of 0.25 was used in this study to classify high and low suitability land areas.

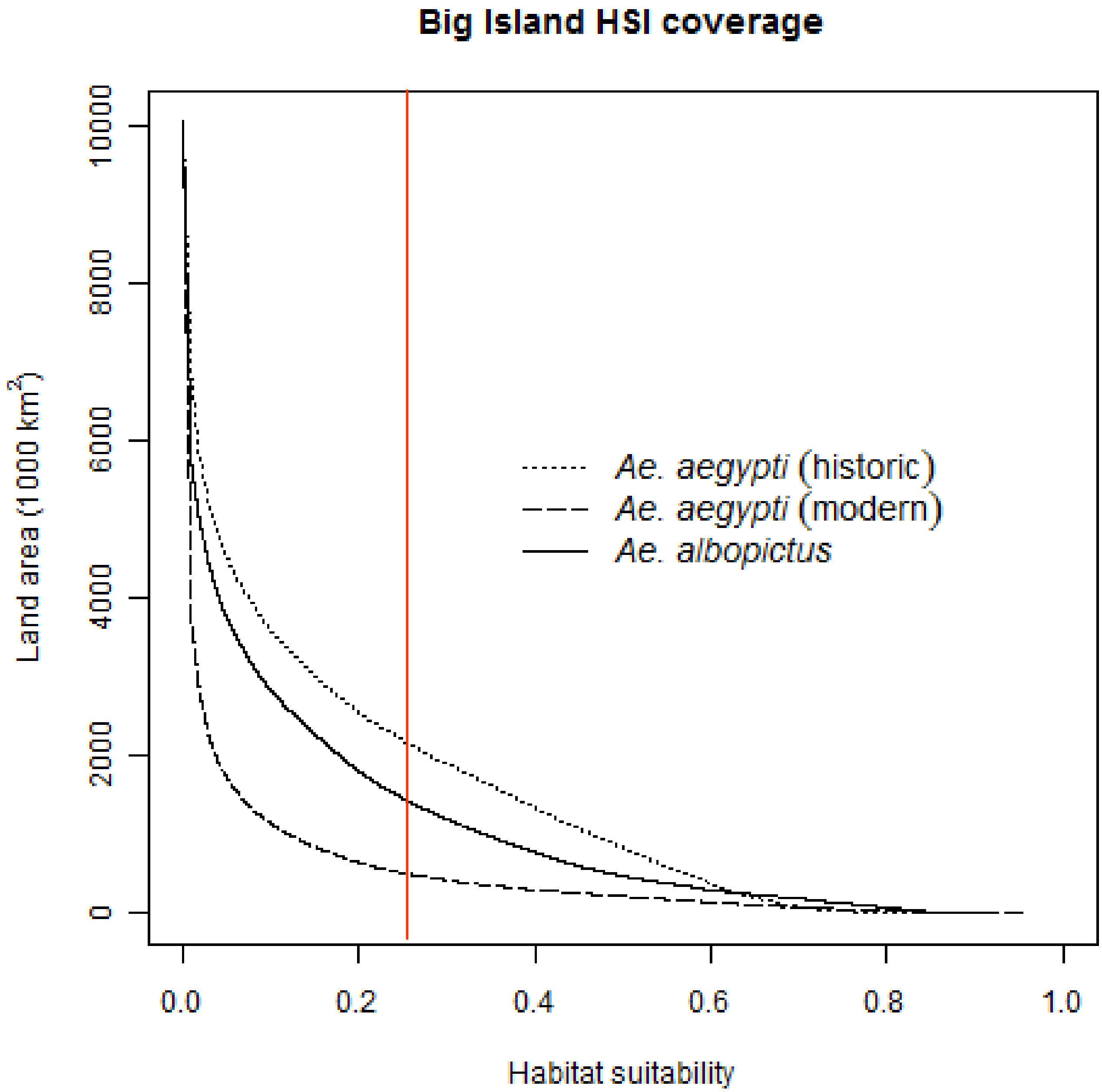


Figure 2.12. Intersection of high probability habitat on main islands (2.12a: not to scale; 2.12b: by island). The portions of blue circles (i.e., high HSI for modern Ae. aegypti) that do not intersect the red circles (i.e., low HSI for Ae. albopictus) may be refugia where Ae. aegypti faces little competition from Ae. albopictus. The portions of the green circles (i.e., high HSI for historic Ae. aegypti) which do not intersect the blue circles (i.e., modern Ae. aegypti) represent areas of decrease for Ae. aegypti. Both in absolute terms and as a fraction of good mosquito habitat, the Big Island has more potential refuge for Ae. aegypti than any other island, Maui Nui has an intermediate amount, and Oahu and Kauai almost none. Because Oahu has little high mountain land, nearly half of its land area is high suitability mosquito habitat.

Figure 2.12a.

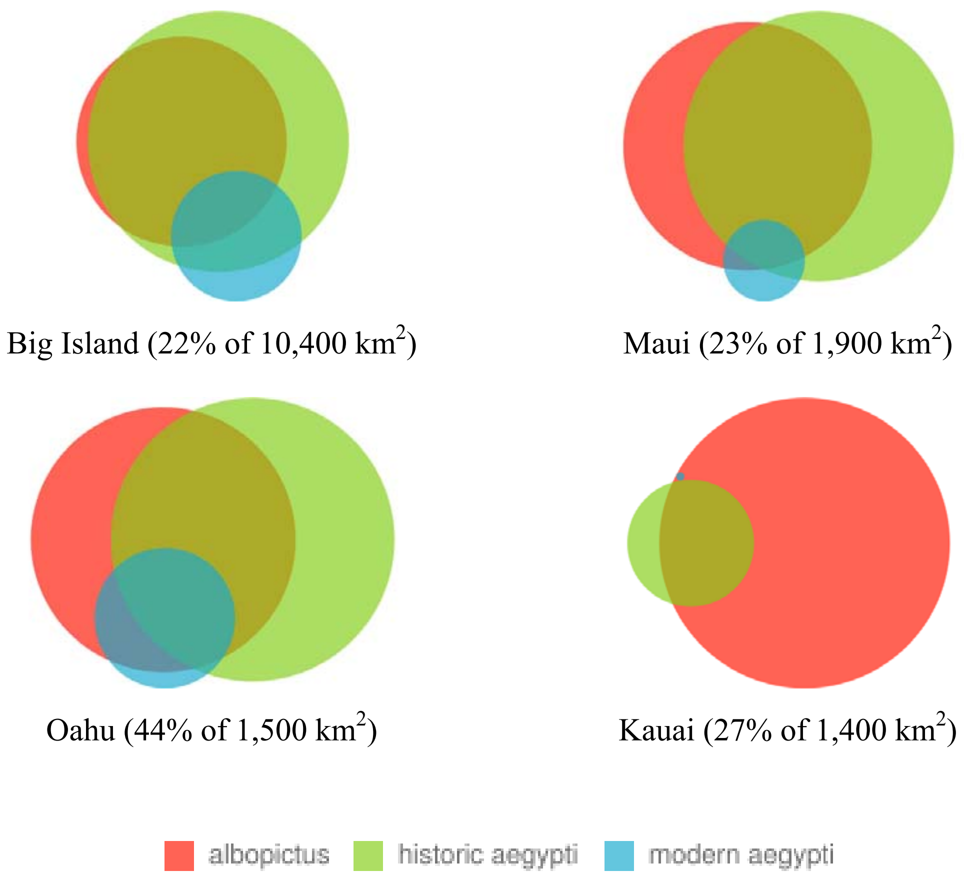
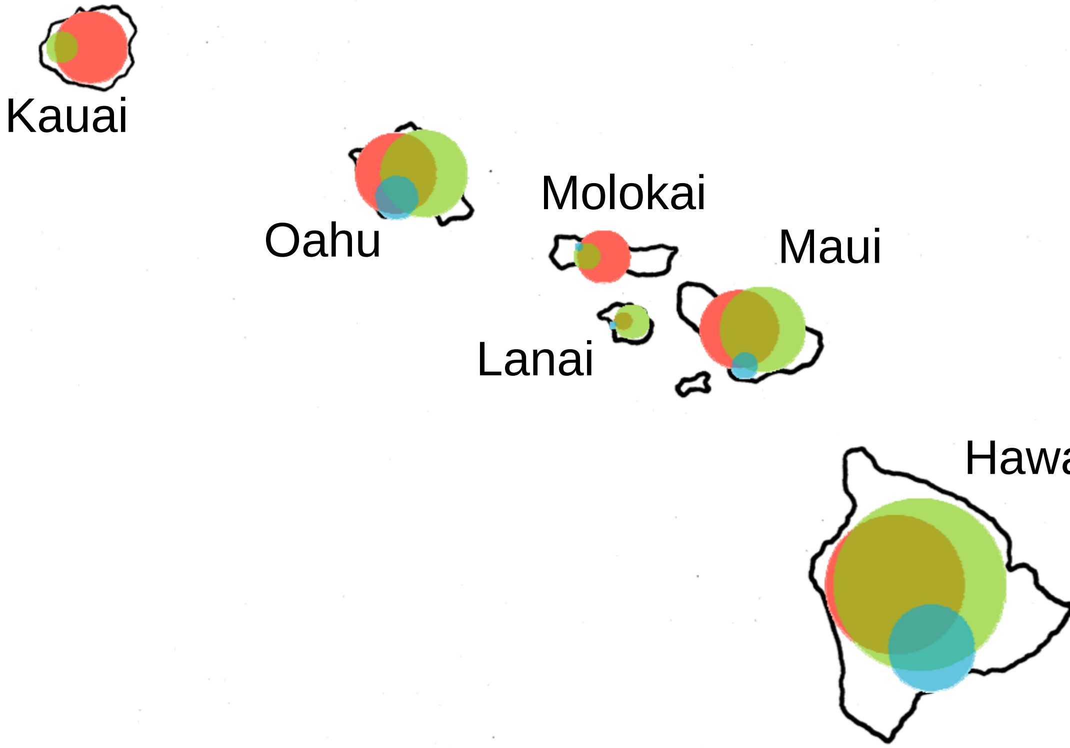


Figure 2.12b. Hawaiian Islands summary figure.



CHAPTER 3. FIELD SURVEY OF MOSQUITOES

3.1. Introduction

Dengue virus is a globally emerging arthropod-borne pathogen with a history of activity in Hawaii since 1903 until as recently as 2011 with notable outbreaks in 1943 and 2001. Its main vectors, *Aedes aegypti* and *Ae. albopictus*, were introduced to Hawaii in the 1890s, with *Ae. aegypti* several years prior to *Ae. albopictus* (Chapter 1). Both species were widespread during the first half of the 20th century, but the more dangerous vector, *Ae. aegypti*, is now largely restricted to a small area on the Kona Coast of the Big Island of Hawaii (Chapters 1 and 2). Both historic data (Chapter 1) and modeled distributional data (Chapter 2) point to synergy between control efforts and human habitation patterns as the primary drivers of *Ae. aegypti* distribution. However, the fact that *Ae. aegypti* has neither spread fully around the Big Island nor reinvaded most of the other islands where habitat appears to be suitable (both from historic comparisons, and by projecting current distributions on the Big Island to the remaining islands) suggests that competition from *Ae. albopictus* may play a role in restricting *Ae. aegypti* to a subset of its previous range.

Competition between larvae to exploit limited food resources in small ephemeral aquatic habitat is the most important interaction between the species (Juliano 1998). *Ae. albopictus* tends to outperform *Ae. aegypti* in this competition across a wide variety of conditions (Braks et al. 2004; Chan et al. 1971; Ho et al. 1989). In fact, *Ae. albopictus* has recently displaced *Ae. aegypti* across much of the southern United States (Chapter 1). Because *Ae. aegypti* eggs survive longer without water, their success in areas where *Ae. albopictus* is present may depend on the length of dry periods that allow water containers to dry out and kill *Ae. albopictus* eggs (Lounibos et al. 2010). Thus a major question has been to what extent the two species co-exist and/or compete on the Kona coast, the driest area of the Big Island and *Ae. aegypti*'s last remaining stronghold in Hawaii.

In areas that are hot and dry, especially urban areas with little vegetation cover, generally *Ae. albopictus* does not dominate over *Ae. aegypti*, while in forested, rural or suburban areas *Ae. aegypti* is not dominant (Chapter 1), and many intermediate situations exist where the two species are sympatric, year after year (Chapter 1). As reviewed in

Chapter 1, this pattern is supported in Hawaii's zones where *Ae. albopictus* is dominant in numbers, but there are no known areas where *Ae. aegypti* dominates in Hawaii. In Chapter 2 I modeled the regional scale distribution of *Ae. albopictus* and *Ae. aegypti* across the landscape of the Hawaiian Islands; the results of this modeling indicate overlapping habitat ranges in Kona. While useful for generating broad geographic predictions from existing data, a thorough understanding of how these mosquitoes interact at the local scale requires more detailed field surveillance.

My aim in this chapter is to discover the extent to which coexistence or exclusion takes place between these mosquitoes at the local scale across diverse sites and seasons in the Kona region of the Big Island of Hawaii. Two possible outcomes are hypothesized: either the two species exist in broad sympatry in the areas where *Ae. aegypti* populations persist; or there is micro-allopatry, that is, some sites will show clear dominance by *Ae. aegypti*. To investigate this I chose sites that varied in suitability for each species based on habitat and historical range (as modeled in Chapter 2) from within the area where *Ae. aegypti* was found in the most recent surveys, then surveyed them periodically across portions of Hawaii's two major seasons (dry and wet) to quantify the relative abundance of *Ae. albopictus* and *Ae. aegypti*. To determine whether and how the two species differ in their response to climatic factors (because drying interval differentially affects egg survival), weather variation was recorded at study sites.

Because areas with long drying intervals are expected to have poor egg survival for *Ae. albopictus* but not for *Ae. aegypti* (Chapter 1), a secondary aim of the survey is to find evidence that the proportion of these mosquito species varies with climatic factors, so weather variation was recorded at study sites across portions of Hawaii's two major seasonal phases (dry and wet).

3.2. Methods

3.2.1. Study area

Hawaii Island (usually referred to as the Big Island to avoid confusion with the state name), located at the southeastern extreme of the Hawaiian island chain, is the largest as well as newest of the group, and is still growing with three active volcanoes. Temperature is near tropical at sea level, and generally declines with elevation. A consistent northeasterly trade wind creates a precipitation gradient from windward to leeward (Giambelluca 1998), so that moisture is highest on the windward facing mountains, and the east and north facing slopes are most favorable for mosquitoes. The length and frequency of dry periods, an important variable for container-breeding mosquitoes, are impacted by Hawaii's seasonal regime, where the period from November to March is relatively wetter and cooler than the summer months.

Because human settlements provide an important source of food and larval habitat for mosquitoes, patterns of development are important to consider for their potential influence on *Aedes* populations. Human density on the Kona coast of the Big Island is relatively modest, although growth has increased sharply since 1970 (Anon. 2002). Coastal areas tend to be dry and warm, with isolated beach resorts and fishing villages separated by arid stretches of scrub and lava. Outside of developed areas with landscaping and irrigation, the age of most recent lava flow is the major influence on presence and type of vegetation. Buildings in remote coastal areas may not be connected to any city infrastructure and therefore have water catchment basins or tanks. Large areas consist of recent lava flows that have scrubby vegetation and little topsoil; small rock basins (often underground) and tree crotch holes in the alien species kiawe (*Prosopis pallida*) or haole koa (*Leucaena leucocephala*) can create localized and ephemeral habitat patches that may be important in sustaining *Ae. aegypti* populations.

The Big Island's western districts of North and South Kona make up the central portion of the leeward coast, located at 156 degrees west longitude, with latitude ranging from 19 to 20 degrees north, and having a combined area of 2119 km^2. Mean annual rainfall on the western side of the Big Island varies considerably, from 25 cm on the coast of North Kona, to 200 cm in the "cloud band" from 600 m – 900 m above sea level, about 8 km inland up the western slope of Mauna Loa. Seasonality is also variable: some areas receive peak rainfall in the summer, while others match the predominant Hawaii pattern of wet winters and dry summers (Giambelluca et al. 1986). The region has been in a drought for several years, with 2010 rainfall gauges measuring 30-80% of the 1970-2000 long term average (Pacific Islands Water Science Center, 2011).

A total of ten study sites were established at elevations from sea level to 650 meters; the locations are mapped in Figure 3.1 and key characteristics are listed in Table 3.1. The sites were chosen to include a range of elevations and land cover conditions in North and South Kona. Since a primary goal of the study was the characterization of sites dominated by *Ae. aegypti*, an important selection criterion was the presence of potential habitat for this species, as determined by initial surveys, including man-made features such as residential buildings and boats, as well as refuse or plants that would trap water for larval habitat. Finally, the practical concerns of landowner permission and an area large enough to place 8-12 traps with low probability of disturbance or theft were considered.

At these sites, land use included residential, agricultural, rangeland and forest, with predominant land cover of grassland, scrub, forest and bare rock. The study sites ranged in habitat suitability, as modeled by Maxent (Chapter 2), from 0.02 to 0.91 (least to most suitable) for modern *Ae. aegypti*, and from 0.08 to 0.67 for *Ae. albopictus*.

3.2.2. Study Period

Sites were sampled approximately every twenty days from their establishment (May – August 2010) until the final sampling in December 2010, resulting in 3-8 sampling events per site (Table 3.2).

3.2.3. iButtons

Data loggers were used at each site to periodically record temperature and humidity during the entire study period (Maxim Hygrochron iButton, Model DS1923, Figure 3.2). At each site the instrument was placed 1-2 m above ground level and sheltered from direct sun and precipitation. Readings were taken every 20 minutes and aggregated for hourly means. Besides temperature and humidity, vapor pressure deficit (VPD) was calculated as a one-dimensional measure of the drying pressure experienced by small organisms (Anderson 1936). VPD (in the equation below, D in units kPa), given T as temperature in degrees Celsius and H as relative humidity, was calculated by the following formulae (Environmental and Water Resources Institute 2005):

Saturation Vapor Pressure (E_s) $\quad E_s = 0.6108 / \exp((17.27 * T) / (T + 237.3))$

Actual Vapor Pressure (E_a) $\quad E_a = H / 100 * E_s$

Vapor Pressure Deficit (D) $D = E_s - E_a$

Higher values of D represent higher amounts of drying effect.

3.2.4. Mosquitraps

Oviposition traps (ovitraps) monitor the presence of mosquito species using containers of water as an attractant to gravid females, with an oviposition substrate to retain eggs (Pratt and Jakob 1967). Because *Ae. aegypti* and *Ae. albopictus* eggs are indistinguishable except by electron microscopy (Darsie 1981), eggs collected by ovitrap are typically reared to 4th instar larvae or adult stage for identification. Preserving, transporting, rearing and identifying mosquitoes from eggs is a labor-intensive process, which limits the utility of oviposition traps for large (or frequent) surveys.

To overcome these limitations we employed the MosquiTrap®, an oviposition style trap (Ecovec, Brazil, described in Gama et al. 2007, Eiras 2002, Figure 3.3). In this trap females are retained on a sticky surface before or after oviposition. Use of these traps eliminates the need to count eggs and rear larvae for identification, allowing more sampling for a given amount of effort. The MosquiTrap® was developed to trap parous female *Ae. aegypti* mosquitoes that could then be tested for dengue or other pathogens. There was no information available on trap performance for *Ae. albopictus* in Hawaii, but results from Brazil suggests the traps are biased in favor of *Ae. aegypti* (A. Eiras, pers. comm.). This difference in trappability for the two species will result in observed proportions that differ from the true proportions of each species. Although the traps were designed in shape and appearance to match visual cues that female *Aedes* use in oviposition site selection, mosquito site choice is also influenced by olfactory cues. Water that contains fermenting organic material provides the best food for larvae, and hay infusion water is a typical bait for oviposition traps (Reiter et al. 1991). In place of hay infusion water MosquiTraps use a small plastic capsule of chemical lure called Atr*Aedes* (Ecovec) that is produced by Ecovec, which is replaced at every trapping session. Because attractiveness of artificial lures varies between species and strains (Kawada 2007; Farajollahi and Kesavaraju 2009), I tested the effectiveness of MosquiTraps with and without Atr*Aedes*, and with and without hay infusion water, at three Kona sites. For four trapping visits at three initial sites from May to July 2010, four traps were placed at each site, with the four possible combinations of two factors: fresh water with/without a modified hay infusion, and MosquiTrap with/without a capsule of Atr*Aedes* lure. At the conclusion of this trial, the results of a Fisher's exact test did not indicate any effect of bait method on mosquito catch rates for either species (*Ae. aegypti*: p=0.33; *Ae. albopictus*: p=0.51) or for total mosquitoes (p=0.75), so I conducted the main survey according to the trap developer's guidelines: plain tap water with Atr*Aedes* lures.

At each study site, traps were positioned to use the widest possible variety of appropriate locations, with a minimum separation of 15 m. This distance was chosen to minimize trap interactions while using the maximum number of traps per site. Strategies for trap placement followed Pratt and Jakob (1967) such as "shaded from direct sunlight."

Because *Ae. aegypti* is anthropophilic and is associated with human habitations, this factor could be important in explaining variation among traps and sites. Each trap location was categorized according to three levels of relative abundance of indoor human-inhabited space, and individual trap locations at a site were selected to range from locations completely indoors (when available) to the opposite extreme of outdoor shaded sites near dense vegetation. While each site had a different distribution of possible trap placement locations, the majority of traps were near or under buildings (level 2), with relatively few "indoor" (level1) and even fewer "outdoor" (level 3).

3.3. Results

3.3.1. Weather variation

Vapor pressure deficit by site is charted in Figure 3.4; an example of VPD variation over the year 2010 is shown at one site (Figure 3.5). One iButton at a coastal site (Kawaihae) was faulty and the four measurements at that site were excluded from analysis. Vapor pressure deficit, calculated for the 50 remaining site visits, was significantly different by site (Kruskal-Wallis chi-squared = 22.22, df = 8, p = 0.0045), but not by visit, suggesting that seasonality was low. The three coastal sites (Hookena, Keoua, Honokohau) had higher mean VPD than the seven non-coastal sites (Welch two-sample t test, p < 0.005). Although non-coastal sites were significantly wetter than coastal sites, there was no linear trend by elevation, as the highest three sites (584 m - 645 m) had higher mean VPD than the three middle elevation sites (191 m - 462 m) and the single driest (VPD) measurement was at one of the highest sites.

3.3.2. Trap Counts

The count for each species was recorded in 373 traps deployed events during 54 site visits from May-December 2010 (summarized in Table 3.2). *Ae. aegypti* were found at most sites, with the exception of the three highest-elevation sites, where only one individual was found during the entire study. *Ae. albopictus* were found at every site during every visit. The count data were overdispersed (i.e., having greater variance than would be expected based on a Poisson distribution), with mean 0.85 mosquitoes per trap and variance 4.15. Candidate variables to explain mosquito count were species elevation, indoor level, temperature, humidity, VPD (calculated as the mean vapor pressure deficit during the two weeks prior to trap collection), and the Maxent habitat suitability index (HSI) values from Chapter 2. To account for spatial and temporal autocorrelation and repeated measures, we used missed effects modeling (Zuur et al. 2009). Random effects were considered for site, visit, site-visit, trap, and per-observation.

The mosquito count data was modeled using the R package lme4 (Bates et al. 2011) as a generalized linear mixed model (GLMM) with Poisson distribution. The optimal model was selected based on Akaike Information Criterion (AIC, Burnham and Anderson 2002). The model selection process for mixed effects models is described by Zuur (2009). The overall maximum likelihood model included significant fixed effect parameters species (Wald test, $p < 0.001$), elevation (Wald test, $p < 0.001$) the species-elevation interaction (Wald test, $p < 0.001$), and a per-observation random effect. No other variables were significant. Mosquito counts by elevation are shown in Figure 3.6 and Figure 3.7. The modeling software was unable to include more than one random effect in a model, and the per-observation random effect explained more of the variation (according to AIC) than random effects for site, trap, or visit. Each species has fixed-effect parameters for the slope and intercept of a straight line describing the relationship between count and elevation; the slope for *Ae. aegypti* counts is negative (i.e., highest counts are at sea level), while *Ae. albopictus* was more common everywhere, and showed a positive relation to elevation (Figure 3.8). The slope sign and covariate significance were not changed by fitting the same model with two outliers removed (two separate traps at the same site, each with ten *Ae. aegypti*, during one coastal site visit after heavy rains in December).

The frequency of *Ae. aegypti* as a proportion of total mosquitoes at each site visit was fitted as a GLMM with binomially distributed errors, with the maximum likelihood model including a random effect for site. None of the covariate terms or their interactions were significant.

3.4. Discussion

Here I present the first systematic field survey of competitive dengue vector species in Hawaii. I show that the distributions of the two species vary significantly with elevation, and that elevation has opposite effects on the two species. The negative relationship between *Ae. aegypti* trap counts and elevation is not unexpected, given that the highest numbers of *Ae. aegypti* were at coastal sites, and none were found at the three highest elevations surveyed. This is consistent with observations that *Ae. aegypti* does relatively better at hot, dry locations (sites with higher VPD) when in competition with *Ae. albopictus*.

Ae. albopictus were found at all sites, and their trap counts had a significant positive relationship to elevation. Since the Kona landscape is hot and dry at sea level, becoming moister and cooler with altitude, this also is not unexpected. There is a "cloud band" between 600 m - 900 m elevation in North and South Kona, below and above which the climate is relatively drier. Because this band creates a climate that has some similarities to the windward areas where *Ae. albopictus* is dominant, it may create an "*albopictus* band." Rather than a linear relationship between mosquito counts and elevation, it would be reasonable to see a relationship that has highest values for *Ae. albopictus* at this high-moisture area. However, this pattern was not evident from the data, possibly because of the limited number of mid-elevation sites surveyed. In any case, elevation is merely a proxy for environmental variables, such as temperature and humidity, that impact mosquito populations, and any elevation pattern that applies to *Ae. albopictus* in Kona should not be extrapolated to other parts of the state until the underlying relationships with other environmental variables are better understood.

Although a middle-altitude "*albopictus* band" is broadly visible in the Maxent prediction map for that species (Chapter 2), in this survey there was no significant correlation between the relative abundance of either species and its Maxent prediction (HSI) at a given study site. There are several explanations for this. The input rasters for the Maxent predictions are necessarily inaccurate, given that they are themselves models interpolated from observations over many years (climate data) or at a single point in time (LANDSAT data). Furthermore, the raster data is aggregated into grid cells of approximately 19 hectares, so that each cell represents the aggregate value for a spatial area, whereas mosquito populations respond to the micro-climate and precise local conditions. The Maxent predictions estimate the probability that habitat for the species exists somewhere in a map cell, and each trap is only detectable by mosquitoes in a very limited area. Essentially, the Maxent models and the survey models are making predictions about two different things: the probability of mosquito habitat existence somewhere in a large spatial area (given the same environmental conditions under which the original presence records were obtained), versus the trappability of mosquitoes at a very precise location and time.

My results do not support the hypothesis that recent weather (two-week lagged mean of temperature, humidity, or the combination of the two as vapor pressure deficit) influence the counts of the two species. This was surprising, given mosquitoes' sensitivity to desiccation and dependence on water for larval habitat. While it is possible that a different aggregation time window would provide better explanatory power, the two-week period was chosen to include weather observations across the time the trap was active. Seasonal weather changes in Hawaii are relatively small, compared to intra-day variation (Figure 3.4) and site-by-site variation (Figure 3.5), and the time period of this study did not cover the yearly extremes of dryness at every site.

MosquiTraps have been shown to be more sensitive to detecting *Ae. aegypti* than other trap methods, even while catching lower absolute numbers (Gama et al. 2007). Because my trapping effort was significantly higher than any previous Kona surveys, my observation that a mosquito species was absent at a particular time and place is equally or more valid than earlier DOH and CDC observations from 1957, 1966 and 2002, and there is little chance that a continuous presence of *Ae. aegypti* exists at the sites where I did not find it.

3.5. Conclusion

The survey of North and South Kona across several months of weather variation did not reveal any sites of *Ae. aegypti* dominance in spite of our attempt to locate sites favorable to that species, and therefore tends to support the hypothesis that *Ae. aegypti* and *Ae. albopictus* are broadly sympatric. With the exception of the highest elevation sites, *Ae. aegypti* maintains consistent low populations throughout the Kona area, where it was always found with *Ae. albopictus*. Variations in the species with respect to geographic gradients in temperature and precipitation are consistent with the observations elsewhere that *Ae. albopictus*' advantage over *Ae. aegypti* is lowest, or even reversed, in hot, dry areas.

We know from Chapter 1 that *Ae. aegypti* can live in the range of environmental conditions found throughout the state's temperate elevations, and that the two species were once sympatric (at least at the landscape scale) in areas like Honolulu and Hilo. In this chapter we have seen that the co-existence continues, across temporal weather variation, at individual sites. Because the co-existence is largely restricted to Kona, the reasonable conclusion is that *Ae. aegypti*'s ability to persist in spite of the pervasive presence of *Ae. albopictus* depends on some combination of environmental factors that are unique to Kona, such as generally low humidity and infrequent flooding intervals for containers. Although similar conditions exist elsewhere in the state, the slopes of Mauna Loa, the world's largest shield volcano, provide large contiguous expanses of relatively homogeneous landscape, with relatively few of the shaded forested valleys that are common on the older islands.

Dispersal limitation is one possible explanation for the failure of *Ae. aegypti* to return to its former habitat. The presence of the species at dry coastal areas on the east side of the Big Island (Chapter 2) implies that the Mauna Loa mountain range and northeasterly trade winds are not effective in limiting *Ae. aegypti* to the western (leeward) side of the island. Although *Ae. aegypti* can be detected via long-term trapping at many places on the Kona Coast, it is far from universally common there, and likely exists as a metapopulation in patches that vary seasonally. Without population genetic studies it is not clear how isolated these patches are. Because adult mosquitoes generally do not move more than a few hundred meters, the majority of dispersal is probably by automobiles. We often observed adult females in our parked vehicle during site visits, and eggs can be present on the surface of any vehicle that is parked long enough to collect water, as well as on items that are transported by vehicle, such as coolers, potted plants, tires, or rubbish.

Inter-island dispersal limitation is likely to be much stronger than intra-island limitation, and it is reasonable to suppose there is currently a low probability of immigration between the Kona Coast and a dry (i.e., invasible) area on Oahu. An increase in inter-island boat traffic, such as would be caused by an automobile ferry service, could create a new connection between mosquito habitats and increase the probability of re-invasion, especially if the ferry terminals were located where *Ae. aegypti* occurs, or habitat is suitable. On the other hand, a decrease in boat transportation, such as might have happened with increased commercial air traffic in the mid-twentieth century, could decrease the rate of successful mosquito immigration.

This study does not clarify exactly how the relative abundance of the two species varies with weather. Although they vary in opposite directions with elevation, and among the study sites the elevation gradient is very strongly correlated (inversely) with annual temperature and (positively) with precipitation, short term (two-week) temperature and humidity variations had no significant effect on mosquito counts in this study.

In this survey we found no evidence for site-by-site segregation or exclusion. I conclude, given the assumption that the two species are in competition, and the historically observed ability of *Ae. aegypti* to exist everywhere in the state, that some threshold level of *Ae. albopictus*, probably combined with low immigration rates, keeps *Ae. aegypti* from establishing via priority effect (Chapter 2). From the perspective of vector borne disease in Hawaii, *Ae. albopictus* is a mixed blessing: while sometimes supporting low levels of dengue transmission, it appears to exclude a more efficient dengue vector, *Ae. aegypti*. However, there is clearly still the potential for *Ae. aegypti* to re-gain a foothold given its history of returning after full or near-eradication (e.g., Gubler and Clark 1995). The situation could be changed by an increase in mosquito immigration rates (such as might be caused by an inter-island ferry, or a new market in used tires), and also by a weakening of the prophylaxis effect of *Ae. albopictus* (e.g., by the dry weather predicted under some climate change scenarios, or by pesticide use that decreases forest insect populations). This highlights the importance of a robust and consistent vector control program in providing continuous and expert monitoring in areas of good habitat for *Ae. aegypti* and areas with high immigration risk, such as airports and harbors.

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Tables

Table 3.1. Site characteristics. Modeled species distribution probabilities are from Chapter 2. Predominant land cover is calculated for the entire cell (19 hectares) and often differs from cover at the immediate site.

	Ae. aegypti (historic)	*Ae. aegypti* (modern)	*Ae. albopictus*	Elev (m)	Latitude	Longitude	Land Cover (site / predominant)
CTAHR	0.46	0.65	0.67	445	19.53	-155.93	scrub / scrub
CTAHR-mauka	0.36	0.41	0.47	645	19.50	-155.90	scrub / grassland
Dragonfly	0.51	0.74	0.27	191	19.44	-155.89	grassland / grassland
Greenwell	0.55	0.91	0.22	462	19.49	-155.91	scrub / scrub
Hao Place	0.23	0.28	0.16	619	19.70	-155.96	low intens. devel. / evergreen
Honokohau	0.73	0.41	0.27	4	19.67	-156.02	low intens. devel. / bare land
Hookena	0.74	0.32	0.39	2	19.38	-155.90	scrub / water
Keoua	0.63	0.31	0.08	2	19.42	-155.91	grassland / water
Kawaihae	0.45	0.51	0.36	31	20.04	-155.83	scrub / bare land
Mitchell	0.27	0.39	0.16	584	19.55	-155.92	scrub / grassland

Table 3.2. Total traps and mosquitoes caught by species at each site visit.

Site Name (elev) visit	# Traps	count (*aeg.*)	count (*albo.*)
CTAHR (450 m)			
5	4	1	5
6	8	1	15
7	8	1	8
8	8	2	7
9	8	0	3
CTAHR-mauka (650 m)			
6	4	0	1
7	4	0	2
8	4	0	2
9	4	0	1
Dragonfly (190 m)			
2	4	1	27
3	4	3	7
4	4	0	10
5	5	4	16
6	11	2	14
7	11	0	9
8	11	0	16
9	11	0	23
Greenwell (460 m)			
2	4	1	20
3	4	0	19
4	4	0	7
5	4	0	22
6	11	2	21
7	11	4	7
8	11	3	21
9	11	0	4
Honokohau (4 m)			
6	8	0	7
7	8	2	12
8	8	2	8
9	8	0	12
Hookena (2 m)			
2	4	2	10
3	4	0	6
4	4	1	2
5	6	0	4
6	10	3	9
7	10	1	10
8	10	0	11
Hao Place (620 m)			
3	3	1	23
4	3	0	17
5	4	0	27
6	4	0	3
7	4	0	5
8	4	0	9
9	4	0	5
Kawaihae (20 m)			
6	13	2	7
7	11	3	25
8	11	4	13
9	11	4	19
Keoua (2 m)			
6	12	3	1
7	12	1	5
8	12	2	33
9	12	24	11
Mitchell (580 m)			
6	9	0	7
7	8	0	1
8	8	0	2

Figures

Figure 3.1. Big Island map with sites

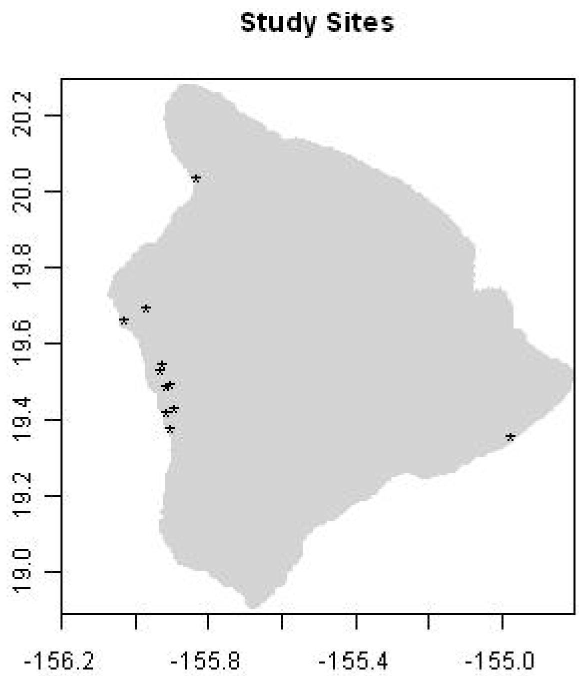

Figure 3.2. DS1923 iButton. Picture by Maxim.

Figure 3.3. MosquiTrap. Picture by Ecovec.

Figure 3.4. During the study period, dryness (VPD) was significantly higher at the coastal sites, but did not show a uniform declining trend with elevation.

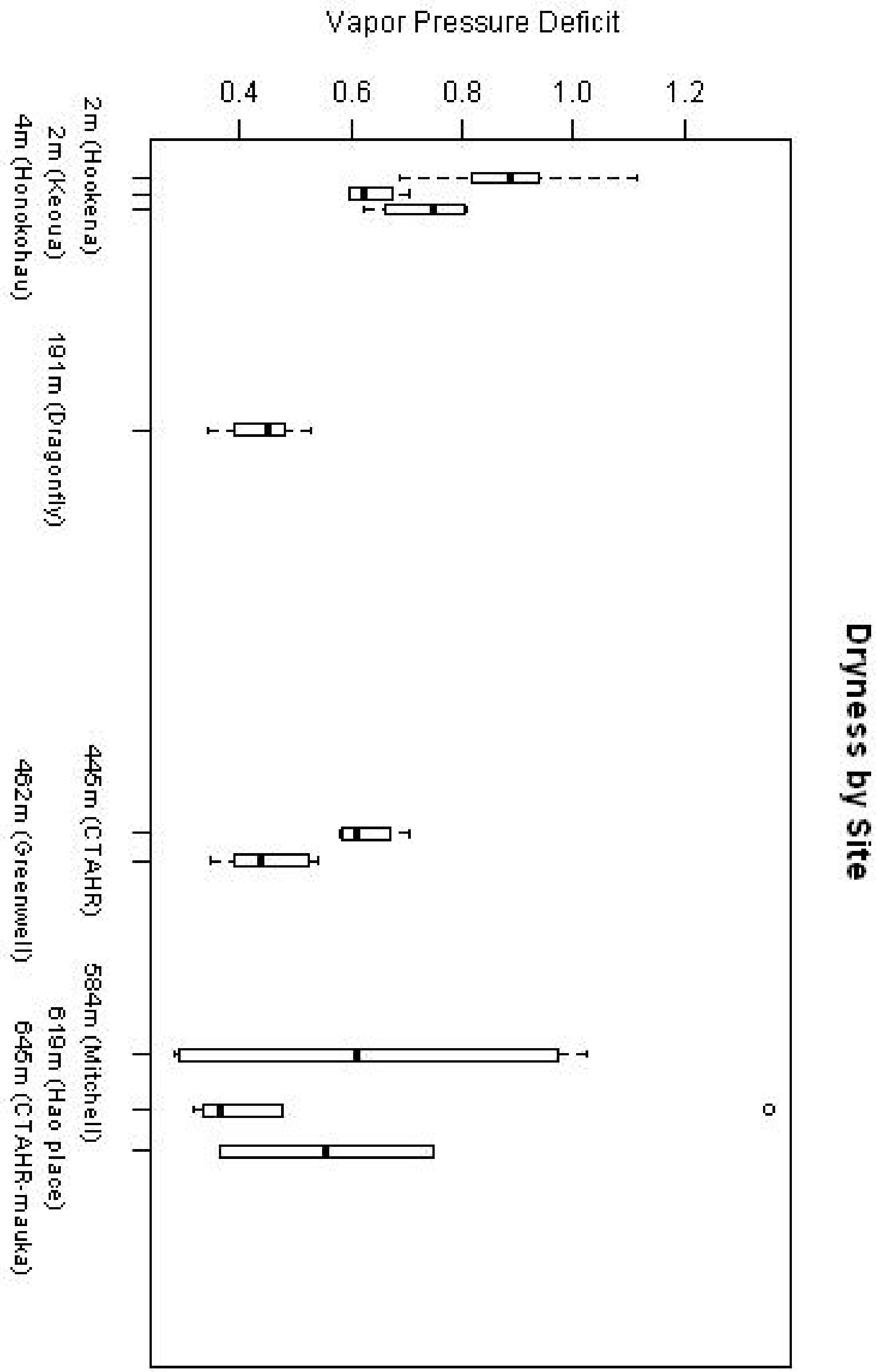

Figure 3.5. A full year of weather observations from the permanent USDA weather station near the CTAHR study site in Captain Cook, Hawaii, show one site's variation in dryness (VPD) at different time scales. Although Hawaii generally has cooler weather and higher precipitation in winter, seasonal trends are often small compared with multi-week variation and site-by-site differences.

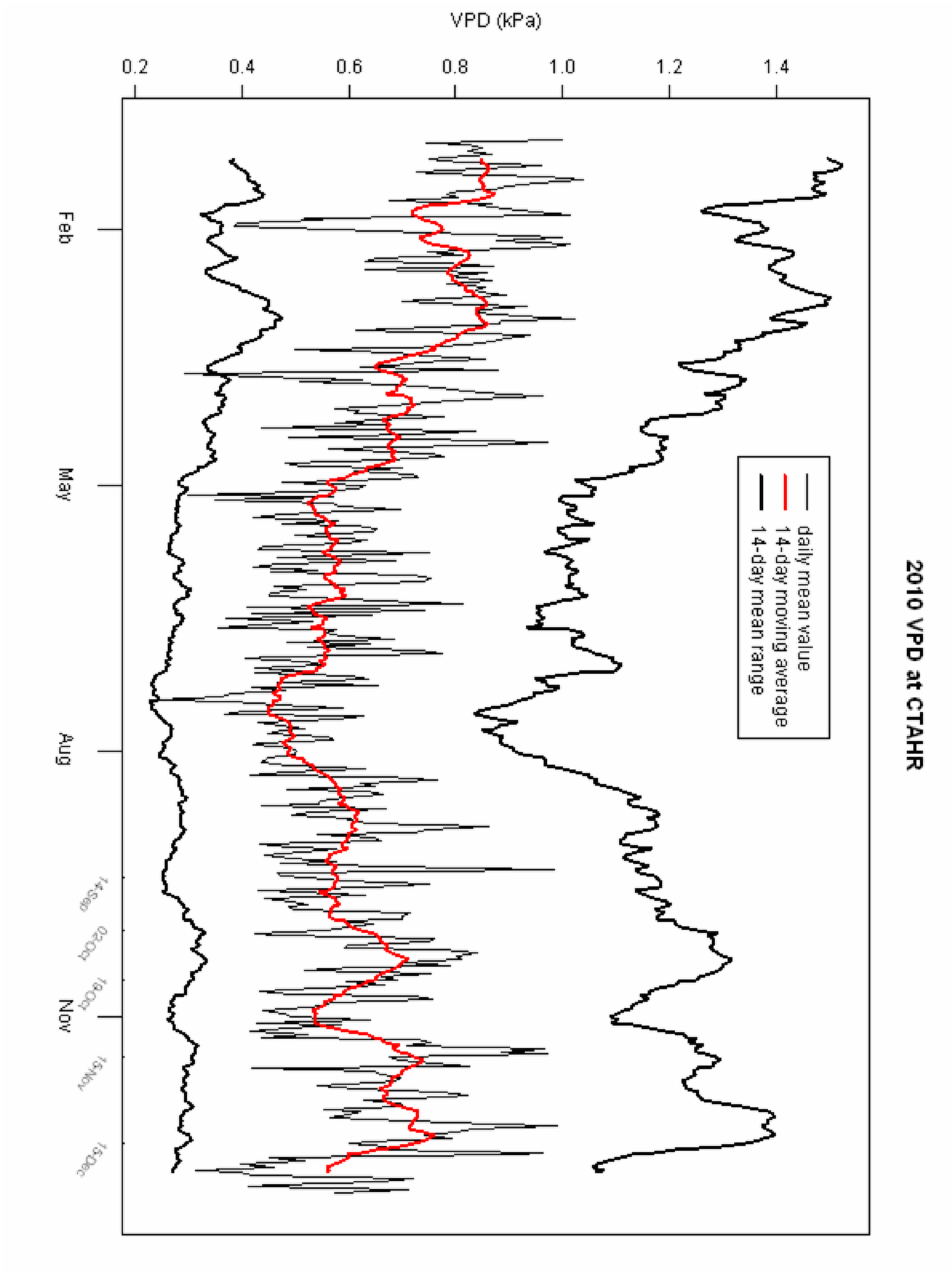

Figure 3.6. Ae. aegypti counts were highest at the coastal sites, and were not found at the three highest elevations surveyed.

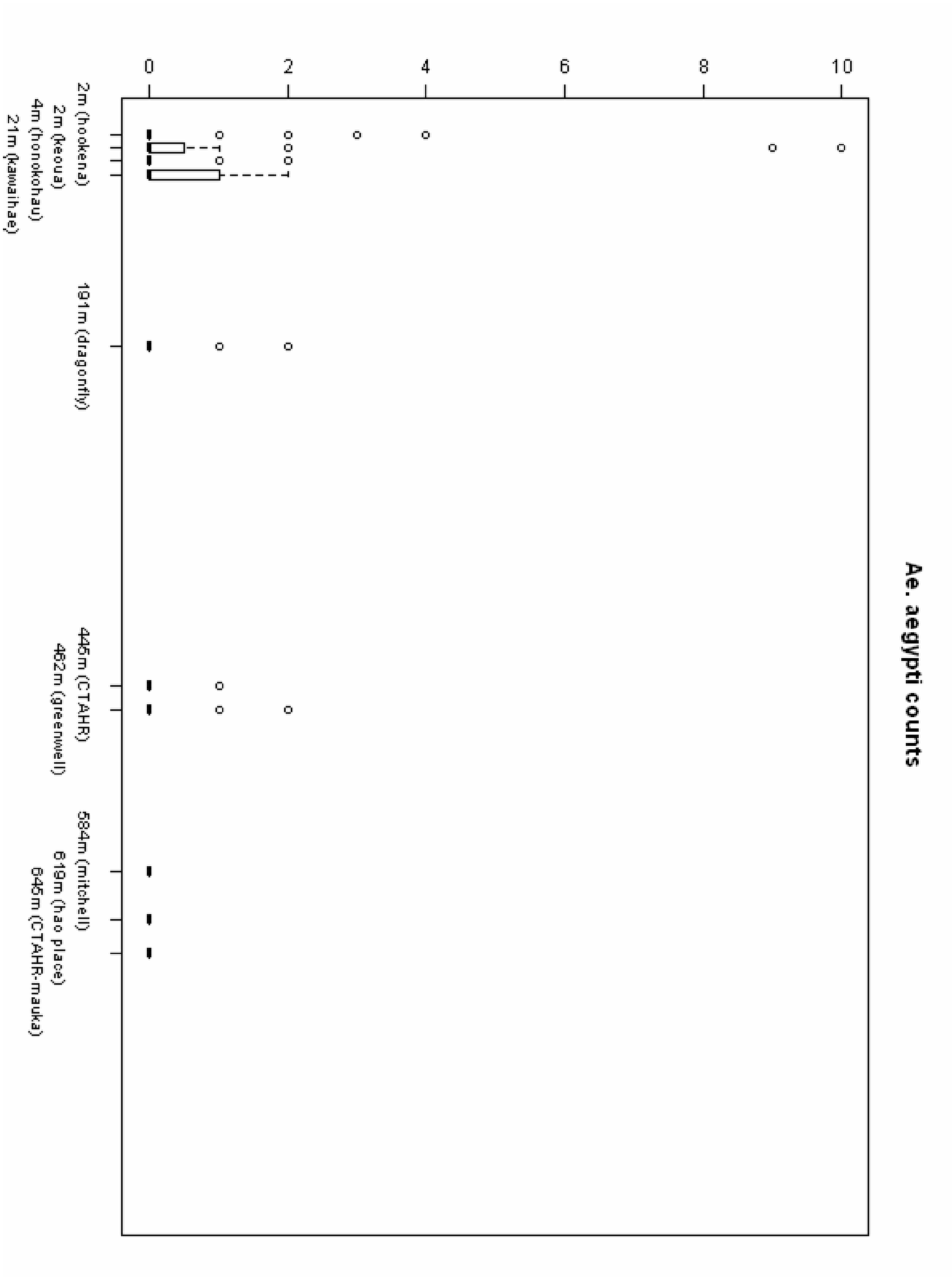

Figure 3.7. Ae. albopictus were more common than Ae. aegypti at every site surveyed, although the difference was smallest at the coastal sites.

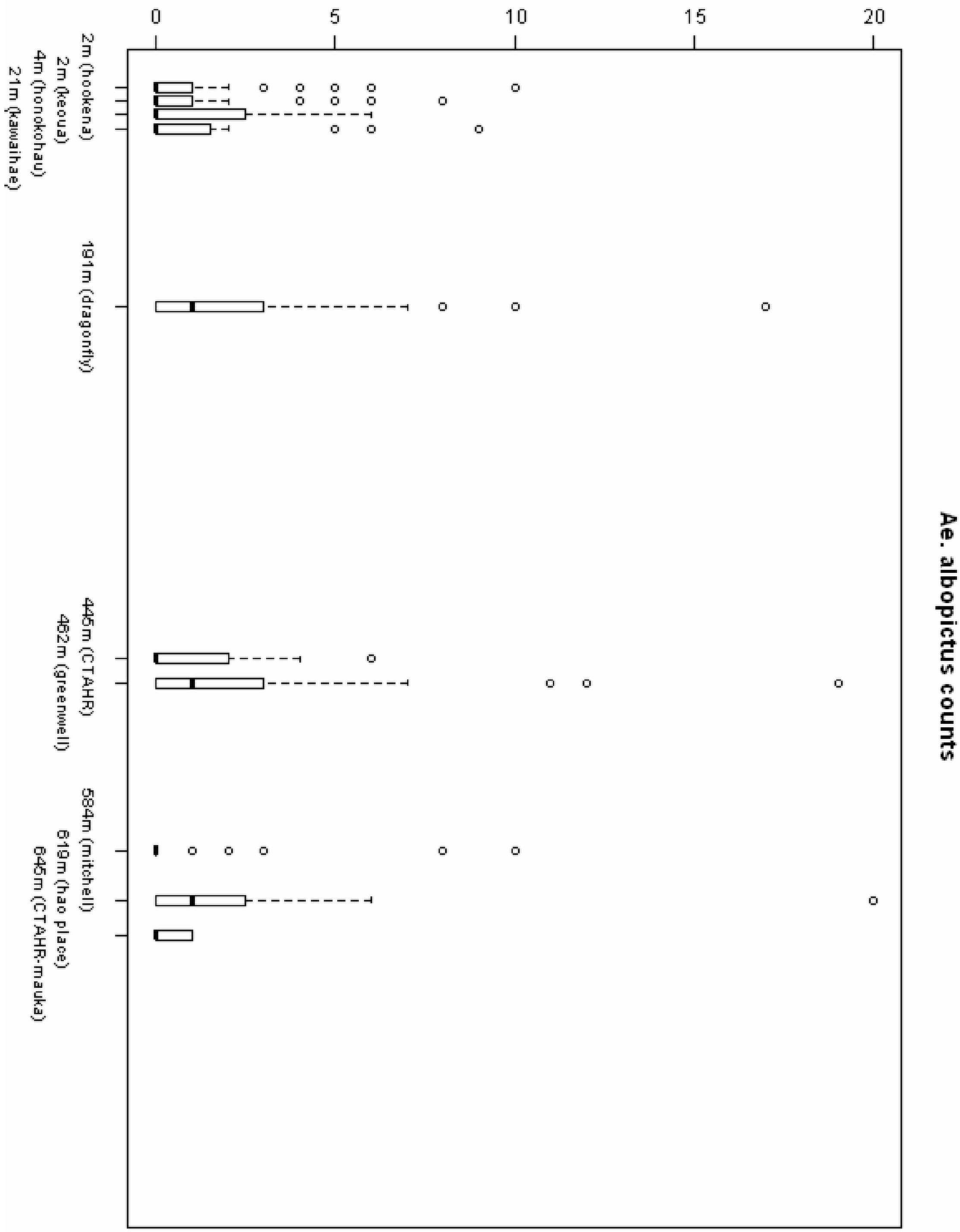

Figure 3.8. Mosquito counts for the two species show opposing correlations with elevation (a strong proxy for annual mean coolness and precipitation); this is consistent with observations elsewhere that Ae. albopictus' competitive advantage over Ae. aegypti is weakest in hot dry areas.