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# PREVALENCE AND DISTRIBUTION OF POX-LIKE LESIONS, AVIAN MALARIA, AND MOSQUITO VECTORS IN KĪPAHULU VALLEY, HALEAKALĀ NATIONAL PARK, HAWAII, USA

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**ABSTRACT:** We determined prevalence and altitudinal distribution of introduced avian malarial infections (*Plasmodium relictum*) and pox-like lesions (*Acipoxvirus*) in forest birds from Kīpahulu Valley, Haleakalā National Park, on the island of Maui, and we identified primary larval habitat for the mosquito vector of this disease. This intensively managed wilderness area and scientific reserve is one of the most pristine areas of native forest remaining in the state of Hawaii, and it will become increasingly important as a site for restoration and recovery of endangered forest birds. Overall prevalence of malarial infections in the valley was 8% (11/133) in native species and 4% (4/101) in nonnative passerines; prevalence was lower than reported for comparable elevations and habitats elsewhere in the state. Infections occurred primarily in 'Apapane (*Himatione sanguinea*) and Hawaii 'Amakihi (*Hemignathus virens*) at elevations below 1,400 m. Pox-like lesions were detected in only two Hawaii 'Amakihi (2%; 2/94) at elevations below 950 m. We did not detect malaria or pox in birds caught at 1,400 m in upper reaches of the valley. Adult mosquitoes (*Culex quinquefasciatus*) were captured at four sites at elevations of 640, 760, 915, and 975 m, respectively. *Culex quinquefasciatus* larvae were found only in rock holes along intermittent tributaries of the two largest streams in the valley, but not in standing surface water, pig wallows, ground pools, tree cavities, and tree fern cavities. Mosquito populations in the valley are low, and they are probably influenced by periods of high rainfall that flush stream systems.

**Key words:** Avian malaria, avian pox, *Acipoxvirus*, *Culex quinquefasciatus*, habitat management, *Plasmodium relictum*.

## INTRODUCTION

The introduction of mosquito vectors, avian pox (*Acipoxvirus*) and avian malaria (*Plasmodium relictum*) to the Hawaiian Islands has played a significant role in the continuing decline and extinction of native Hawaiian forest birds (Warner, 1968c; van Riper et al., 1986). Limited historical records suggest that the primary vector of these diseases, *Culex quinquefasciatus*, was first introduced on Maui (Hardy, 1960), but it is not known when avian pox or malaria reached the island. Of 10 species of endemic passerines that were recorded from Maui historically, four are now thought to be extinct (Jacobi and Atkinson, 1995). Remaining species are limited to a narrow band of high-elevation

rain forest that encircles the northern and eastern slopes of Haleakalā Volcano. These areas are extremely steep, rugged, and isolated. Although a few of the most critically endangered species of honeycreepers may still persist, their long-term future is uncertain given continuing threats posed by invasive plants, diseases, insects, and climate change (Loope, 1998; Benning et al., 2002).

Kīpahulu Valley, on the southeastern slopes of Haleakalā Volcano, is one of the most pristine areas remaining in the state of Hawaii. The valley is an intensively managed scientific reserve in Haleakalā National Park that is now largely fenced and free of feral ungulates from 300-m elevation to the summit. This area was first explored by the Kīpahulu Valley Scientific

Expedition of 1967, and its value as an intact native forest ecosystem is widely recognized (Warner, 1968a). Scientists on the expedition rediscovered Maui Nukupu'u (*Hemignathus lucidus affinis*) at the upper reaches of this valley. They also noted the virtual absence of native species below approximately 850 m, and they described gradually increasing numbers of native honeycreepers as elevation increased. These changes were inversely related to the presumed distribution of *Culex quinquefasciatus* in the valley, leading Warner (1968b) to suggest that avian pox and malaria were the primary limiting factors for native birds at lower elevations.

We conducted surveys for avian malaria and pox-like lesions at five locations in Kīpahulu Valley and at one location on a ridgeline adjacent to the valley to obtain baseline data on disease prevalence. Terrestrial habitats and stream margins also were surveyed to identify primary mosquito larval habitat. Findings are discussed in relation to the possibility that reduction of mosquito larval habitat may represent a long-term management strategy for reducing disease transmission in Haleakalā National Park.

## MATERIALS AND METHODS

### Study sites

Study sites were chosen based on their accessibility and elevation. Kīpahulu Valley is extremely rugged and accessible only by foot or helicopter (Fig. 1). The valley is isolated by sheer walls and divided into an upper and lower shelf by a vertical wall. The lower shelf is drained by Palikea Stream and its tributaries, whereas the upper shelf is drained by Kaukau'ai Stream that flows in a deep canyon along the western wall of the valley. Our study sites were located at a series of Haleakalā National Park management camps at 640 m (BRAVO), 945 m (GINGER), and 1,430 m (CHARLIE) on the upper shelf, and 760 m (FERN) and 915 m (DELTA) on the lower shelf of the valley. We also visited a park management camp on a ridgeline west of the valley at 975 m (LOST). Each location was visited for up to seven consecutive days between August and Decem-

ber 2002. FERN and BRAVO sites were each visited a second time for 3–4 days during this 5-mo period for additional sampling.

### Mist netting and blood collection

At each study site, mist nets were set up in areas frequented by native birds and adjacent to fruiting or blooming trees, particularly 'Ōhi'a (*Metrosideros polymorpha*). Forest birds were captured using 6–18-m-long, 36-mm mesh mist nets, supported by 5–6-m collapsible fishing poles. From 10 to 18 nets were operated from sunrise to sunset for 4–5 days at each location. Nets were closed whenever persistent rain or wind occurred. A sample of blood no more than 1% of the bird's body weight was collected via jugular venipuncture with a heparinized 28 gauge needle and insulin syringe. A blood smear was immediately made, air-dried, and fixed for several minutes with absolute methanol. The remaining blood sample was transferred to microhematocrit tubes, centrifuged, and separated into plasma and packed blood cells. Plasma was frozen for serologic analysis, and packed red blood cells were mixed with 100  $\mu$ l of Tris-EDTA lysis buffer (0.1 M Tris-HCl, pH 8.0, 0.1 M EDTA, and 2% sodium dodecyl sulfate) and frozen for future genetic studies.

### Adult mosquito trapping

Adult mosquito traps were placed from 150 to 200 m apart along existing trails and fence lines. Because of steep slopes in the valley, traps were located along contour lines whenever possible. Adult mosquitoes were trapped using battery-powered Centers for Disease Control and Prevention (CDC) miniature light traps operated without a light bulb and CDC gravid female traps (J. W. Hoch Company, Gainesville, Florida, USA). The CDC miniature light traps were modified with ABC trap lids (American Biophysics, East Greenwich, Rhode Island, USA) and baited with CO<sub>2</sub> gas (henceforth referred to as CO<sub>2</sub> traps) from a 2.3- or 4.5-kg CO<sub>2</sub> tank connected to the trap with vinyl tubing. Flow rates were adjusted to 200 to 500 ml/min with a regulator. CO<sub>2</sub> traps were hoisted to mid-canopy in the forest with a small pulley that was suspended from a branch. Gravid female traps were paired with CO<sub>2</sub> traps and baited with alfalfa infusions. Both CO<sub>2</sub> and gravid female traps were set at dusk and checked in the morning. Live adult mosquitoes were aspirated from the traps and shipped alive to Kilauea Field Station, Pacific Island Ecosystems Research Center at Hawai'i Volcanoes National Park. Midguts and salivary glands were dissected

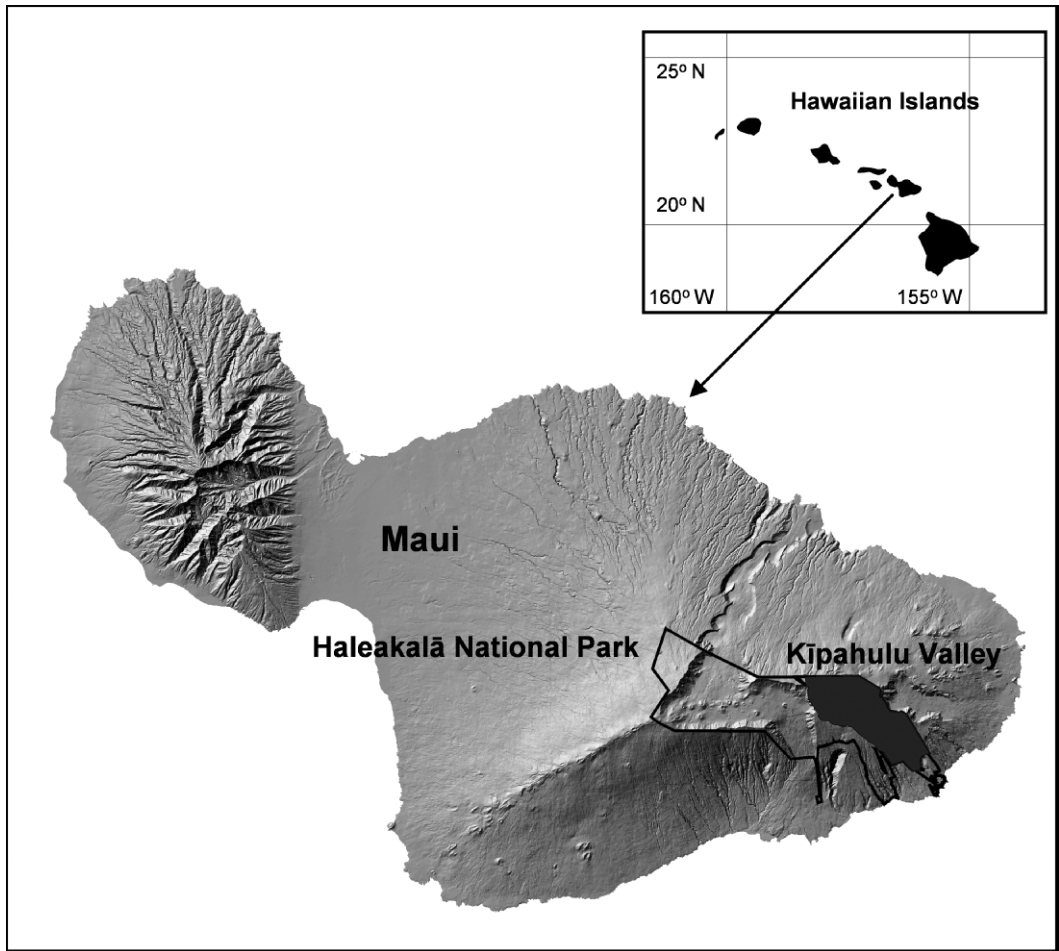


FIGURE 1. Location of Kīpahulu Scientific Reserve (20°42'N, 156°02'W) and Haleakalā National Park on the Island of Maui.

from surviving mosquitoes, and slide preparations were examined by either phase-contrast or Normaski contrast interference microscopy to detect oocysts and salivary gland sporozoites (Garnham, 1966).

#### Mosquito larval surveys

Two 1-km transects were traversed at each site to locate mosquito larval habitat and to detect the presence of feral ungulates. Transects were chosen based on existing transects and trails to minimize forest disturbance. One transect was oriented to follow altitudinal contours, and the other transect was oriented to cross contour lines and cover a significant elevation range at each study site. Transects were divided into 10-m segments, and data were recorded for each segment. All standing surface water was examined within 2.5 m of

either side of the transect by dipping water sources with a standard mosquito dipper or turkey baster. We searched for ground pools, rock holes, bogs, tree fern cavities, tree holes, ponds, seeps, small permanent and intermittent streams, and pig wallows that were identified by the presence of freshly disturbed soil. Feral ungulate activity was noted as fresh, intermediate, or old using the procedure and criteria of Anderson and Stone (1993).

We were able to conduct stream surveys at BRAVO and FERN where access to stream canyons and smaller tributaries was possible. Streams were separated into 10-m segments and followed as long as possible before reaching an impassible waterfall. Rock pools and stream margins were surveyed by dipping a maximum of 10 dips per 10-m section. Flowing water was not sampled. Mosquito larvae were identified in the field. Represen-

tative samples also were placed in 70% ethanol for verification in the laboratory.

### Malarial and pox diagnostics

Blood smears were stained with 6% phosphate-buffered Giemsa, pH 7.0, for 1 hr, rinsed with tap water, air-dried, and examined by microscopy to diagnose acute and chronic malarial infections. One hundred 500 $\times$  fields were examined on each slide, which was equivalent to a search effort of approximately 30,000 erythrocytes per smear. Plasma samples were screened by immunoblotting (Atkinson et al., 2001a, 2005) to detect antibodies to *P. relictum*. We used both microscopy and results from immunoblotting to establish infection status for each individual. Birds that tested positive by either smear or serology were classified as positive. Birds were classified as negative when both smears and serology were negative. These criteria allowed us to identify birds with both acute and chronic malarial infections, but we were unable to detect infections that were less than 2–3 days old, when parasites were undergoing initial rounds of multiplication in fixed tissues of the bird and numbers of parasites in the peripheral circulation are extremely low (Garnham, 1966).

Birds were examined carefully in the field for swollen or crusty lesions on exposed skin or missing toes, which may be indicative of active or old pox-like infections. Active pox-like lesions were identified by presence of swellings or crusty scabs. Inactive pox-like lesions were identified by presence of missing toes that were otherwise healed (van Riper et al., 2002; Atkinson et al., 2005).

### Statistical analyses

Binary logistic regression in the statistical program Systat 11.0 (Steinberg and Colla, 2004) was used to test for association between presence or absence of malarial infection and the independent covariates of species origin (native or nonnative) and elevation for all study sites. Results were considered significant when  $P < 0.05$ .

## RESULTS

### Malarial and pox prevalence

We captured 247 birds in total belonging to 10 species during the study (Table 1), but we collected sufficient blood from only 234 individuals for diagnostic analyses by both blood smear and serology. Native species (Hawai'i 'Ama-

kahi, 'Apapane, 'Iiwi [*Vestiaria coccinea*], and Maui 'Alauahio [*Paroreomyza montana*]) made up 55.5% (137 of 247) of the total, ranging from 14% (1 of 7) of the catch at BRAVO (640 m) to 76% (31 of 41) of the total catch at GINGER (945 m). Of the 234 individuals that were tested by both microscopy and serology for malaria, 15 birds (6.4%) were positive by microscopy or serology, with most infections detected in Hawai'i 'Amakihi ( $n=8$ ), followed by 'Apapane ( $n=3$ ), Nutmeg Mannikin (*Lonchura punctulata*) ( $n=2$ ), Japanese White-eye (*Zosterops japonicus*) ( $n=1$ ), and Red-billed Leiothrix (*Leiothrix lutea*) ( $n=1$ ). Eight of the 15 infections (53%) were positive by both microscopy and serology, and an additional seven birds tested positive for malaria by serology alone. Only two birds with pox-like lesions were captured during the sampling period—a Hawai'i 'Amakihi from FERN (760 m) with active, crusty lesions on one foot; and a Hawai'i 'Amakihi from DELTA (915 m) with a healed missing toe.

Prevalence of malarial infection ranged from 14% at BRAVO (760 m) to 0% at CHARLIE (1,430 m) (Table 1), but there was no significant association between elevation and malarial infection ( $P=0.115$ ). There were no significant differences in prevalence of malarial infection between native (8%; 11/133) and nonnative species (3.9%; 4 of 102) ( $P=0.785$ ), and there were no significant interactions between elevation and status as native or nonnative species ( $P=0.972$ ).

### Adult mosquito trapping

*Culex quinquefasciatus* were captured in CO<sub>2</sub> traps at elevations up to 975 m, ranging from 0.07 captures/trap night at DELTA to 2.54 captures/trap night at BRAVO (Table 2). Because of logistical difficulties in shipping live mosquitoes between islands, only a fraction of captured mosquitoes survived shipping, and they were dissected to determine infection status. Prevalence of malarial infection in host-seeking mosquitoes in CO<sub>2</sub> trap catches at



TABLE 1. Total captures and percent prevalence of malaria and pox by species at each study site<sup>a</sup>. Malarial prevalence is based on a sample of 234 individuals that were tested by both microscopy and serology. Pox prevalence is based on physical examination of all captured individuals (*n*=247) for pox-like lesions.

Site	Elevation (m)	Variable	HAAM	APAP	IIWI	MAAL	JAWE	RBLE	JABW	MELT	NOCA	NUMA	Total	
BRAVO	640	Total captures	1	NC <sup>b</sup>	NC	NC	2	3	NC	NC	1	NC	7	
		Malaria	0% (0/1)	NC	NC	NC	0% (0/2)	33% (1/3)	NC	NC	0% (0/1)	NC	14% (1/7)	
		Pox	0% (0/1)	NC	NC	NC	0% (0/2)	0% (0/3)	NC	NC	0% (0/1)	NC	0% (0/7)	
FERN	760	Total captures	25	1	NC	NC	8	10	1	NC	NC	3	48	
		Malaria	22% (5/23)	0% (0/1)	NC	NC	0% (0/6)	0% (0/9)	0% (0/1)	NC	NC	NC	33% (1/3)	14% (6/43)
		Pox	4% (1/25)	0% (0/1)	NC	NC	0% (0/8)	0% (0/10)	0% (0/1)	NC	NC	NC	0% (0/3)	4% (2/48)
DELTA	915	Total captures	22	8	2	NC	8	10	5	NC	NC	3	58	
		Malaria	5% (1/21)	25% (2/8)	0% (0/2)	NC	13% (1/8)	0% (0/10)	0% (0/5)	NC	NC	NC	33% (1/3)	9% (5/57)
		Pox	5% (1/22)	0% (0/8)	0% (0/2)	NC	0% (0/8)	0% (0/10)	0% (0/5)	NC	NC	NC	0% (0/3)	3% (2/58)
GINGER	945	Total captures	12	15	4	NC	4	5	NC	NC	NC	1	41	
		Malaria	0% (0/12)	0% (0/15)	0% (0/4)	NC	0% (0/3)	0% (0/5)	0% (0/5)	NC	NC	NC	0% (0/1)	0% (0/40)
		Pox	0% (0/12)	0% (0/15)	0% (0/4)	NC	0% (0/4)	0% (0/5)	0% (0/5)	NC	NC	NC	0% (0/1)	0% (0/41)
LOST	975	Total captures	27	6	NC	NC	16	9	4	2	1	NC	65	
		Malaria	7% (2/27)	20% (1/5)	NC	NC	0% (0/14)	0% (0/8)	0% (0/8)	0% (0/4)	0% (0/2)	0% (0/1)	NC	5% (3/61)
		Pox	0% (0/27)	0% (0/6)	NC	NC	0% (0/16)	0% (0/9)	0% (0/4)	0% (0/4)	0% (0/2)	0% (0/1)	NC	0% (0/65)
CHARLIE	1,430	Total captures	7	2	1	4	2	10	1	NC	NC	1	28	
		Malaria	0% (0/7)	0% (0/2)	0% (0/1)	0% (0/4)	0% (0/1)	0% (0/10)	0% (0/1)	0% (0/1)	NC	NC	0% (0/1)	0% (0/27)
		Pox	0% (0/7)	0% (0/2)	0% (0/1)	0% (0/4)	0% (0/2)	0% (0/10)	0% (0/1)	0% (0/1)	NC	NC	0% (0/1)	0% (0/28)
Total	Total	Total	94	32	7	4	40	47	11	2	2	8	247	
		Malaria	9% (8/91)	10% (3/31)	0% (0/7)	0% (0/4)	3% (1/34)	2% (1/45)	0% (0/11)	0% (0/2)	0% (0/2)	0% (0/2)	25% (2/8)	6% (15/234)
		Pox	2% (2/94)	0% (0/32)	0% (0/7)	0% (0/4)	0% (0/40)	0% (0/47)	0% (0/11)	0% (0/2)	0% (0/2)	0% (0/2)	0% (0/8)	1% (2/247)

<sup>a</sup> Species are indicated by codes as follows: HAAM = Hawaii 'Amakihi (*Hemignathus virens*), APAP = 'Apapane (*Himatione sanguinea*), IIWI = 'Iiwi (*Vestiaria coccinea*), MAAL = Maui 'Alauahio (*Parozomiza montana*), JAWE = Japanese White-eye (*Zosterops japonicus*), RBLE = Red-billed Leiothrix (*Leiothrix lataea*), JABW = Japanese Bush Warbler (*Cettia diphone*), MELT = Melodious Laughing Thrush (*Garrulax canorus*); NOCA = Northern Cardinal (*Cardinalis cardinalis*), NUMA = Nutmeg Mannikin (*Lonchura punctulata*). Numbers in parentheses indicate number positive over total number examined for each species.

<sup>b</sup> NC = none captured.

BRAVO and FERN, the two lowest elevation study sites, was 4 and 3%, respectively. We did not detect malarial infections in the small numbers of adult *Culex* from LOST that were dissected. Adult mosquitoes were not captured or observed at GINGER or CHARLIE.

### Larval surveys

Small ground pools and tree fern cavities were the two primary sources of standing water along the 12 km of terrestrial transects that we sampled. Small ground pools made up more than 90% of the water bodies that were found, but their abundance was relatively low, ranging from 1.5 to 15 pools/km of transect. *Culex* larvae were not found in any of these terrestrial water sources.

Intermittent streambeds and associated rock holes were the most abundant sources of standing water in the valley. Because of difficulties safely accessing larger stream drainages in the upper reaches of the valley, we were able to complete stream surveys at only BRAVO and FERN. We surveyed 360 m of streambed along two dry intermittent streams at BRAVO, and we found *C. quinquefasciatus* larvae. However, we did not find mosquito larvae along 200 m of the larger perennial Kaukaūai Stream. Mosquito larvae were found in rock pools and puddles of stagnant water containing decomposing leaves and tree fern (*Cibotium* spp.) fronds. They were relatively common in the two streambeds without flowing water, occurring in 61% (22 of 36) of the 36 10-m segments that were sampled. At FERN, we sampled short segments of three streams (160, 110, and 110 m). Larvae were found in 18% (2 of 11) of the 11 10-m segments that were sampled in one large streambed without flowing water, but not in the two segments of the larger flowing Palikea Stream. As observed at BRAVO, larvae were present in rock pools containing stagnant water and organic debris.

TABLE 2. Mosquito captures per trap night for CO<sub>2</sub> and gravid female traps.

Location	BRAVO <sup>a</sup>	FERN	DELTA	GINGER	LOST	CHARLIE
Elevation (m)	640	760	915	945	975	1,430
CO <sub>2</sub> catches (no./trap night)	2.54 (n=37)	2.44 (n=34)	0.07 (n=14)	0 (n=15)	0.59 (n=17)	0 (n=12)
Malarial prevalence	4% (1/26)	3% (1/33)	ND	ND	0% (0/5)	ND
Gravid female catches (no./trap night)	0.06 (n=65)	0 (n=42)	0 (n=24)	0 (n=24)	0.04 (n=24)	0 (n=21)
Malarial prevalence	0% (0/4)	ND <sup>b</sup>	ND	ND	ND	ND

<sup>a</sup> Traps were operated in both August and December.

<sup>b</sup> ND = no dissections.

## DISCUSSION

Prevalence and transmission of avian malaria and avian pox in Hawai'i are dependent on vector populations that are, in turn, regulated by altitudinal variations in temperature and rainfall as well as availability of suitable larval habitat (LaPointe, 2000; Ahumada et al., 2004). In Hawai'i, vector populations and transmission rates become more seasonal as altitude increases; declining temperatures limit larval development and place thermal constraints on development of malarial parasites within adult mosquitoes (LaPointe, 2000; Benning et al., 2002). These conditions generally lead to declines in prevalence and transmission of these diseases as elevation increases (van Riper et al., 1986, 2002; Atkinson et al., 2005). *Culex quinquefasciatus* populations typically peak in late summer and early fall in the Hawaiian Islands, and we timed our fieldwork so that it encompassed the presumed peak time for disease transmission on Haleakalā Volcano (van Riper et al., 1986; LaPointe, 2000).

We found a low prevalence of both pox-like lesions and malarial infections in Kīpahulu Valley relative to other locations in Hawai'i where comparable methodologies have been used (Atkinson et al., 2005). This suggests that these two introduced diseases are not well established in this area. In studies on windward and leeward Hawai'i Island, prevalence of malarial infection in native species ranged from 70 to 100% at elevations below 600 m, to less than 15% at 1,800 m on Mauna Loa and Kilauea Volcanoes (Atkinson et al., 2005; Woodworth et al., 2005). In forest habitats on leeward Mauna Loa Volcano that were comparable with sites sampled in this study (600–1,000 m), prevalence of malarial infection in native species was 34%, more than 4 times higher than what we observed in Kīpahulu (Atkinson et al., 2005).

Possible factors explaining lower prevalence of malaria/pox in Kīpahulu Valley

include decreased host or vector susceptibility or lower numbers of mosquitoes due to insufficient or unsuitable breeding habitat. Results from experimental studies (Atkinson et al., 1995, 2000, 2001b; Yorinks and Atkinson, 2000) indicate that all four species of native honeycreepers sampled in this study are highly susceptible to avian malaria. Because of this and the recovery of naturally infected *Culex* mosquitoes in lower portions of the valley, very low population densities or sporadic occurrence of *C. quinquefasciatus* may be the primary factor responsible for lower disease transmission in the valley. Although Kīpahulu Valley and southeastern Haleakalā Volcano are climatically similar to other high mountains in the archipelago, they are unique relative to other areas in Hawai'i because of their physical isolation, large area of relatively undisturbed native habitat, and extremely low numbers of feral pigs and other introduced ungulates that can create standing water sources in rain forest habitats (Atkinson et al., 1995). The absence of habitat fragmentation caused by ranching and suburban development, the absence of lava flows that create natural corridors for movement of mosquito vectors into forest habitats, and the absence of artificial water sources (e.g., stock ponds, cattle troughs, water tanks, and irrigation systems) that create larval habitat for *C. quinquefasciatus* are also likely to be important factors that may limit mosquito numbers.

Isolated on the southeastern coast of Maui Island, Kīpahulu Valley receives typical tradewind weather and heavy rainfall associated with orographic effects of Haelakalā Volcano. Estimates of precipitation range from 2 to 6 m annually (Giambelluca and Schroeder, 1998). The valley has two large perennial streams along its eastern and western walls, and it is dissected by numerous smaller drainages. These are especially well developed on the geologically older, lower shelf of the valley, creating abundant larval habitat along the rocky margins of streambeds



where water can collect and stagnate in potholes during dry spells when stream levels are low. Mosquito larvae were found relatively easily in rock holes along the margins of smaller streams at BRAVO and FERN that contained stagnant water that was rich in organic detritus. Larvae were not detected on terrestrial transects in spite of intensive efforts to locate and sample standing water sources. The detection of larvae from the smaller gulches that are not as subject to flood conditions suggests that such sites may provide the majority of mosquito breeding areas within Kīpahulu Valley.

Our highest capture rates for adult mosquitoes were at BRAVO and FERN and in both areas significant numbers of larval *C. quinquefasciatus* were found in rock holes along stream margins. Although mosquito dissections were limited due to their death during transport to the laboratory, we were able to detect salivary gland infections or midgut oocysts in up to 4% of host-seeking mosquitoes collected from CO<sub>2</sub> traps at both sites. The presence of infected mosquitoes documents malaria transmission at these sites, but low prevalence of infection in the host population suggests that transmission may be sporadic and dependent on weather conditions that create habitat for mosquito larvae. In this case, such habitat is associated with rock holes along stream margins. In support of this hypothesis and in contrast to other areas in Hawai'i where malarial prevalence is higher (Atkinson et al., 2005), we found no significant interaction between elevation and host status (native or nonnative species) and no significant association between elevation and malarial infection. Our sampling was conducted during an abnormally dry fall, and during this 5-mo study, we experienced only one episode of torrential rain with flash flooding that would have scoured stream margins. The majority of mosquito larvae were collected within several weeks after this flash flood when larval habitat along stream margins was still abundant and before rock holes

had time to dry. This may suggest that control of malaria transmission in Kīpahulu will be difficult. Stream margins are often located in deep, rocky canyons with frequent waterfalls and cliffs, and they are difficult to reach throughout most of the valley. Application of biopesticides such as toxins derived from *Bacillus thuringiensis israelensis* (BTI) may be feasible at some of these sites, but the effects of BTI on native aquatic fauna have not been tested.

The periodic mosquito habitat availability may explain why pox and malaria are not better established in forest bird populations in the valley and why prevalence of infection relative to other locations in the state is so low. Long-term monitoring of disease transmission coupled with collection of climatic data and measurements of stream levels at this site and others will be necessary to test this hypothesis. If such a relationship exists, increased transmission of malaria to forest birds may be predictable base on climatic events, such as the periodic recurrence of El Niño, southern oscillation events in the central Pacific (Chu, 1995).

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