Vector movement underlies avian malaria at upper elevation in Hawaii: implications for transmission of human malaria

Leonard A. Freed · Rebecca L. Cann

Abstract With climate warming, malaria in humans and birds at upper elevations is an emerging infectious disease because development of the parasite in the mosquito vector and vector life history are both temperature dependent. An enhanced-mosquito-movement model from climate warming predicts increased transmission of malaria at upper elevation sites that are too cool for parasite development in the mosquito vector. We evaluate this model with avian malaria (*Plasmodium relictum*) at 1,900-m elevation on the Island of Hawaii, with air temperatures too low for sporogony in the vector (*Culex quinquefasciatus*). On a well-defined site over a 14-year period, 10 of 14 species of native and introduced birds became infected, several epizootics occurred, and the increase in prevalence was driven more by resident species than by mobile species that could have acquired their infections at lower elevations. Greater movement of infectious mosquitoes from lower elevations now permits avian malaria to spread at 1,900 m in Hawaii, in advance of climate warming at that elevation. The increase in malaria at upper elevations due to dispersal of infectious mosquitoes is a real alternative to temperature for the increased incidence of human malaria in tropical highlands.

Introduction

Malaria at upper elevations is an emerging infectious disease of increasing importance for both humans and birds (Patz et al.

L. A. Freed (✉)
Department of Biology, University of Hawaii at Manoa, Honolulu, HI 96822, USA
e-mail: lfreed@hawaii.edu

R. L. Cann
Department of Cell and Molecular Biology, University of Hawaii at Manoa, Honolulu, HI 96822, USA
e-mail: rcann@hawaii.edu

1996, 2005; Epstein et al. 1998; Lindsey and Martens 1998; Kovats et al. 2001; Benning et al. 2002; Harvell et al. 2002), largely because temperature limits population growth and development of sporozoites (sporogony) in mosquito vectors, making them infectious to host species (Martens et al. 1995; Patz 1998; LaPointe et al. 2010). Time for completion of sporogony can be reduced with rising mean air temperatures (LaPointe et al. 2010), expected with global warming, and even with diurnal fluctuations in air temperature under certain conditions (Paaijmans et al. 2009). Air temperature also affects larval development, biting rate, and survival of mosquitoes (Martens et al. 1995; Patz 1998; Rueda et al. 1990; Rogers and Randolph 2000), increasing their effectiveness as vectors. Prevalence of malaria has increased in upper elevation tropical populations of humans in Africa, Madagascar, South America, and New Guinea (Peters et al. 1958; Matola et al. 1987; Loevinsohn 1994; Mouchet et al. 1998; Hay et al. 2002; Rutar et al. 2004), as well as birds in Hawaii (Freed et al. 2005), but the link with climate warming at these elevations has been debated. Alternative explanations include greater number of mosquitoes from clearing of forest for agriculture, greater surveillance, resistance to DDT, resistance to anti-malarial drugs, reduced use of mosquito netting, and migration of infected hosts or infectious mosquitoes from lower elevation (Reiter 2001, 2008; Reiter et al. 2004; Plowright et al. 2008; Lafferty 2009; Ostfeld 2009; Freed et al. 2005). The only upper elevation location where temperature change explains a significant portion of the increased prevalence of malaria is in resident people in the highlands of Kenya (Zhou et al. 2004; Pascual et al. 2006), but even these studies acknowledge that other factors may be involved.

A simple model can combine dispersal of infectious mosquitoes with climate warming at lower elevation (Fig. 1). Mosquitoes readily move as diverse studies on continents and islands with different mosquito species have documented (Service 1997). Mosquitoes may disperse by rising from the
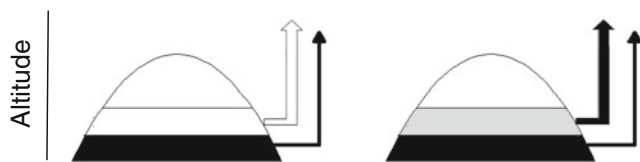
Fig. 1 The enhanced-mosquito-movement model. Shown are two identical profiles of mountains. Both mountains have low elevations (in *black*) that support sporogony of malarial parasites in the mosquito vector. The next elevation is *white on the left* but *gray on the right* mountain. The *white area* does not support sporogony but the *gray area*, with climate warming, has recently warmed to the point that it supports sporogony. In both mountains, there are *arrows* extending upward that represent movement of mosquitoes. The *inner arrows* are wider because studies of mosquito dispersal indicate a drop off with distance (Service 1997; LaPointe 2008). The *black arrows* on both mountains have some infectious mosquitoes. The *white arrow* at mid-elevation on the *left mountain* has just non-infectious mosquitoes. The *right mountain* has more infectious mosquitoes moving to upper elevations than the *left mountain* because some infectious mosquitoes move from the *gray elevation*. This model indicates that climate warming at mid-elevations can influence malaria at cool upper elevations. The model accounts for the increase of avian malaria at upper elevation in Hawaii, and likely accounts for the incidence of human malaria in tropical highlands where air temperatures do not support sporogony, or where movement of infectious mosquitoes adds to prevalence even when air temperatures support sporogony

boundary layer of air near the ground where they may be carried by prevailing wind, covering distances up to 5 km (Service 1997). That may be an underestimate because many species of mosquitoes have been captured in nets dragged by aircraft. Ten species of mosquitoes, including known vectors for human and avian malaria, have been captured in nets 150 m above the ground in northeast India with estimated dispersal distances of 70–110 km in the wind (Reynolds et al. 1996). Kite traps operated in New South Wales, Australia, captured six species of mosquitoes at altitudes up to 310 m with estimated flight range of 594–648 km (Kay and Farrow 2000). Experimental placement of mosquitoes at higher altitude is required to determine if there is a physical or biological limit to how high they fly and survive. Nevertheless, such dispersal may be adaptive because individuals can avoid competition in crowded larval habitats. Consistent with this hypothesis, more than twice as many female mosquitoes as male mosquitoes were captured by Kay and Farrow (2000). In addition, dispersal by mosquitoes may enable adults to find mates, additional hosts, and oviposition sites.

Climate warming on a mountain, relative to the threshold for sporogony, will progress from lower to upper elevations. This means that elevations that previously were too cool for sporogony can warm sufficiently to support the process (Fig. 1). Some proportion of mosquitoes from that elevation, before warming, may move to upper elevations. If so, when that elevation warms enough to support sporogony, some of those moving mosquitoes will be infectious. Through this mechanism, there will be increasing numbers of infectious mosquitoes at upper elevation, as warming progresses up the

mountain, even while air temperatures at upper elevation are too low to support sporogony. The rate of overall mosquito movement may change with warmer temperatures, especially if the warming creates shorter larval development and higher survival of adults, which will increase population density and competition. But even if the rate of movement does not change, more mosquitoes that move will be infectious and the effect will be cumulative at cool upper elevations. The consequence is that the prevalence of malaria at upper elevation will increase over time even while air temperatures are too low to support sporogony (Fig. 1). We propose this alternative as the enhanced-mosquito-movement (EMM) model.

We use native birds in old-growth forest at Hakalau Forest National Wildlife Refuge at 1,900-m elevation on the windward slope of Mauna Kea, Island of Hawaii, to evaluate the model. The refuge weather station at 1,941 m indicates only 3 months out of the year with mean temperatures greater than 13 °C (Freed et al. 2005; Freed and Cann 2012a), the threshold temperature for sporogony of the avian malarial parasite *Plasmodium relictum* (LaPointe et al. 2010), and field studies of sporogony of *P. relictum* indicate no sporozooite development in the infected mosquito vector (*Culex quinquefasciatus*) held at 1,800-m windward elevation with comparable temperatures (LaPointe et al. 2010). In the same general time frame, climate warming has occurred in Hawaii (Giambelluca et al. 2008), which means that higher mid-elevations now sometimes or always support sporogony.

We first documented increased community-wide prevalence of malaria in Hawaiian birds at 1900 m between 1988–1992 and 2001–2002, based on an epizootic in 2001 (Freed et al. 2005). Most of the cases detected by the limited sampling were in mobile native species that could have moved to lower elevation where they were bitten by an infectious mosquito and returned to upper elevation where tested. Here, we analyze many more birds over many more years, and additional species. We prove definitively that transmission occurs on-site in resident species.

Materials and methods

We analyzed 737 additional samples and add them to the 1007 previously reported (Feldman et al. 1995; Freed et al. 2005) for a total sample size of 1744 in this analysis. All samples were collected in the same 0.25-km^{-2} 1,900-m site in old-growth forest at Hakalau (Freed 2001). The total sample includes adult and young of the eight native passerine birds and six species of introduced birds. Young birds were sexed by plumage or shorter wings that persist until their first prebasic moult of their second year (Freed and Cann 2009). One of the introduced species was the Kalij pheasant (*Lophura leucomelana*), a ground-nesting bird present on the refuge. Blood samples were taken from five young chicks

present in a discovered nest, importantly, because they could not yet fly with their underdeveloped wings.

Blood samples from the brachial vein were immediately put in non-lysis buffer and frozen within 8 h, and total genomic DNA was extracted using standard biochemical methods (Feldman et al. 1995; Freed et al. 2005). Many detailed studies are required to determine which species or lineages of *Plasmodium* occur in a given location (Ortego et al. 2007; Valkiūnas et al. 2007; Illera et al. 2008; Baillie et al. 2012; Ventim et al. 2012; Ferrer et al. 2012; Howe et al. 2012). In Hawaii, only one lineage of *P. relictum* exists (Beadell et al. 2006), and a micrograph of blood stages is shown in Savage et al. (2005).

We used the PCR protocol based on a primer for the larger ribosomal subunit, which has an insertion in *Plasmodium* relative to vertebrates (Feldman et al. 1995). Both fragments from the PCR were sequenced to show that the larger fragment was related to other *Plasmodium* species, and blind samples were correctly identified in birds judged infected by *P. relictum* in Hawaii by smear diagnostics (Feldman et al. 1995). This protocol ties alternative primers for the highest accuracy compared to thin blood smear-positive microscopy (Freed and Cann 2006). Positive and negative controls were used for each sample. Accuracy can be attributed to the use of non-lysis buffer rather than the particular primer because this buffer preserves DNA for downstream analysis. The high copy number of target genes from nucleated red blood cells in birds compared to those of mammals requires extensive optimization of amplification protocols for high accuracy (Freed and Cann 2006).

To test the prediction of the EMM model, specifically that malaria would increase in an upper elevation site too cool to support sporogony, we used the community of resident and mobile birds. Mobile species are represented by the nectarivorous iiwi (*Vestiaria coccinea*) and apapane (*Himatione sanguinea*), which are less seen during the post-breeding months than insectivorous species. Movements of these species have been documented at Hakalau (Kuntz 2008) and elsewhere on the Island of Hawaii (Ralph and Fancy 1995). The six resident species of native birds consisted of the Hawaii amakihi (*Hemignthus virens virens*), Hawaii akepa (*Loxops coccineus coccineus*), Hawaii creeper (*Oreomysis mana*), akiapolaau (*Hemingnthus monroi*]), Hawaii elepaio (*Chasiempis sandwichensis ridgwayi*), and omao (*Myadestes obscurus*). In addition, the introduced red-billed leiothrix (*Leiothrix lutea*) and Japanese white-eye (*Zosterops japonicus*) are resident species.

We used logistic regression in all analyses unless otherwise indicated. We initially tested prevalence among months to investigate seasonality. Then we tested variation in prevalence among years and between the mobile and resident species. We treated year as a linear trend in one model. We used time period as a factor that modeled a stepwise increase in the second model. The early period consisted of years 1988–1994, the later period of years 1995–2002, which roughly split the interval

1988–2002. The interaction between species set and year, and between species set and period, determined if prevalence of malaria increased in parallel in the mobile and resident species. In addition, we used logistic regression on each species set, with period as a factor, to determine which set increased more.

Included among the samples were 84 cases of birds which were sampled in 2 years. Fifty-two of these cases were before 2000 and 32 were after 1999. There were three cases when the bird went sequentially from negative to positive, all were after 1999. We conducted a Fisher exact test to determine if these cases contributed to the increased prevalence of malaria.

Results

Monthly patterns of infection varied between young birds and adults, suggesting seasonal patterns of parasite transmission, as previously documented (Atkinson and Samuel 2010). The breeding season for most bird species occurs from January through June, when temperatures are initially coldest (Freed et al. 2005) but arthropod food is abundant (Freed et al. 2007). All 141 young birds of native species sampled during January to May tested negative for malaria. In contrast, 13 of 358 young birds captured during June to December tested positive (test of proportions, $P=0.02$). Therefore, young birds from January to May were dropped from further analysis. In contrast, adults tested positive during each month of the year, with differences among months (Fig. 2, logistic regression, $P=0.034$). Evidence of seasonality is indicated from the highest prevalence in September following the month of highest average air temperature (Freed and Cann 2012a). This is not due to chance since there are 24 ways the month with highest prevalence can coincide with or follow a random warmest month (combinatorial test, $P=0.04$). August and September are the months of highest temperature at lower elevations (Nullet and Sanderson 1993). Thirteen of the 16 birds testing positive during these 2 months were mobile species, so seasonality may be stronger at lower elevations.

In total, 10 of 14 species of native and introduced bird species had at least one individual that tested positive (Fig. 3). While significant variation in prevalence existed among species ($P<0.0001$), the Hawaii elepaio stands out as a potential resident reservoir of malaria at upper elevation. Prevalence in elepaio was low during 1988–1991 (0.08, $n=13$), and increased an order of magnitude to 0.82 ($n=11$) by 1999–2002 (test of proportions, $P<0.0001$). Two individual birds that tested positive in 2001 also tested positive in 2002 upon recapture and resampling.

Formal proof of transmission on the study site comes from introduced Kalij pheasants. Four of five chicks sampled from the same nest on the ground in June 1995 tested positive. Year 1995 also had the highest prevalence of malaria and the greatest number of species testing positive (Hawaii akepa,

Fig. 2 Prevalence of malaria by month during 1988–2002. Sample size *above bars*
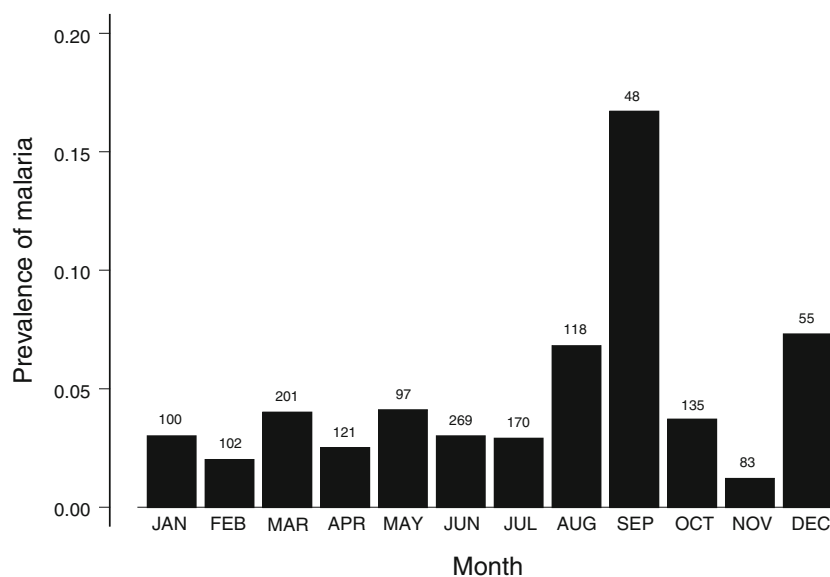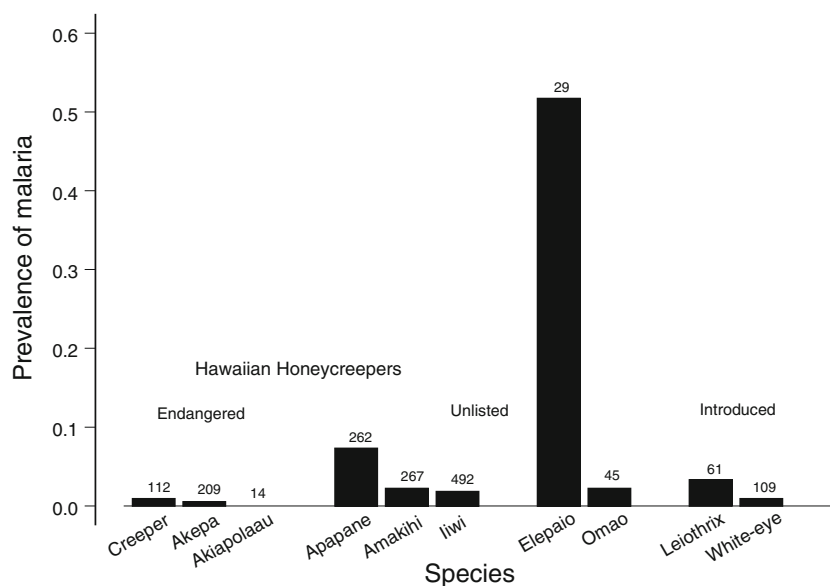
apapane, Hawaii amakihi, Hawaii elepaio, and the introduced red-billed leiothrix, along with the pheasant). All these species except the apapane are year-round residents at the 1,900-m site.

Epizootics at upper elevation must be based on resident species more than mobile species. Two years, 1995 and 2001, had high prevalence of malaria in resident birds (Fig. 4a) and may be considered epizootics. There was high prevalence in mobile species in 2001 but not 1995.

There was increased prevalence over time in both mobile and resident species (Fig. 4a, b). This was not indicated as a linear trend ($p = 0.09$), but as a stepwise increase ($p = 0.01$). There was no species set by period interaction ($p = 0.45$). However, separate analyses indicate that the increase was driven more by resident species ($p = 0.02$) than by mobile species ($p = 0.41$) (Fig. 5).

The Fisher exact test for repeated samples was barely significant ($p = 0.05$). There were no cases of change before 2000 and 0.09 of birds with repeated samples were positive on the second sample. Although the sample is small, both resident and mobile species changed after 1999, consistent with the rest of the samples.

Discussion

This study demonstrates that malaria is transmitted at upper elevations that are too cool for on-site sporogony, several epizootics were documented, and prevalence increased over time. There was greater increase in resident than in mobile species, and one species, the elepaio, had prevalence expected of a reservoir. Kuntz (2008) radio tracked iiwi during 2003–

Fig. 3 Prevalence of malaria by species. The introduced northern cardinal (*Cardinalis cardinalis*) (*n* = 4), house finch (*Carpodacus mexicanus*) (*n* = 24), and nutmeg mannikin (*Lonchura punctulata*) (*n* = 1), not shown, all tested negative. Sample size *above bars*

Fig. 4 Prevalence of malaria in resident species (**a**) and mobile species (**b**) by year. Zero prevalence reflects the corresponding sample size, which are generally *above bars*. Note the several cases of non-zero prevalence in resident species but zero prevalence in mobile species
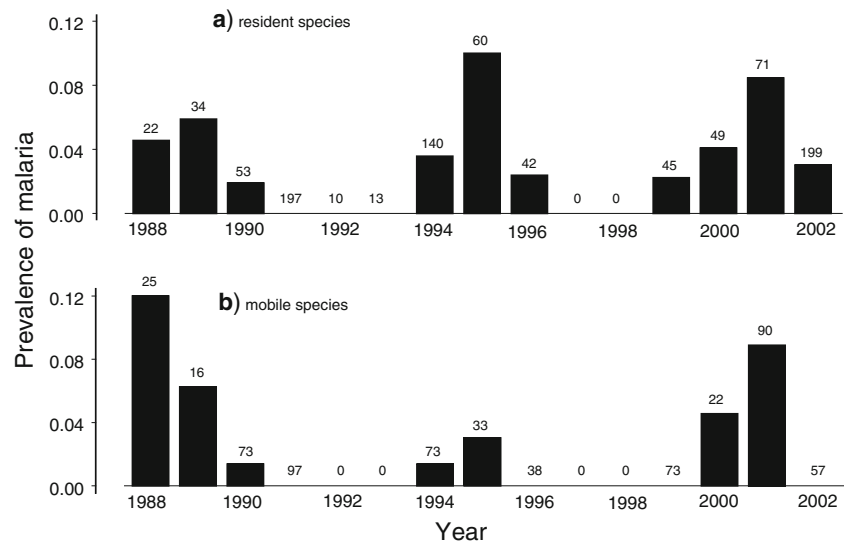
2004 and showed that 45 % of 111 remote locations were at or below 1,475 m and individuals were regularly travelling to elevations closer to 1,200 m where they were following the bloom of ohia-lehua (*Metrosideros polymorpha*) trees, showing they were moving to areas where malaria was endemic. By July the bloom had shifted back to 1,400 m (Kuntz 2008). Iiwi thus had limited exposure to areas where malaria was endemic. In addition, rainfall may have occurred too infrequently to promote mosquito breeding. Here, we summarize the evidence for mosquito movement in Hawaii and evaluate the EMM model for both avian malaria in Hawaii and human malaria in tropical highlands.

Mosquito movement in Hawaii is indicated from two sources. A mark/recapture study at 900-m elevation forest on Mauna Loa indicated that *C. quinquefasciatus* can move up to 3 km, but that was the limit of attempts to recapture mosquitoes (LaPointe 2008). The movements generally followed prevailing winds which are usually seaward at night. However, winds can be upslope during strong trade winds, El Nino storms, and rare hurricanes, reversing prevailing dispersal patterns (Schroeder 1993). A population genetic study of mosquitoes provides indirect evidence of greater movement. Although upper elevation sites produced few samples, mosquitoes from 1,786-m elevation were assigned by genetic markers to a source site close to sea level (Keyghobadi et al. 2006). That distance was 50 km, more than an order of magnitude greater than the 3 km constrained distance of the mark/recapture study, and consistent with long movements of the mosquito in northeast India (Reynolds et al. 1996) and in New South Wales, Australia (Kay and Farrow 2000).

Mosquito movement has been assumed to occur in Hawaii for many years (Goff and van Riper 1980; Scott et al. 1986; Benning et al. 2002; LaPointe 2008). Our long-term study reveals extensive movement of infectious mosquitoes. The

Fig. 5 Prevalence of malaria of resident and mobile species by time period, indicating greater increase in resident than in mobile species. Sample size *above bars*
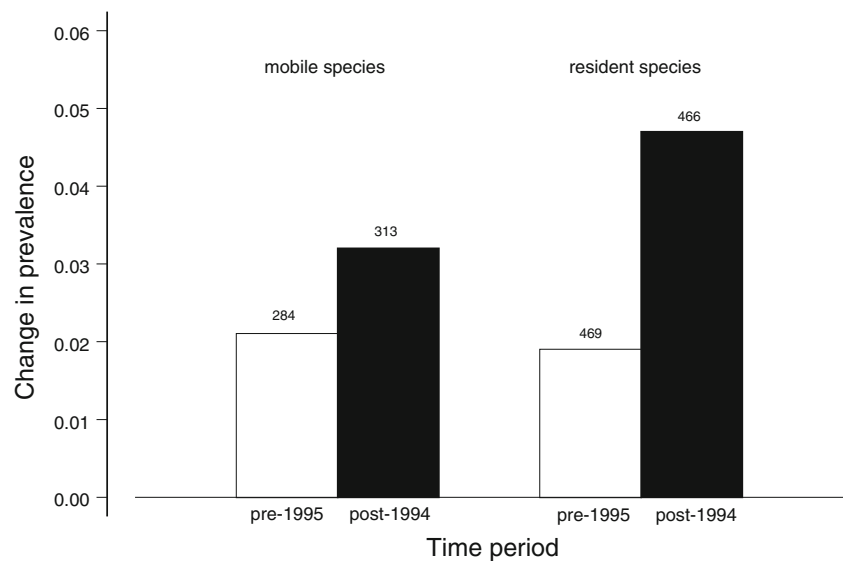
high prevalence in resident species we detected in 1995 and 2001 must have involved many infectious mosquitoes that moved to upper elevations where they survived long-enough to obtain a blood meal. This could result from repeated single female immigrants or many insects blown up by storm or high-wind conditions.

Modeling of mosquito populations in Hawaii, based solely on air temperature and precipitation (Ahumada et al. 2004), cannot account for the results of our study at 1,900-m elevation or observation of malaria in resident birds on Maui at comparable elevation (Feldman et al. 1995). The elevational limits of *C. quiquefasciatus* in Hawaii were identified at 1,475 m for self-sustaining populations and 1,715 m for temporary summer populations (Ahumada et al. 2004). The self-sustaining elevation is essentially identical to previous studies of malaria and mosquito abundance (Goff and van Riper 1980; van Riper et al. 1986). Mosquitoes become rarer above 1,500 m (Goff and van Riper 1980), while native birds become more common (Scott et al. 1986). The 1,715-m elevation for summer populations reflects the higher summer air temperatures (Freed et al. 2005; Freed and Cann 2012a). But our study shows that climate warming at lower elevations (Giambelluca et al. 2008) has increased the number of infectious mosquitoes that now disperse to higher elevations, as evidenced by infection in rare endangered birds and 82 % of individuals in the elepaio, a hyperdispersed territorial species spanning our 0.25-km^{-2} study site (VanderWerf 2004).

Mosquito movement has also been documented for *Anopheles* mosquito vectors of human malaria (Service 1997; Reynolds et al. 1996; Kay and Farrow 2000). Our study implies that that the statistical association between warming and incidence of malaria in the African highlands (Zhou et al. 2004) may be based on warming at mid-elevations and movements of infectious mosquitoes from those elevations. Research in human malaria would benefit from determining if such movements underlie the link between climate warming and increased incidence of malaria in tropical highlands (Peters et al. 1958; Matola et al. 1987; Loevinsohn 1994; Mouchet et al. 1998; Hay et al. 2002; Rutar et al. 2004). Attention focused on mosquito population dynamics at mid-elevations, and capture rates of infectious mosquitoes at different elevations, would further public health discussions. The more equatorial high elevation tropics have higher air temperatures than 1,900-m Hawaii at 19.8° north latitude, which means that air temperatures are closer to the threshold for sporogony, 14.9 to 20.5 °C at Kericho in Kenya (Paaijmans et al. 2009), potentially combining on-site sporogony with movement of infectious mosquitoes from lower elevations.

While the fate of infectious mosquitoes after biting a host species at upper elevation is not known, mosquito breeding on site and surrounding areas is extremely rare. A search for larvae in 1,024 pools of water over 3 months found no evidence (Woodworth et al. 2001). A longer-term experimental approach involves oviposition buckets with an infusion of brewer's yeast and dead grass. Over a time period of 2 years and 8 months, beginning in October 2001, there was only a single case of mosquito breeding (Freed et al. 2005). This is not consistent with local attraction of cryptic insects to breeding sites. It is more likely that infectious mosquitoes are blown up by the wind, survive long enough to bite a bird, and then die because of the cool temperatures or predation by birds or other insects.

The recent establishment of a resident disease reservoir at upper elevation is totally unprecedented for both human and avian malaria, and requires examination of characteristics of this particular vertebrate host. The elepaio, a monarchine flycatcher, colonized the Hawaiian Islands much more recently than the Hawaiian honeycreepers (VanderWerf et al. 2009; Lerner et al. 2011), and its ancestors from Southeast Asia and low-lying Pacific islands had ample exposure to malaria (Jarvi et al. 2003). The elepaio species on the lower Island of Oahu also has high prevalence (0.87) of malaria (VanderWerf et al. 2006). The range of the Hawaii elepaio on Mauna Kea, from the Hawaiian Forest Bird Survey conducted in 1977 (Scott et al. 1986), extends well below the limit of self-sustaining mosquito populations at 1,475 m. We identified elepaio during an expedition to that elevation in 2003. This species may roost at night lower in the tree canopy than other native birds, perhaps making it among the first native hosts detected by night-biting infectious mosquitoes. The elepaio has features of a reservoir in that it tolerates the disease with high annual survival (VanderWerf 2004), and is the main native bird species at elevations below 1,500 m (Scott et al. 1986). It is now likely that the parasite in elepaio exists continuously from at least that elevation to 1,900 m. The role of the elepaio as a reservoir involved in the transmission of malaria to other birds remains to be studied.

A latitudinal gradient in air temperatures may be similar to an elevational gradient in temperature. The EMM model may apply to the former. Higher latitudes that could not support sporogony may, with warming, become able to produce infectious mosquitoes. Mosquito movement may be involved in even higher latitudes if competition for breeding sites increases. This mechanism predicts that birds at higher latitudes should exhibit an increased prevalence of malaria even if local air temperatures are too low to support sporogony.

Penguins transported to temperate zoos may be a good system for evaluating the model. Malaria has long been known to occur in penguins housed in temperate zoos (Griner 1974), as well as from wild birds in temperate locations in South Africa, Australia, New Zealand, and Gough Island in the South Atlantic (Jones and Shellam 1999a). It is penguins from the Antarctic and sub-Antarctic, where blood parasites have not been detected (Jones and Shellam 1999b), that are expected to have increased prevalence of malaria in more northern latitude zoos. If these northern latitude zoos exist where air temperatures do not support sporogony and the

transport to the zoos precludes transmission, then movement of infectious mosquitoes from less northern latitudes would be the cause of infection.

Hawaiian honeycreepers are extremely susceptible to malaria (van Riper et al. 1986; McCallum and Dobson 1995; Atkinson et al. 1995; Atkinson and Samuel 2010), having evolved in the absence of vector-borne disease for over 3 million years (Lerner et al. 2011). Conservation efforts now focus on remaining populations that are restricted in range to upper elevation forests (Scott et al. 1986). These forests are assumed to be characterized by year-round cold temperatures unsupportive of the vector and development of the parasite in the vector (Atkinson and LaPointe 2009), reasons why conservation efforts focus on preserving and restoring these habitats. This study anticipates an increasingly complex problem when sufficient climate warming supporting sporogony advances higher on Mauna Kea. Temperature per se increasing in elevation and enhanced storm activity with warming will jointly increase opportunities for mosquito movement.

Food limitation in wild birds is comparable to malnourishment in humans, increasing their susceptibility to malaria (el Samani et al. 1987). Malaria for the birds on the refuge may now interact synergistically with other threats that have created food limitation (Pimm 1996). The birds are increasingly food limited, possibly from competition with an invasive introduced bird, the Japanese white-eye (Freed and Cann 2012b; Freed and Cann 2013), and have also become hosts for an explosive increase in chewing lice (Freed et al. 2008), which increase food requirements to replace the additional heat lost through degraded plumage. The food limitation is evidenced by stunted skeletal growth of all native species measured (Freed and Cann 2009) and extended length of the molting season for both young birds and adults (Freed and Cann 2012a). Tens of thousands of native birds have been lost from the normalizing selection associated with these life history changes (Freed and Cann 2009, 2012a, b). All evidence indicates that the population collapse of the endangered Hawaii creeper during 2002–2007 was based on starvation (Freed and Cann 2013). We noted a potential cost of tolerance to malaria of iiwi and apapane during the 2001 epizootic, evidenced by broken head feathers in birds that tolerated malaria but not in birds of the species that tested negative (Freed et al. 2005). Without adaptive management efforts, food-limited birds will succumb eventually to increasing malaria at upper elevations. Their ability to thermoregulate and provide oxygen to their tissues will be further compromised (Hayworth et al. 1987), even if disease-tolerant bird genotypes eventually emerge from their now limited gene pools.

References

Ahumada JA, LaPointe D, Samuel MD (2004) Modeling the population dynamics of *Culex quinquefasciatus* (Diptera: Culicidae), along an elevational gradient in Hawaii. J Med Entomol 41(6):1157–1170

Atkinson CT, LaPointe DA (2009) Introduced avian diseases, climate change, and the future of Hawaiian honeycreepers. J Avian Med Surg 23(1):53–63

Atkinson CT, Samuel MD (2010) Avian malaria *Plasmodium relictum* in native Hawaiian forest birds: epizootiology and demographic impacts on apapane *Himotione sanguinea*. J Avian Biol 41(4):357–366

Atkinson CT, Woods KL, Dusek RJ, Sileo LS, Iko WM (1995) Wildlife disease and conservation in Hawaii: pathogenecity of avian malaria (*Plasmodium relictum*) in experimentally infected iiwi (*Vestiaria coccinea*). Parasitology 111(Suppl):S59–S69

Baillie S, Gudex-Cross D, Barraclough R, Blanchard W, Brunton D (2012) Patterns in avian malaria at founder and source populations of an endemic New Zealand passerine. Parasitol Res 111(5):2077–2089. doi:10.1007/s00436-012-3055-y

Beadell JS et al (2006) Global phylogeographic limits of Hawaii's avian malaria. Proc R Soc B Biol Sci 273(1604):2935–2944

Benning TL, LaPointe D, Atkinson CT, Vitousek PM (2002) Interactions of climate change with biological invasions and land use in the Hawaiian Islands: modeling the fate of endemic birds using a geographic information system. Proc Natl Acad Sci U S A 99(22):14246–14249

el Samani FZ, Willett WC, Ware JH (1987) Nutritional and socio-demographic risk indicators of malaria in children under five: a cross-sectional study in a Sudanese rural community. J Trop Med Hyg 90(2):69–78

Epstein PR, Diaz HF, Elias S et al (1998) Biological and physical signs of climate change: focus on mosquito-borne diseases. Bull Am Meteorol Soc 79(3):409–417

Feldman RA, Freed LA, Cann RL (1995) A PCR test for avian malaria in Hawaiian birds. Mol Ecol 4(6):663–673

Ferrer E, García-Navas V, Sanz J, Ortego J (2012) Molecular characterization of avian malaria parasites in three Mediterranean blue tit (*Cyanistes caeruleus*) populations. Parasitol Res 111(5):2137–2142. doi:10.1007/s00436-012-3062-z

Freed LA (2001) Significance of old-growth forest to the Hawaii akepa. Stud Avian Biol Ser 22:173–184

Freed LA, Cann RL (2006) DNA quality and accuracy of avian malaria PCR diagnostics: a review. Condor 108(2):459–473

Freed LA, Cann RL (2009) Negative effects of an introduced bird species on growth and survival in a native bird community. Curr Biol 19(20):1736–1740

Freed LA, Cann RL (2012a) Changes in timing, duration and symmetry of molt are associated with extensive decline of Hawaiian forest birds. PLoS One 7:e29834

Freed LA, Cann RL (2012b) Increase of an introduced bird competitor in old-growth forest associated with restoration. NeoBiota 13:43–60

Freed LA, Cann RL (2013) Females lead population collapse of the endangered Hawaii creeper. PLoS One 8:e67914

Freed LA, Cann RL, Goff ML, Kuntz WA, Bodner GR (2005) Increase in avian malaria at upper elevation in Hawaii. Condor 107(4):753–764

Freed LA, Fretz JS, Medeiros MC (2007) Adaptation in the Hawaii akepa to breed and moult during a seasonal food decline. Evol Ecol Res 9: 157–167

Freed LA, Medeiros MC, Bodner GR (2008) Explosive increase in ectoparasites in Hawaiian forest birds. J Parasitol 94(5):1009–1021

Giambelluca TW, Diaz HF, Luke MSA (2008) Secular temperature changes in Hawaii. Geophys Res Lett 35(12), L12702

Goff ML, van Riper IIIC (1980) Distribution of mosquitoes (Diptera: Culicidae) on the east flank of Mauna Loa Volcano, Hawaii. Pac Insects 22:178–188

Griner LA (1974) Avian malaria in penguins. Adv Vet Sci Comp Med 18: 251–271

Harvell CD et al (2002) Climate warming and disease risks for terrestrial and marine biota. Science 296(5576):2158–2162

Hay SI et al (2002) Climate change and the resurgence of malaria in the East African highlands. Nature 415(6874):905–909

Hayworth AM, van Riper IIIC, Weathers WW (1987) Effects of Plasmodium relictum on the metabolic rate and body temperature in canaries (Serinus canarius). J Parasitol 73(4):850–853

Howe L, Castro I, Schoener E, Hunter S, Barraclough R, Alley M (2012) Malaria parasites (Plasmodium spp.) infecting introduced, native and endemic New Zealand birds. Parasitol Res 110(2):913–923. doi: 10.1007/s00436-011-2577-z

Illera J, Emerson B, Richardson D (2008) Genetic characterization, distribution and prevalence of avian pox and avian malaria in the Berthelot's pipit (Anthus berthelotii) in Macaronesia. Parasitol Res 103(6):1435–1443. doi:10.1007/s00436-008-1153-7

Jarvi SI et al (2003) Detection of avian malaria (Plasmodium spp.) in native land birds of American Samoa. Conserv Genet 4(5):629–637

Jones HI, Shellam GR (1999a) Blood parasites in penguins, and their potential impact on conservation. Mar Ornithol 27:181–184

Jones HI, Shellam GR (1999b) The occurrence of blood-inhabiting protozoa in captive and free-living penguins. Polar Biol 21(1):5–10

Kay BH, Farrow RA (2000) Mosquito (Diptera: Culicidae) dispersal: implications for the epidemiology of Japanese and Murray Valley encephalitis viruses in Australia. J Med Entomol 37(6):797–801

Keyghobadi N, LaPointe D, Fleischer RC, Fonsecda DM (2006) Fine-scale population genetic structure of a wildlife disease vector: the southern house mosquito on the island of Hawaii. Mol Ecol 15(13): 3919–3930

Kovats RS, Campbell-Lendrum DH, McMichael AJ, Woodward A, Cox JSH (2001) Early effects of climate change: do they include changes in vector-borne disease. Philos T Roy Soc B 356(1411):1057–1068

Kuntz WA (2008) The importance of individual behavior to life history and conservation: breeding and movement biology in the iiwi (Vestiaria coccinea). University of Hawaii at Manoa

Lafferty KD (2009) The ecology of climate change and infectious diseases. Ecology 90(4):888–900

LaPointe DA (2008) Dispersal of Culex quinquefasciatus (Diptera: Culicidae) in a Hawaiian rain forest. J Med Entomol 45(4):600–609

LaPointe DA, Goff ML, Atkinson CT (2010) Thermal constraints to the sporogonic development and altitudinal distribution of avian malaria Plasmodium relictum in Hawaii. J Parasitol 96(2):318–324

Lerner HRL, Meyer M, James HF, Hofreiter M, Fleischer RC (2011) Multilocus resolution of phylogeny and timescale in the extant adaptive radiation of Hawaiian honeycreepers. Curr Biol 21(21): 1838–1844

Lindsey SW, Martens WJM (1998) Malaria in the African highlands: past, present and future. Bull World Health Organ 76(1):33–45

Loevinsohn ME (1994) Climatic warming and increased malaria incidence in Rwanda. Lancet 343(8899):714–718

Martens WJM, Jetten TH, Rotmans J, Niessen LW (1995) Climate change and vector-borne diseases: a global modelling perspective. Glob Environ Chang 5(1):195–209

Matola YG, White GB, Magayuka SA (1987) The changed pattern of malaria endemicity and transmission at Amani in the eastern Usambara mountains, north-eastern Tanzania. J Trop Med Hyg 90(3):127–134

McCallum HI, Dobson AP (1995) Detecting disease and parasite threats to endangered species and ecosystems. Trends Ecol Evol 10(5):190–194

Mouchet J et al (1998) Evolution of malaria in Africa for the past 40 years: impact of climatic and human factors. J Am Mosq Contr Assoc 14(2):121–130

Nullet D, Sanderson M (1993) Radiation and energy balances and air temperature. In: Sanderson M (ed) Prevailing trade winds. University of Hawaii Press, Honolulu, pp 37–55

Ortego J, Calabuig G, Cordero P, Aparicio J (2007) Genetic characterization of avian malaria (Protozoa) in the endangered lesser kestrel, Falco naumanni. Parasitol Res 101(4):1153–1156. doi:10.1007/s00436-007-0575-y

Ostfeld RS (2009) Climate change and the distribution and intensity of infectious diseases. Ecology 90(4):903–905

Paaijmans KP, Read AF, Thomas MB (2009) Understanding the link between malaria risk and climate. Proc Natl Acad Sci U S A 106(33):13844–13849

Pascual M, Ahumada JA, Chaves LF, Rodo X, Bouma M (2006) Malaria resurgence in the East African highlands: temperature trends revisited. Proc Natl Acad Sci U S A 103(15):5829–5834

Patz JA (1998) Predicting key malaria transmission factors, biting and entomological inoculation rates, using modelled soil moisture in Kenya. Trop Med Int Health 3(10):818–827

Patz JA, Epstein PR, Burke TA, Balbus JM (1996) Global climate change and emerging infectious diseases. J Am Med Assoc 275(3):217–223

Patz JA, Campbell-Lendrum DH, Holloway T, Foley JA (2005) Impact of regional climate change on human health. Nature 438(7076):310–317

Peters W, Christian S, Jameson J (1958) Malaria in the highlands of Papua and New Guinea. Med J Aust 2:409–416

Pimm SL (1996) Lessons from a kill. Biodivers Conserv 5(9):1059–1067

Plowright RK, Sokolov SH, Gorman ME, Daszak P, Foley JE (2008) Causal inference in disease ecology: investigating drivers of disease emergence. Front Ecol Environ 6(8):420–429. doi:10.1890/070086

Ralph CJ, Fancy SG (1995) Demography and movements of apapane and iiwi in Hawaii. Condor 97(3):727–742

Reiter P (2001) Climate change and mosquito-borne disease. Environ Health Perspect 109(Supp. 1):141–161

Reiter P (2008) Global warming and malaria: knowing the horse before hitching the cart. Malar J 7(Suppl 1):S3

Reiter P et al (2004) Global warming and malaria: a call for accuracy. Lancet Infect Dis 4(6):323–324

Reynolds DR et al (1996) Atmospheric transport of mosquitoes in northeast India. Med Vet Entomol 10(2):185–186

Rogers DJ, Randolph SE (2000) The global spread of malaria in a future, warmer world. Science 289(5485):1763–1766

Rueda LM, Patel KJ, Axtell RC, Stinner RE (1990) Temperature-dependent development and survival rates of Culex quinquefasciatus and Aedes aegypti (Diptera: Culicidae). J Med Entomol 27(5):892–898

Rutar T, Salgueiro EJB, Maguire JH (2004) Introduced Plasmodium vivax malaria in a Bolivian community at an elevation of 2,300 meters. Am J Trop Med Hyg 70(1):15–19

Savage AF, Ariey F, Greiner EC (2005) A new species of Plasmodium from Malagasy vangas. J Parasitol 91(4):926–930

Schroeder T (1993) Climate controls. In: Sanderson M (ed) Prevailing trade winds: weather and climate in Hawaii. University of Hawaii Press, Honolulu, pp 12–36

Scott JM, Mountainspring S, Ramsey FL, Kepler CB (1986) Forest bird communities of the Hawaiian Islands: their dynamics, ecology, and conservation. Stud Avian Biol Ser 9:1–431

Service MW (1997) Mosquito (Diptera: Culicidae) dispersal—the long and short of it. J Med Entomol 34(6):579–588

Valkiūnas G, Zehtindjiev P, Hellgren O, Ilieva M, Iezhova T, Bensch S (2007) Linkage between mitochondrial cytochrome b lineages and morphospecies of two avian malaria parasites, with a description of *Plasmodium (Novyella) ashfordi* sp. nov. Parasitol Res 100(6): 1311–1322. doi:10.1007/s00436-006-0409-3

van Riper IIIC, van Riper SG, Goff ML, Laird M (1986) The epizootiology and ecological significance of malaria in Hawaiian land birds. Ecol Monogr 56(4):327–344

VanderWerf EA (2004) Demography of Hawaii elepaio: variation with habitat disturbance and population density. Ecology 85(3):770–783

VanderWerf EA, Burt MD, Rohrer JL, Mosher SM (2006) Distribution and prevalence of mosquito-borne diseases in Oahu elepaio. Condor 108(4):770–777

VanderWerf EA, Young LC, Yeung NW, Carlon DB (2009) Stepping stone speciation in Hawaii's flycatchers: molecular divergence supports new island endemics within the elepaio. Conserv Genet 11(4): 1283–1298. doi:10.1007/s10592-009-9958-1

Ventim R, Ramos J, Osório H, Lopes R, Pérez-Tris J, Mendes L (2012) Avian malaria infections in western European mosquitoes. Parasitol Res 111(2):637–645. doi:10.1007/s00436-012-2880-3

Woodworth BL et al (2001) Breeding productivity and survival of the endangered Hawaii creeper in wet forest refuge on Mauna Kea, Hawaii. Stud Avian Biol Ser 22:164–172

Zhou G, Minakawa N, Githeko AK, Yan G (2004) Association between climate variability and malaria epidemics in the East African highlands. Proc Natl Acad Sci U S A 101(8):2375–2380