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Original Contribution

Experimental Evidence for Evolved Tolerance to Avian Malaria in a Wild Population of Low Elevation Hawai'i 'Amakihi (*Hemignathus virens*)

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Abstract: Introduced vector-borne diseases, particularly avian malaria (*Plasmodium relictum*) and avian pox virus (*Avipoxvirus* spp.), continue to play significant roles in the decline and extinction of native forest birds in the Hawaiian Islands. Hawaiian honeycreepers are particularly susceptible to avian malaria and have survived into this century largely because of persistence of high elevation refugia on Kaua'i, Maui, and Hawai'i Islands, where transmission is limited by cool temperatures. The long term stability of these refugia is increasingly threatened by warming trends associated with global climate change. Since cost effective and practical methods of vector control in many of these remote, rugged areas are lacking, adaptation through processes of natural selection may be the best long-term hope for recovery of many of these species. We document emergence of tolerance rather than resistance to avian malaria in a recent, rapidly expanding low elevation population of Hawai'i 'Amakihi (*Hemignathus virens*) on the island of Hawai'i. Experimentally infected low elevation birds had lower mortality, lower reticulocyte counts during recovery from acute infection, lower weight loss, and no declines in food consumption relative to experimentally infected high elevation Hawai'i 'Amakihi in spite of similar intensities of infection. Emergence of this population provides an exceptional opportunity for determining physiological mechanisms and genetic markers associated with malaria tolerance that can be used to evaluate whether other, more threatened species have the capacity to adapt to this disease.

Keywords: avian malaria, Hawai'i 'Amakihi, *Plasmodium relictum*, honeycreeper, climate change, adaptation, tolerance

Introduction

The Hawaiian Islands are home to one of the most imperiled avifaunas in the world. The extreme isolation of the archipelago and a remarkable range of environmental gradients and habitat types created conditions favorable for radiation of a handful of avian colonists from Asia, other Pacific Islands, and North America to more than 100 species representing 13 avian families from seven avian orders (Pratt 2009). The Hawaiian honeycreepers are perhaps the

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best example of this, radiating from a Eurasian rosefinch ancestor of Asian origin (Lerner et al. 2011) to the more than 50 species and subspecies that have been documented both from historical collections and the subfossil record (Pratt 2009). Of the 47 species and subspecies of forest birds recorded after western contact with the islands, only 33 survived into the second half of the twentieth century (Banko and Banko 2009). Today, 24 species and subspecies are federally listed as endangered and many of these have not been seen in more than a decade (Banko and Banko 2009; U.S. Fish and Wildlife Service 2006, 2010).

Habitat modification by invasive species, human development, introduced predators, introduced competitors, stochastic events, and introduced diseases have played significant roles in these declines (Scott and van Riper 2001), but the relative importance of these factors has been difficult to assess. The idea that introduced diseases may have played a key role was introduced by Richard Warner in his classic work that identified avian malaria (*Plasmodium relictum*) and pox virus (*Avipoxvirus* spp.) as two primary culprits limiting native forest birds to cooler, high elevation forests on the islands (Warner 1968), where temperatures limit mosquito numbers and extrinsic development of malarial parasites in the vector (van Riper et al. 1986; LaPointe et al. 2010). These high elevation refugia have remained strongholds for remaining species of endangered forest birds and are the only locations in the islands, where more common native species reach their greatest densities and diversity (Gorresen et al. 2009). Given current rates of global warming, however, these refugia are unlikely to persist into the second half of this century (Benning et al. 2002; Samuel et al. 2011).

The recent emergence of a rapidly expanding low elevation population of Hawai'i 'Amakihi on the southeastern corner of Hawai'i Island (Woodworth et al. 2005; Spiegel et al. 2006), plus observations of small, but persistent low elevation populations of 'Apapane (*Himatione sanguinea*) (Nielsen 2000; Atkinson et al. 2005), Oahu 'Amakihi (*Hemignathus chloris*) (Shehata et al. 2001; Krend 2011), I'iwi (*Vestiaria coccinea*) (Vander Werf and Rohrer 1996), and Oahu 'Elepaio (*Chasiempis ibidis*) (Vander Werf et al. 2006) is an encouraging trend. Adaptation to malaria has been predicted given the intense selection pressure of this disease on naïve native species (van Riper et al. 1986; Cann and Douglas 1999), but neither the mechanisms involved nor the relative contributions of migration, demographic factors, and genetics are clearly understood (Woodworth et al. 2005). Genetic differences have been detected between low and high elevation populations of Hawai'i 'Amakihi with microsatellites (Eggert et al. 2008) and nuclear and mitochondrial markers (Foster et al. 2007), but differences appear to be based on a variety of haplotypes and we do not know how these neutral markers relate to disease susceptibility. Given the widespread emergence of this phenomenon across the archipelago, it is possible that both tolerance and resistance mechanisms may have emerged independently in different locations given the complex interplay of host genetics, varying selective pressure from disease, and environmental and other limiting factors that affect host demography.

Both the recovery of Hawai'i 'Amakihi in lowland areas with high rates of disease transmission and the availability of unexposed high elevation populations for comparison provide an unusual opportunity to understand how mechanisms of disease tolerance or resistance may evolve in wild populations. Tolerance and resistance mechanisms can significantly affect transmission dynamics given higher prevalence and intensity of infection that is predicted in tolerant hosts, can lead to different selective pressures for parasite virulence, and can differ in their relative benefits and costs to host fitness (Råberg et al. 2009; Sorci 2013). While high prevalence of infection and high transmission rates of malaria on Hawai'i Island suggest that Hawai'i 'Amakihi may have evolved tolerance to infection (Sorci 2013), the significantly lower prevalence of infection in Oahu 'Amakihi suggests that either the two species may have taken different evolutionary paths in response to malaria (Krend 2011) or that persistence in the lowlands of Oahu is dependent on lower transmission rates or other demographic factors. We took advantage of the altitudinal differences in selective pressure to avian malaria on Hawai'i Island to compare physiological responses in Hawai'i 'Amakihi from both low and high elevation populations to acute infection with *P. relictum* under controlled experimental conditions. Our goal was to clarify whether tolerance or resistance mechanisms have contributed to the recent low elevation population expansion of this species.

Methods

Juvenile, hatch-year Hawai'i 'Amakihi were captured with mist nets between October 2002 and October 2003 on the island of Hawai'i in the Upper Waiakea Forest Reserve near Powerline Road (19°40′N, 155°22′W, elevation 1,750 m) and at Malama Ki Forest Reserve (19°26′N, 155°52′W,

elevation 16 m) less than 1 km from the coastline. ʻAmakihi were captured, banded, weighed, and aged by plumage characteristics (Lindsey et al. 1998) and transported within several hours of capture to a mosquito-proof aviary at Hawaii Volcanoes National Park. Birds from Upper Waiakea Forest Reserve were moved immediately into the aviary because of the low prevalence of malaria (<5%) from this site and screened for infection with *P. relictum* within one month of capture. Birds from Malama Ki were bled by jugular venipuncture with a heparinized 28 gauge insulin syringe soon after arrival at the aviary. Heparinized blood was used to make a blood smear that was then dried and fixed immediately with 100% methanol. Blood was transferred to microhematocrit tubes and spun with a microhematocrit centrifuge. Tubes were scored with a file immediately above the buffy coat, broken, and plasma and packed cells were collected with a pipette, transferred to separate 0.5 ml vials, and transported on wet-ice to the laboratory for malarial diagnostics. Birds from Malama Ki Forest Reserve were held overnight in a separate, mosquito-proof quarantine room; while blood samples were tested by microscopy or serology to diagnose malarial infections. Thin smears were stained for 1 h with 6% phosphate buffered Giemsa, pH 7.0, and scanned at 400× for 10 min to confirm that birds did not have patent malarial infections. Plasma was used in a modification of the indirect ELISA described by Graczyk et al. (1993) to test for antibodies to *P. relictum* (Woodworth et al. 2005). Birds that tested positive by either test were returned to their capture site and released the following morning. Birds that tested negative were moved into the aviary. The birds were housed for approximately 1 week within the aviary in individual cages measuring 60 × 30 × 30 cm with supplement heat from 500 W heat lamps, and fed a diet of Nectar Plus (Nekton Corporation, Guenter Enderle, Tarpon Springs, FL, USA), fresh orange slices, scrambled eggs, frozen corn, and mealworm larvae. The birds were exposed to natural lighting through a translucent corrugated fiberglass roof on the aviary to maintain natural light cycles. After approximately 1 week or as soon as it was evident that birds had adapted to their diet, they were released into a large flight cage (6 m × 3 m × 3 m) until sufficient numbers for all experimental groups were collected. Because of the high prevalence of malaria at Malama Ki Forest Reserve (Woodworth et al. 2005), it took almost 12 months to capture and acclimate 12 uninfected ʻAmakihi to captivity. Blood samples were collected from all birds a second time within several months of capture and processed as described above. Blood smears were screened and plasma from the second bleed was tested by an immunoblot technique described by Atkinson et al. (2001) for antibodies to a crude erythrocyte extract of *P. relictum* to confirm that birds did not have early acute infections when first brought into captivity.

Based on capture location, ʻAmakihi were assigned to either a low elevation treatment group (n = 12) or randomly assigned to either a high elevation treatment group (n = 10) or a high elevation control group (n = 9). We were unable to capture enough uninfected low elevation ʻAmakihi within the time allotted for the experiment to include a separate low elevation control group. Each bird in the treatment group was exposed individually to the bite of a single *P. relictum*-infected *Culex* mosquito using colonized *Culex quinquefasciatus* and procedures described by Atkinson et al. (1995). Birds in the control group were exposed to the bite of a single uninfected mosquito. Mosquitoes were infected from a Pekin Duckling (Metzer Farms, Gonzales, CA, USA) that had been inoculated with a thawed, deglycerolized aliquot of an Apapane isolate of *P. relictum* (KV115) that was collected at Kilauea Iki Crater in Hawaiʻi Volcanoes National Park and used in prior experimental studies (Atkinson et al. 2000). The aliquot that was used was passaged once in a canary after initial isolation in 1992 and stored frozen in liquid nitrogen to minimize the potential effect of multiple passages on parasite virulence. Prior to the start of the experiment, an aliquot was thawed and passaged seven more times in Pekin Ducklings before parasitemia was high enough for exposure to mosquitoes.

We measured mortality, changes in food consumption, changes in weight, parasitemia, reticulocytemia, and heterophil/lymphocyte ratio to assess infection dynamics and physiological responses in the low and high experimental groups relative to each other and to the uninfected high elevation control group. ʻAmakihi were weighed and bled via the brachial vein for preparation of thin blood smears every two days beginning on the day they were exposed to mosquitoes (day 0) and continuing for 36 days. Thin blood smears were fixed and stained as described earlier and parasitemia was quantified by counting the number of infected erythrocytes per 1,000 erythrocytes (Gering and Atkinson 2004). Reticulocytemia was calculated from stained blood smears by counting number of reticulocytes per 100 erythrocytes.

Heterophil/lymphocyte ratios were calculated at three points during the infection by counting number of heterophils and lymphocytes per 100 white blood cells and

calculating the ratio of the cells prior to infection, at peak parasitemia, and 2 weeks after the peak parasitemia. Nectar consumption was recorded daily between 0700 and 0900 for each bird.

Based on prior experimental studies (Atkinson et al. 2000), birds were removed from the experiment, classified as fatalities, and subsequently treated with oral chloroquine (10 mg/kg) as per our approved Institutional Animal Care and Use Committee Protocol during acute phases of infection when parasitemia exceeded 20%, food consumption fell below 5 ml of Nekton over the prior 24-h-period, and individuals appeared moribund. Birds that died during the course of the experiment in spite of chloroquine treatment were refrigerated and necropsied within 24 h of death.

Data on food consumption, weight, parasitemia, reticulocytemia, and heterophil/lymphocyte ratio were analyzed using repeated measures ANOVAs where responses for each bird were measured daily (food consumption), at 2- or 4-day intervals (parasitemia, weight, reticulocytemia) or by period (heterophil/lymphocyte ratio). To adjust for possible differences in size and food consumption between birds from low and high elevation sites, we collected daily data on food consumption and biweekly weights for 1 month prior to the start of the experiment. We used these values to calculate a pre-infection average for each bird and then converted food and weight measurements during the course of the experiment as a percent increase or decrease from these pre-infection means. In each analysis, treatment groups (low elevation, high elevation, control) and their interaction were analyzed as among-block (bird) effects. Changes in responses over time and the interaction of time with treatment groups were analyzed as within-block effects. Significant among-block effects were analyzed using *t* tests for multiple comparisons. All analyses were conducted using general linear model procedures with Type III sums of squares (Systat Version 11 2004). Survival distributions between the two treatment groups were analyzed using a Kaplan–Meier estimator and the log-rank test (Systat Version 11 2004). Results were considered statistically significant when $P < 0.05$.

Results

Mortality and Parasitemia

All birds that were exposed to an infective mosquito bite developed patent infections with *P. relictum*, with most (19/22) becoming positive by blood smear 4–6 days post-infection (PI). Control birds, exposed to the bite of single uninfected mosquitoes, remained uninfected for the duration of the experiment. Patent infections in three birds (two low elevation and one high elevation) were not evident until 8, 14, and 16 days PI for undetermined reasons, but infection dynamics resembled those from other birds and they were retained in the analysis. Five birds in the high elevation group (5/10, 50%) and two birds in the low elevation group (2/12, 16.7%) died or were removed from the experiment when they reached a pre-determined end point between 12 and 28 days PI. Parasitemia for fatalities in both groups followed similar dynamics and increased until death (Fig. 1). Mean survival times (±SD) for the fatalities in both groups were similar (low elevation = 21 ± 9.9 days; high elevation = 20 ± 6.8 days) and fatalities in both groups had similar mean parasitemia at day of death (low elevation = 155.3 ± 92.7 parasites/1000 RBC; high elevation = 150.5 ± 104.6 parasites/1000 RBC). Kaplan Meier Survival curves differed significantly between the low and high elevation groups based on the a priori prediction that mortality would be lower in ‘Amakihi from low elevations (log-rank test, $\chi^2 = 2.804$, $df = 1$, $P = 0.047$) (Fig. 2).

Parasitemia did not differ significantly between low and high elevation ‘Amakihi ($F = 0.945$, $df = 1$, $P = 0.352$) (Fig. 3a). While parasitemia varied significantly by day ($F = 2.895$, $df = 14$, $P = 0.001$) with a rapidly increasing acute phase and a declining chronic phase, there were no significant treatment × day effects ($F = 0.407$, $df = 14$, $P = 0.971$), indicating that treatment had no effect on these day to day changes. Soon after peak parasitemia, numbers of reticulocytes increased significantly relative to uninfected control birds in both low and high elevation ‘Amakihi ($F = 36.59$, $df = 2$, $P < 0.0001$). Reticulocytemia varied significantly by day ($F = 32.616$, $df = 8$, $P < 0.0001$), with significant treatment × day effects ($F = 8.629$, $df = 16$, $P < 0.0001$). Reticulocytemia reached a peak at 18 days PI in survivors of both experimental groups. High elevation birds had significantly higher reticulocytemia than low elevation birds at day 10 PI ($P = 0.002$) and day 18 PI ($P = 0.022$) (Figure 3b). Similarly, the heterophil/lymphocyte (H/L) ratio increased significantly in both low and high elevation ‘Amakihi relative to control birds during the course of infection ($F = 7.458$, $df = 2$, $P = 0.005$). These differences varied significantly by period ($F = 7.536$, $df = 2$, $P = 0.002$) but there was no significant treatment × period interaction ($F = 2.126$, $df = 4$, $P = 0.100$), indicating that treatment did not have an effect on how these values changed over time (Fig. 4).

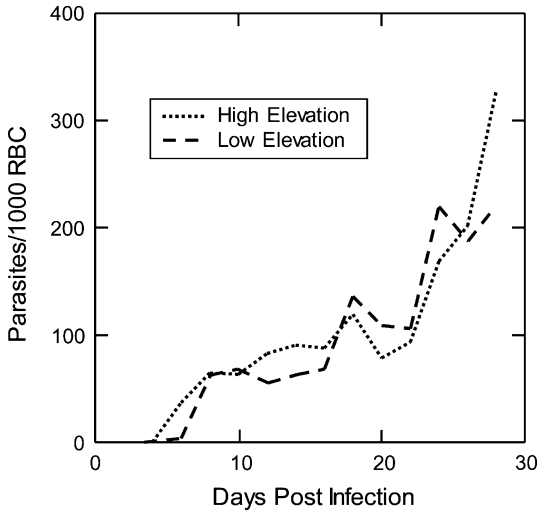

Figure 1. Parasitemia in low elevation ($n = 2$) and high elevation ($n = 5$) Hawai'i 'Amakihi that either died or were classified as fatalities and treated with oral chloroquine on the day they met criteria for removal from the experiment.

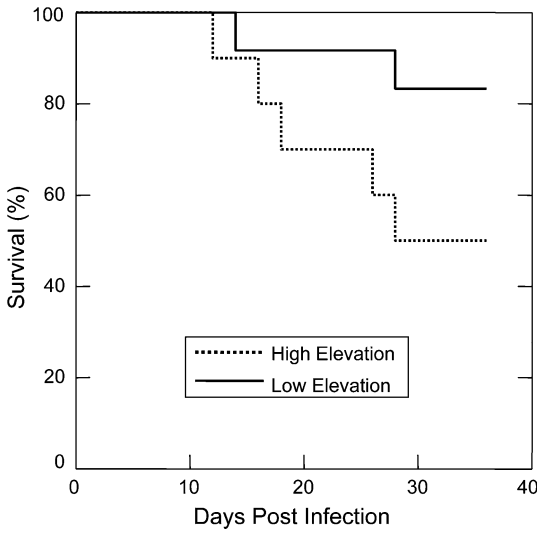

Figure 2. Kaplan Meier survival curves for low and high elevation Hawai'i 'Amakihi with experimental malarial infections.

Infected birds that died during the course of the experiment had gross lesions characteristic of acute malarial infection, including thin, watery heart blood and enlarged, darkly pigmented livers and spleens (Atkinson et al. 2000).

Food Consumption and Weight

Food consumption differed significantly between experimental groups ($F = 6.344$; $df = 2, 21$; $P = 0.007$) with significant day to day fluctuations ($F = 5.440$; $df = 36, 756$; $P < 0.0001$). Birds in the high elevation treatment group had an early and rapid drop in food consumption within 6 days of infection ($P = 0.025$) and consumed less food than either control or low elevation treatment birds for the remainder of the experiment (Fig. 5). Treatment × day interactions were not statistically significant, suggesting that treatment did not have an effect on how the experimental groups changed over time ($F = 1.076$; $df = 72, 756$; $P = 0.318$).

Weight varied significantly among all three experimental groups, with significant declines in both the low and high elevation treatment groups relative to uninfected control birds ($F = 13.189$, $df = 2, 21$, $P < 0.0001$). Significant differences were evident among all three groups by day 16 PI ($P < 0.047$). Both day ($F = 59.254$, $df = 18, 378$, $P < 0.0001$) and treatment × day ($F = 12.711$, $df = 36. 378$, $P < 0.0001$) interactions were significant, indicating that treatment had a significant effect on how the experimental groups changed over time (Fig. 6).

Discussion

Host defense mechanisms to infectious disease can involve resistance mechanisms, tolerance mechanisms or a combination of both (Read et al. 2008). Resistance mechanisms directly affect parasite burden or intensity. For example, in human malarial infections, defined resistance mechanisms lower parasitemia by limiting erythrocyte invasion (Duffy blood group receptors), parasite reproduction processes (sickle cell anemia, G6PD deficiency), or lead to increased phagocytosis of infected erythrocytes (Verra et al. 2009). By contrast, host-tolerance defense mechanisms minimize physiological damage caused by both the parasite itself and the ensuing immunopathology to infection without directly affecting parasite burden or intensity (Roy and Kirchner 2000; Read et al. 2008). While tolerance has been documented in several host/parasite systems (Pamplona et al. 2007; Råberg et al. 2007; Ayres et al. 2008; Ayres and Schneider 2008, 2009; Seixas et al. 2009), the physiological mechanisms are poorly understood and there are few well documented natural systems in which emergence and evolution of this phenomenon has been thoroughly investigated (Råberg et al. 2009; Sorci 2013).

Taken together, similar intensities of infection in high and low elevation experimental groups and fewer acute effects of infection in low elevation birds support the

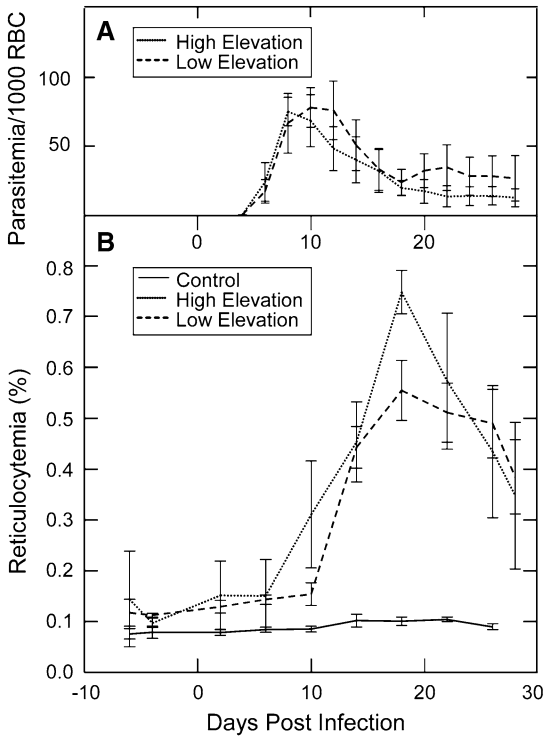

Figure 3. Parasitemia (**a**) and % reticulocytemia (**b**) in surviving low elevation (n = 10) and high elevation (n = 5) Hawai'i 'Amakihi with experimental malarial infections. Note similarity of parasitemia curves between low and high elevation birds (**a**) and significant increases in numbers of circulating reticulocytes relative to uninfected control birds (**b**).

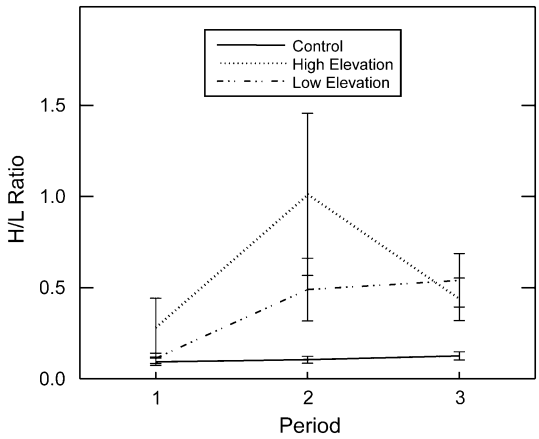

Figure 4. Changes in heterophil/lymphocyte ratio (H/L ratio) over time for uninfected control (n = 9) and surviving low elevation (n = 10) and high elevation (n = 5) Hawai'i 'Amakihi. Samples were collected prior to infection (*Period 1*), at the crisis when parasitemia reached a peak (*Period 2*), and two weeks after the crisis (*Period 3*) when birds were recovering from infection.

hypothesis that Hawai'i 'Amakihi have acquired tolerance rather than resistance to this introduced disease. Both groups were equally susceptible to single infective mosquito bites, had similar survival times and parasitemia among birds that succumbed to infection (Fig. 1), and had similar peak parasitemia at approximately 12 days post-infection (Fig. 3a). By contrast, low elevation birds had significantly lower mortality (Fig. 2), significantly lower reticulocyte counts on specific days both prior to and after peak parasitemia (Fig. 3b), no significant reduction in food consumption (Fig. 5) and less weight loss over the course of the experiment (Fig. 6). These multiple lines of evidence suggest that low elevation 'Amakihi suffered fewer pathological effects from acute infection and were able to maintain better physiological condition than their high elevation counterparts in spite of similar intensities of infection.

Several factors have probably contributed to emergence of tolerance in this population of 'Amakihi on Hawai'i Island, including presence of several forest reserves and ongoing volcanic activity from the SE rift zone of Kilauea Volcano that has protected large areas of lowland native forest and discouraged residential and agricultural development (Steinberg et al. 2010), favorable demographic characteristics associated with high nest productivity of Hawai'i 'Amakihi relative to other species of honeycreepers (Kilpatrick et al. 2006), high genetic diversity of this lowland 'Amakihi population (Foster et al. 2007; Eggert et al. 2008), and high selective pressures from year-round malarial transmission (Samuel et al. 2011). We do not know the specific combination of factors that led to emergence and rapid spread of this trait in the 1990s after decades where populations remained virtually undetectable at this elevation (Spiegel et al. 2006). Kilpatrick (2006) used a modeling approach to demonstrate that malaria resistance can spread through a native forest bird population when resistance is determined by a single locus, selective pressures from disease transmission are moderate, and host survival and reproduction are increased through rodent control or other management actions that improve survival of adults and recruitment of juvenile birds into the breeding population. In the case of lowland 'Amakihi, the dominance of unique haplotypes indicates that emigration from mid- and high-elevation habitats is not important in augmenting the expanding population (Foster et al. 2007; Eggert et al. 2008). This suggests that demographic rates may have been high enough in this population (Kilpatrick 2006) to allow for spread of tolerance traits in spite of high rates of nest predation (Klein et al. 2004) and impacts on invertebrate food resources by alien parasitoids (Peck et al.

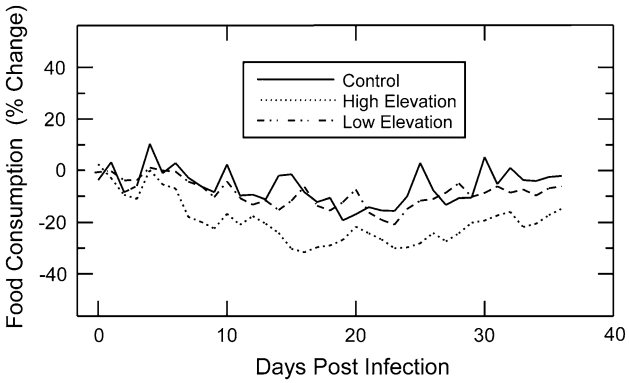

Figure 5. Changes in food consumption relative to pre-infection averages for uninfected controls and experimentally infected low (n = 12) and high (n = 10) elevation Hawai'i 'Amakihi.

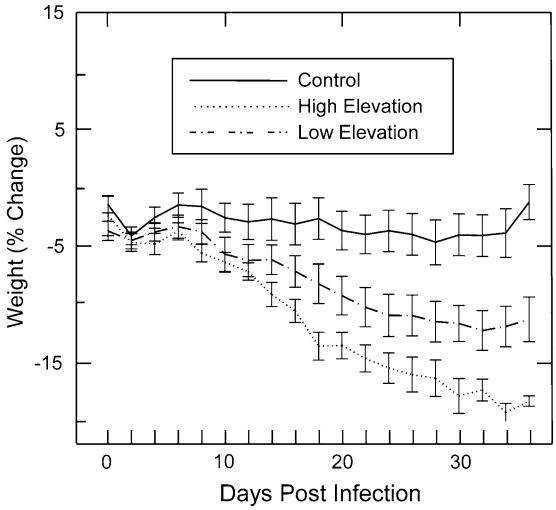

Figure 6. Changes in weight relative to pre-infection averages for uninfected controls and experimentally infected low (n = 12) and high (n = 10) elevation Hawai'i 'Amakihi.

2008). More detailed studies of this system coupled with comparative studies of O'ahu 'Amakihi where prevalence of malarial infection is low and resistance mechanisms might be playing a role in persistence of lowland populations (Krend 2011) may help to identify the specific combination of factors that led to emergence of tolerance. This question is highly relevant to conservation of remaining species of native birds given the threat that rapidly changing climatic conditions poses to high elevation disease refugia.

The avian immune response to malarial infection is complicated and involves a combination of both the cellular and humoral arms of the adaptive immune response (van Riper et al. 1994). Proteins encoded by the major histocompatibility complex (*Mhc*) (Jarvi et al. 2001, 2004; Bonneaud et al. 2006; Sepil et al. 2013) and both specific and non-specific inflammatory processes (Sorci and Faivre 2009; Bichet et al. 2012; Christe et al. 2012) may play significant roles in tolerance or resistance to infection. Innate immune responses may also be important in avian host–parasite interactions (Grueber et al. 2012; Hellgren and Sheldon 2011), but their role in mediating immune responses to malaria has not yet been explored. Two clues from our limited experimental data suggest that tolerance mechanisms in low elevation 'Amakihi may be related to mechanisms that reduce immunopathology associated with acute infection. The significantly higher reticulocyte counts in high versus low elevation birds at day 18 PI may reflect a compensatory response to non-specific inflammatory processes and removal of both infected and uninfected erythrocytes from the circulation as parasitized erythrocytes were removed from the circulation by the immune system and replaced with these immature red blood cells (Graham et al. 2005; Totino et al. 2010). By contrast, clearance of infected erythrocytes in low elevation birds may be more targeted, leading to lower anemia and improved ability to recover from acute infection. Lower trends in heterophil/lymphocyte ratios in low elevation birds (Fig. 4) are also suggestive of a reduced cell-mediated response to infection that might moderate immunopathology associated with acute infections, but these trends were not statistically significant and additional experimental work with larger sample sizes is needed.

Other ongoing work also supports a connection between both innate and adaptive immunity and tolerance mechanisms in these birds. Preliminary evaluation of *Mhc* diversity in experimentally infected low and high elevation 'Amakihi using 454 sequencing techniques has revealed six clusters of alleles that occur more often in surviving 'Amakihi (Jarvi unpublished data). Their role in antigen presentation and parasite recognition by the cellular immune response may increase specificity of the response and reduce collateral damage to host tissues (Sorci 2013). Some recent preliminary data also suggests that low elevation 'Amakihi have higher titers of natural antibodies than their counterparts from high elevation populations (Atkinson and Paxton 2013). This difference appears to be independent of infection status, but the specific role that innate immunity might play in response to malarial infection is still not known.

While these findings are encouraging for more common native species of honeycreepers such as the 'Apapane that are exposed to selective pressure at the lower edges of their ranges (Nielsen 2000; Atkinson et al. 2005), it is not

clear whether critically endangered species such as the Kiwikiu (Maui Parrotbill, *Pseudonestor xanthophyrys*), Palila (*Loxioides bailleui*), or ʻAkiapōlāʻau (*Hemignathus munroi*) have the numbers, genetic diversity, and demographic characteristics to adapt to this disease on their own. However, availability of both malaria tolerant and malaria susceptible populations of Hawaiʻi ʻAmakihi provides an exceptional opportunity to clarify the physiological mechanisms of tolerance and find candidate genes and molecular markers that might be used to identify individuals capable of surviving infection in closely related species of honeycreepers. For example, preliminary work using AFLP techniques to evaluate differences in band frequencies between experimentally infected low and high elevation ʻAmakihi is promising. There are statistically significant differences in frequency of specific band classes between low and high elevation ʻAmakihi that appear to be associated with at least five candidate genes, but these need to be evaluated in more individuals from both populations to obtain a more accurate estimate of gene frequencies (Jarvi et al. unpublished data). Other possible approaches include the use of transcriptomics and next generation sequencing to identify differences in expression of specific genes during acute infection with malaria and development of panels of single nucleotide markers (SNPs) that might correlate with ability to recover from infection.

Conclusions

Hawaiʻi's endangered honeycreepers have survived into this century largely because cool, high elevation native forest on the highest peaks on Kauaʻi, Maui, and Hawaiʻi Islands have served as refugia from mosquito-transmitted avian malaria. Warming and drying trends associated with global climate change and upslope movement of disease transmission may lead to loss of these refugia by the end of this century. The recent appearance of evolved tolerance to avian malaria has contributed to recovery of low elevation populations of Hawaiʻi ʻAmakihi in the Puna District of Hawaiʻi Island and opens the possibility that other native honeycreepers may also be able to adapt to this disease through processes of natural selection. An experimental approach based on comparing physiological responses to acute malarial infections between malaria-tolerant low elevation Hawaiʻi ʻAmakihi and susceptible high elevation Hawaiʻi ʻAmakihi can help to identify tolerance mechanisms in low elevation birds and allow development of specific genetic or physiological markers that can be used to evaluate susceptibility and tolerance to malaria in remaining populations of endangered honeycreepers.

Acknowledgments

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