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Ecology and conservation biology of avian malaria

Dennis A. LaPointe,[1] Carter T. Atkinson,[1] and Michael D. Samuel[2]

[1]U.S. Geological Survey, Pacific Island Ecosystems Research Center, Hawaii National Park, Hawaii. [2]U.S. Geological Survey, Wisconsin Cooperative Wildlife Research Unit, University of Wisconsin, Madison, Wisconsin

Address for correspondence: Dennis A. LaPointe, U.S. Geological Survey, Pacific Island Ecosystems Research Center, Hawaii National Park, HI 96718. dennis_lapointe@usgs.gov

Avian malaria is a worldwide mosquito-borne disease caused by *Plasmodium* parasites. These parasites occur in many avian species but primarily affect passerine birds that have not evolved with the parasite. Host pathogenicity, fitness, and population impacts are poorly understood. In contrast to continental species, introduced avian malaria poses a substantial threat to naive birds on Hawaii, the Galapagos, and other archipelagoes. In Hawaii, transmission is maintained by susceptible native birds, competence and abundance of mosquitoes, and a disease reservoir of chronically infected native birds. Although vector habitat and avian communities determine the geographic distribution of disease, climate drives transmission patterns ranging from continuous high infection in warm lowland forests, seasonal infection in midelevation forests, and disease-free refugia in cool high-elevation forests. Global warming is expected to increase the occurrence, distribution, and intensity of avian malaria across this elevational gradient and threaten high-elevation refugia, which is the key to survival of many susceptible Hawaiian birds. Increased temperatures may have already increased global avian malaria prevalence and contributed to an emergence of disease in New Zealand.

Keywords: avian malaria; Hawaiian forest birds; *Culex* mosquito; *Plasmodium*; climate change

Introduction

Avian malaria, a disease caused by protozoan parasites in the genus *Plasmodium*, has played a seminal role as a model for human malarial infection since these common intraerythrocytic parasites of wild birds were first recognized.[1–5] Today, avian malaria is more frequently used as a model system to investigate general host–parasite interactions, coevolutionary processes, and the role of parasites in host life-history evolution. The pathogenicity of most avian species of *Plasmodium* is poorly understood but ranges from sublethal effects on host fitness to population decline and extinction. One species, *Plasmodium relictum*, plays an important role as a limiting factor in the distribution and abundance of native Hawaiian forest birds.[6–11] In this paper, we provide a review of the ecology and epidemiology of avian malaria, its impact on avian communities, and how climate and landscape change may alter this disease system. We rely on examples from the Hawaiian Islands, but many interactions are universal and applicable to insular and continental avian malaria, human malaria, and other mosquito-borne diseases enzootic in passerines, such as West Nile virus.

Natural history

Avian malaria is a common mosquito-transmitted disease of wild birds that has a world-wide distribution. The disease is caused by intracellular protozoan parasites in the genus *Plasmodium,* which share morphological and developmental features with closely related haemosporidian parasites in the genera *Haemoproteus* and *Leucocytozoon.*[12,13] Avian malaria is a complex, spatially heterogeneous host–parasite disease caused by more than 40 parasite species that differ widely in host range, geographic distribution, vectors, and pathogenicity.[12] Although the taxonomy of *Plasmodium* is currently in flux and supporting field studies have been limited,[10] diversity of mitochondrial genes suggests that genetic

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structure may underlie differences in host susceptibility, vector competence, and parasite virulence.[14]

The parasites occur in many avian species and families but primarily affect passerine birds.[5] The mosquito vectors of avian malaria are generalist blood feeders that likely transfer parasites among multiple avian species.[15] Although there are numerous reports of individual birds with acute, pathogenic infections with *Plasmodium*, the impacts of these blood parasites on host fitness and host population dynamics in wild birds is poorly understood.[16] Reports of epizootics are rare and mostly associated with birds in zoological collections, introductions of parasites or mosquito vectors to remote islands, or domestic birds that are exposed to sylvatic cycles of malaria outside of their geographic origins.[17–19] At one extreme, avian malaria causes high mortality in naive Hawaiian birds and has been implicated in the extinction, population decline, and restricted distribution of multiple Hawaiian bird species.[6,7,10,17,20] At the other extreme, there is little evidence of overt mortality in wild bird populations that have a long evolutionary association with the parasites. There is increasing evidence, however, that malaria may have significant effects on host fitness, including mate selection, reproductive success, and immune response.[16,21]

The species of *Plasmodium* that infect birds have a cosmopolitan distribution and are found in all major zoogeographic regions of the world with the exception of Antarctica, where mosquito vectors responsible for their transmission do not occur. Reports of *Plasmodium* from the Australian region are notably limited, although it is not clear whether this reflects inadequate sampling or a distributional anomaly.[5,22] Seven species of *Plasmodium* have a cosmopolitan distribution. *Plasmodium relictum* and *P. circumflexum* have the broadest geographic distribution and are reported from the Nearctic, Palearctic, Oriental, Ethiopian, Neotropical, and Australian regions. *P. vaughani*, *P. cathemerium*, *P. nucleophilum*, *P. rouxi*, and *P. elongatum* have been reported from every region except the Australian region.[22]

Plasmodium infections have been reported from all avian orders with the exception of the Struthoniformes (ostriches), the Coliiformes (mousebirds), and the Trogoniformes (trogons and quetzals), but only about half of all avian species have been examined. The greatest diversity of *Plasmodium* species are found in Galliformes, Columbiformes, and Passeriformes.[5] *P. relictum* has one of the widest host ranges, occurring in more than 400 species in 70 different avian families.[5,12,22] The relatively broad host range of *Plasmodium* is characteristic of this genus, but exceptions are common.[5,12] For example, *P. elongatum* is known from as few as 67 avian species and *P. hermani* is recognized only from North American wild turkeys (*Meleagris gallopavo*). Recently, molecular methods revealed a greater complexity in the genetic lineages of *Plasmodium* (and closely related *Haemoproteus*) that is currently difficult to reconcile with traditional morphological species.[23] Multiple lineages can occur in the same host individual, and their occurrence in a wide range of avian orders, families, and species is much broader than previously recognized.[24–27]

Mosquitoes in the genus *Culex* are believed to be the most common vectors of avian *Plasmodium*; however, only *Culex quinquefasciatus*, *Cx. tarsalis*, and *Cx. stigmatasoma* have been identified as natural vectors of *P. relictum* in California and only *Cx. quinquefasciatus* in Hawaii.[28,29] By contrast, more than 60 different species of culicine and anopheline mosquitoes can support experimental development of a variety of *Plasmodium* from avian hosts.[30] *P. relictum*, one of the best studied species of avian malaria, can complete development in at least 26 mosquito species from four different genera (*Culex*, *Aedes*, *Culiseta*, and *Anopheles*) in the laboratory. More recently, molecular assays for avian malaria have identified a number of potential vector species.[31–36] Although few of these studies have confirmed the presence of salivary gland sporozoites or demonstrated transmission, they support the notion that relatively little host specificity among most parasite–vector associations may facilitate switching of vertebrate hosts.[37]

Epidemiology

Although mosquito transmission of *Plasmodium* was discovered by Ross,[38] much of what we know about the life cycle of avian malaria was described 30–40 years later in a series of detailed studies of the blood and tissue stages of *P. elongatum*[39,40] and *P. gallinaceum*.[41,42] Other than scattered reports and descriptions of tissue or exoerythrocytic stages of infection, only a handful of additional species have been studied in any detail.[5] All have an initial cycle of asexual, preerythrocytic reproduction

(preerythrocytic merogony) within host tissues immediately after sporozoites are inoculated by mosquitoes. This is followed by multiple cycles of asexual reproduction within circulating erythrocytes (erythrocytic merogony) and host tissues (exoerythrocytic merogony) that eventually leads to production of gametocytes within circulating blood cells (gametogony). Gametocytes of all species of avian *Plasmodium* remain in circulation and do not continue development until ingested by a vector. Once in the midgut of a mosquito vector, gametocytes undergo gametogenesis to form true gametes. Male gametocytes produce up to eight, flagellated microgametes. One microgamete will fertilize a macrogamete, and within 24 hours a motile zygote develops that is capable of penetrating the midgut wall to begin development as an oocyst. These initial stages of gametogenesis and fertilization exhibit little or no specificity for mosquito vectors and can be completed *in vitro*. It is only during invasion of the membrane surrounding the blood meal and subsequent penetration of the midgut epithelium that development can be blocked.[43]

Oocysts undergo a type of asexual reproduction called sporogony and eventually produce thousands of sporozoites. The duration of sporogony is dependent on temperature, and at optimal temperatures oocysts can mature within seven days. When oocysts reach a diameter of approximately 40 μm, they rupture, releasing sporozoites that move to the salivary glands, penetrate the glandular cells, and eventually gain access to the salivary ducts. At this point, the mosquito is considered infective, and upon subsequent blood feeding, sporozoites pass with the saliva into a new avian host to initiate infection.

Birds typically undergo an acute phase of infection where parasitemia increases steadily to a peak called the crisis, approximately 6–12 days after parasites first appear in the blood. After this acute phase, intensity appears to be influenced by the complex interplay of host immunity, seasonal photoperiod, and hormones associated with reproduction. Infected birds are typically anemic, lethargic, anorexic, and have ruffled feathers. Hematocrits may fall by more than 50%. Disease progression and clinical signs closely parallel the number of parasites in the peripheral blood circulation and the reticuloendothelial system.[44] The hallmark signs of acute infections with *Plasmodium* include thin, watery blood with enlargement and discoloration of

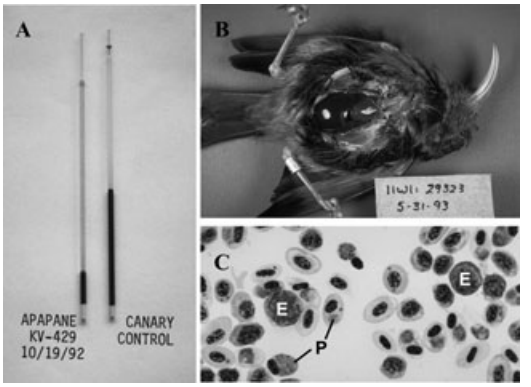

Figure 1. **Hallmark signs of acute *Plasmodium* infections. (A) Severe anemia and low packed cell volume for an apapane infected with *P. relictum*. (B) Gross lesions (enlarged, darken liver) associated with a fatal malarial infection in an iiwi. (C) Blood smear of iiwi with an acute infection of *P. relictum*. Note parasitized erythrocytes (P) and erythroblasts (E).**

the liver and spleen by deposition of malarial pigment in tissue macrophages (Fig. 1). Enlargement of these organs is due to hypercellularity and increased phagocytic activity of macrophages rather than edema.[45] Fatal pathologies may arise from anemia as infected erythrocytes rupture during asexual parasite reproduction and as both infected and uninfected erythrocytes are removed by the spleen. During the acute phase, indirect mortality may occur when birds weakened by anemia succumb to environmental stressors such as predation, starvation, or inclement weather.[46]

In birds that survive infection, the acute phase is followed by a rapid decline in intensity of infection to chronic levels as strong antibody and cell-mediated responses develop to the parasites.[44] Chronic infections likely persist for the lifetime of infected birds at extremely low intensities, and both circulating parasites and persistent exoerythrocytic meronts can serve as a source for recrudescing infections.[3,47,48] When chronically infected birds are rechallenged with homologous strains of the parasite, they may have only brief, low-intensity increases in peripheral parasitemia.[2,49,50] This concept of prolonged chronic infection that stimulates immunity to reinfection is fundamental to the epidemiology of avian malaria, yet supporting experimental evidence is limited to only a handful of studies in a few avian species.[48,51–53]

In temperate climates, a recrudescence, or spring relapse, occurs during the breeding season when

increased corticosterone levels suppress the host's immune system.[54] Recrudescence of chronic infections is believed to facilitate seasonal transmission in temperate climates where vector populations may have corresponding seasonal population increases, but this may not apply to all species of avian *Plasmodium*. In Europe, absence of some lineages of *P. relictum* in hatch-year birds indicates that transmission occurs on the wintering rather than the breeding grounds.[55] Seasonal recrudescence has not been reported in Hawaii, but concomitant infections with immunosuppressing pox virus may trigger malaria recrudescence in the Hawaiian system.[7,12]

Population impacts

There is relatively little evidence that avian *Plasmodium* causes major epizootic die-offs in most natural bird hosts. The most significant reports of pathogenicity from avian *Plasmodium* occur in birds with acute infections, typically captive birds in zoological collections and the avifauna of isolated islands where new host–parasite associations become established. In a thorough review of over 5,000 papers on avian blood parasites, only about 4% reported mortality or pathogenicity, mostly in domestic birds or zoological collections.[22] Avian malaria is particularly pathogenic in captive penguins exposed to mosquito vectors from outside of their natural range.[56,57]At the Rotterdam Zoo, mortality from avian malaria occurred annually among Atlantic puffins (*Fratercula arctica*), common guillemots (*Uria aalge*), and black-legged kittewakes (*Rissa tridactyla*), as well as black-footed penguins (*Spheniscus demersus*).[58] Although mortality from *Plasmodium* has not been reported in wild penguins,[59,60] future spread of new mosquito vectors and potential effects of global climate change may place wild colonies at risk.[61,62]

There are only a handful of documented *Plasmodium* deaths of wild birds in the United States and other parts of the world, and virtually all are associated with acute infections.[5] For example, intense transmission of *Plasmodium* and other haemosporidians in the rookeries of wading birds (ciconiiformes) in Venezuela is suspected to be a cause of high levels of nestling mortality, but it is unclear if other factors are also involved.[5,63] Similarly, acute infections may lead to increased predation on infected hosts.[46,64]

The more significant impacts of these parasites may be subclinical and indirect, with long-term effects on the lifetime reproductive success of their avian hosts. For example, male white-crowned sparrows (*Zonotrichia leucophrys oriantha*) infected with *Plasmodium* sing fewer songs in response to experimental playbacks. Parasite-induced changes in song frequency or quality may affect female mate selection.[65] More direct effects of *Plasmodium* infection on reproductive success have been demonstrated in wild populations of blue tits (*Cyanistes caeruleus*) and great reed warblers (*Acrocephalus arundinaceus*).[66–70]

In contrast to malaria in continental species, introduced avian malaria poses a substantial threat to naive endemic birds on isolated islands. The accidental introduction of *P. relictum* and the southern house mosquito (*Culex quinquefasciatus*) to the Hawaiian Islands has devastated native Hawaiian forest birds[6,7] and continues to play a significant role in limiting the geographic and altitudinal distribution of remaining species.[71,72] Although numerous limiting factors have contributed to extinctions of Hawaiian avifauna, avian malaria is believed to be one of the key components driving population collapse in otherwise suitable, lowland (<900 m above sea level, asl) habitat. Mortality due to acute infection appears to be the major contributor to population effects.[6,7,20,46,71,73] Chronic infections may affect survival in adult Hawaii amakihi (*Hemignathus virens*) but do not affect reproductive success or prevent populations from growing.[74] Although many native species continue to decline in forests where avian malaria is prevalent, the Hawaii amakihi appears to be evolving tolerance to infection, and lowland populations have rebounded dramatically in recent years.[7,8,75]

Much less is known about the impact of avian malaria on the avifauna of New Zealand and the Galapagos Archipelago.[60,62,76,77] Although *Cx. quinquefasciatus* arrived in the North Island of New Zealand (1830s) about the same time as in the Hawaiian Islands (*circa* 1826) and malarial infections were reported in introduced birds as early as 1920,[78] significant deaths from avian malaria among captive native birds have only been reported in the last 15 years (New Zealand dotterel [*Charadrius obscures*], mohua [*Mohoua ochrocephala*], South Island saddlebacks [*Philesturnus carunculatus carunculatus*]).[79–82] Prevalence of *Plasmodium* by

polymerase chain reaction (PCR) in wild nonnative birds ranges from 9% to 26%, suggesting the disease is more prevalent than previously thought.[60,62] Recent surveys of endemic birds have detected chronic malarial infections, as well as cases of acute mortality, in wild, endemic, and indigenous birds, and there is increasing concern that transmission of avian malaria may be limiting success of some reintroduction programs.[82] In the Galapagos, avian malaria has recently been reported in the endemic Galapagos penguins and, while there is no evidence of clinical illness or mortality, there is also concern that spread into the endemic avifauna of this archipelago might parallel what happened to native Hawaiian forest birds after introduction of *Plasmodium*.[60,76,77]

Ecological drivers of transmission

Several common drivers of transmission have been identified in continental studies of avian malaria, including host density, proximity to water, temperature, and host immunocompetence.[83–86] Comprehensive studies of host and vector dynamics and the environmental factors that influence transmission rates have been done in the Hawaiian Islands.[19] Although it has been argued that the Hawaiian system may not be applicable to continental areas because both the vector and parasites have been introduced,[69] its simplicity makes it highly relevant for understanding the complexities of mainland systems where multiple vectors and parasite species, as well as human influences, make it difficult to measure ecological effects on host fitness.[86,87]

The avian malaria system in Hawaii includes many factors that facilitate transmission of both endemic and epizootic disease across a geographically broad landscape, including a novel pathogen in highly susceptible naive hosts,[71,72] broad host range, a widely dispersed and highly competent vector,[7,29,95] favorable climate for vector and parasite,[11,88] and an efficient reservoir of chronically infected native birds.[20,73]

Habitat and human impacts on vector abundance

The geologically young, volcanic landscape of the Hawaiian Islands provides limited natural freshwater habitat for the mosquito vector of avian malaria. On the older islands (Kauai, Oahu, Maui, and Molokai) and volcanoes like Mauna Kea, erosive forces have exposed less permeable substrates, creating perched wetlands or rock pools along streambeds. As a result, Kauai's topography is characterized by numerous perennial streams and vast open bogs, while on the relatively young volcano of Mauna Loa, surface hydrology is limited to a few small bogs, ground pools, and intermittent streams. Depressions in exposed lava substrates along intermittent stream beds and elsewhere impound rainwater and surface drainage to create suitable larval mosquito habitat,[89,90] but there is little evidence of available natural tree holes or other phytotelmata in Hawaiian forests.[91,92] In Hawaii, forest fragmentation by natural and human activities increases the likelihood of vector and native bird interactions. On the slopes of the active Mauna Loa and Kilauea volcanoes, forests are naturally fragmented by lava flows into islands or *kipuka* where native birds and vectors may be concentrated in crucibles of malarial transmission.[93,94] In general, conservation areas in Hawaii exist within a matrix of residential and agricultural areas where roadways and open landscapes may enhance mosquito abundance and dispersal into native forest bird habitat.[95]

Other human activities and alterations of the environment can significantly enhance vector abundance, richness, and disease transmission.[96] Road construction, forestry, agriculture, ranching, and residential development have all contributed to vector abundance. *Cx. quinquefasciatus* is, primarily, a peridomestic mosquito and is found in great abundance in suburban and agricultural areas where artificial containers and impoundments are common larval habitats.[92,97–99] However, this mosquito also occurs in Hawaiian forests where feral pigs are managed as a game species. Feral pigs increase the abundance of mosquitoes by foraging on native tree ferns.[90,100] In forests with minimal surface water, pigs create cavities in tree fern trunks that fill with leaf litter and rainwater to form larval mosquito habitat (Fig. 2). More larval habitat leads to increased vector abundance, an important factor in determining transmission and prevalence rates of avian malaria throughout low- and midelevation (900–1500 m asl) forests.[20]

In New Zealand, there is a positive association between the distribution of *P. relictum* and introduced *Cx. quinquefasciatus*.[62] Anthropogenic landscape change may be facilitating the geographic spread of *Cx. quinquefasciatus,* as deforestation and

Figure 2. Tree fern (*Cibotium glacum*) cavities are created by the feeding behavior of feral pigs in Hawaiian wet forests. In forests where surface hydrology is limited, tree fern cavities are the primary larval habitat for the mosquito vector of avian malaria, *Culex quinquefasciatus*.

agriculture have created nutrient-rich larval habitat. The native New Zealand mosquito, *Cx. pervigilanus,* prefers aquatic habitat with medium detrital loads, leaving habitat with more detritus available for introduced species like *Cx. quinquefasciatus.*[101] In the Galapagos, populations of *Cx. quinquefasciatus* are closely associated with human residence and agriculture.[102] As human populations grow and move among the five inhabited islands, *Cx. quinquefasciatus* populations will likely increase in distribution, abundance, and seasonal occurrence unless peridomestic sources of water are managed for mosquito control.[102,103]

This positive relationship between forest disturbance, vector abundance, and avian malaria prevalence defines the emergent nature of *P. relictum* where *Cx. quinquefasciatus* has been introduced to parasite-depauperate island ecosystems. In the continental forests of Cameroon, West Africa, however, avian malaria prevalence in native rain forest birds may increase or decrease in response to forest disturbance.[104,105] There are many possible nonmutually exclusive explanations for these findings. For example, deforestation and encroaching agriculture may destroy or create larval habitat for specific vectors, affect the general health of hosts, or alter key vector–host interactions through native biodiversity loss or the introduction of livestock.[104]

Reservoirs of infection and the dilution effect

Human activities have greatly altered avian communities in Hawaii through the introduction of at least 17 avian species that have become established in forest habitats. The most abundant and widely dispersed species in Hawaiian forests is the Japanese white-eye (*Zosterops japonicas)*, a species first released in 1929.[106] Our understanding of the role these introduced species play in the avian malaria disease system has changed over time. Warner[6] found malarial infections in two nonnative species, the house finch (*Carpodacus mexicanus)* and the Japanese white-eye, but not in native apapane or amakihi, suggesting that nonnative species were the reservoirs of malaria. Conversely, van Riper *et al.*[7] detected parasitemias of up to 30% in apapane and less than 3% in nonnative species, including the northern cardinal (*Cardinalis cardinalis)*, Japanese white-eye, and red-billed leiothrix (*Leiothrix lutea)*.[73] Recent archipelago-wide surveys using PCR and serological diagnostics[8,107–109] agree with the findings of van Riper *et al.*[7] with the possible exception that prevalence (based on PCR diagnostics) in Oahu amakihi (*Hemignathus chloris)* is lower than nonnative species.[110] Laboratory challenge experiments have shown that native birds surviving infection acquire immunity to reinfection but retain a chronic, low parasitemia, making these individuals efficient, lifetime reservoirs of disease.[7,72] Unfortunately, the malaria reservoir in native Hawaiian birds may well be the Achilles heel for these bird populations.[73] Efforts to control malaria transmission by removal of reservoir hosts would be counterproductive and may hinder natural selection for disease resistance in native species.[8,9]

In laboratory challenge experiments, Japanese white-eye and red-billed leiothrix develop transient malarial infections but are incapable of infecting mosquitoes beyond a brief period following the acute phase, thereby, making them poor reservoirs of disease. In contrast, the nonnative house sparrow (*Passer domesticus*) develops acute parasitemias that are intermediate in intensity and remain infectious to mosquitoes up to one year. House sparrows may serve as disease reservoirs for native birds inhabiting or foraging in the anthropogenic ecotones favored by this species. Isolated house sparrow populations may even allow for the evolution of more virulent strains of *P. relictum* that could ultimately spill over into the forest bird community.[97]

Because of their relative resistance to infection and short-lived parasitemias, Japanese white-eye and red-billed leiothrix are incompetent reservoirs that may serve to buffer or dilute disease

transmission from infective mosquitoes.[99,111] Because Japanese white-eyes and other nonnatives make up a large proportion of some forest bird communities, one might expect a significant dilution effect. However, nonnative species, especially Japanese white-eyes and red-billed leiothrix, are more defensive to mosquito blood feeding and may thereby reduce the potential dilution effect. The potential dilution effect may be more significant when agriculture encroaches on forests and people and livestock become alternate blood hosts for generalist vectors.[104]

Climate

Although habitat and avian communities largely determine the geographic distribution of avian malaria, climatic factors are likely the strongest drivers of transmission.[20,87] Temperature and rainfall in Hawaii are highly variable across different temporal and spatial scales with significant variation across small geographical distances depending on elevation, aspect, and slope.[112] Rainfall occurs throughout the year with the greatest accumulation between October and March, but annual and seasonal rainfall can vary substantially between wet and dry years.[113] Rainfall can have direct and indirect effects on malarial transmission by influencing larval habitat availability and larval and adult survivorship. In Hawaiian rainforests, mean annual precipitation (2000–6000 mm) is sufficient to maintain larval mosquito habitats and keep relative humidity high for adult survival. However, the extended droughts associated with El Niño Southern Oscillation events will reduce available larval habitat and adult survivorship.[88,97,114] Extreme rainfall events ($>$200 mm/day) can also have a negative effect on larval and adult survivorship by flooding larval habitat and causing direct mortality to adults.[88,97,115]

Temperature effects on the disease system are not limited to extreme conditions. Seasonal temperatures have a direct effect on larval mosquito development that influences adult abundance. The link between human malaria transmission and climate has been recognized for a long time,[116] but the significance of temperature to the distribution of avian malaria is just being explored.[11,87] In a spatial analysis of avian malaria prevalence in the olive sunbird (*Cyanomitra olivacea*) across 28 sites in Central and West Africa, Sehgal *et al.*[87] found the maximum temperature of the warmest month to be the most important environmental factor influencing local prevalence. In Hawaii, where annual changes in mean temperature are minimal, elevation accounts for much of the variation in ambient temperatures. Temperature decreases with elevation, and lower temperature slows the development of mosquito larvae.[117] Lowland *Cx. quinquefasciatus* populations consist of year-round, overlapping generations that increase during the most favorable parts of the year. At higher elevations ($\geq$900 m asl), populations consist of fewer cohorts which are seasonal and less abundant.[88,97] Temperature also influences survival of adults and duration of the gonotrophic cycle (time between blood meal and oviposition), factors that contribute to disease transmission.[20,97] Perhaps the most important temperature effect on avian malaria transmission is through control of the parasite extrinsic incubation period, the interval of parasite development in the vector from blood meal acquisition to salivary gland infection. This incubation period lasts 6 to 28 days, is inversely dependent on temperature, and ultimately restricts the altitudinal distribution of infectious mosquitoes and disease transmission.[11] *P. relictum* requires a minimum of 13° C for development; therefore, development is limited during cool seasons or at high elevations. Development is rapid above 28° C, slows considerably below 21° C, and is extended beyond 30 days at temperatures less than 17° C.[11]

Altitudinal, seasonal, and annual patterns of transmission

The intensity and seasonal patterns in avian malaria transmission vary dramatically among low, mid, and high elevations in Hawaiian rainforests.[20,73,97] Key patterns in the Hawaiian malaria–forest bird system are high malaria transmission in low-elevation forests with minor seasonal or annual variation in infection; episodic transmission in mid-elevation forests with site-to-site, seasonal, and annual variation; and disease refugia in high-elevation forests with slight risk of infection only during summer. These transmission patterns are driven primarily by climatic effects on the parasite extrinsic incubation period, mosquito population dynamics across an altitudinal gradient, and landscape variation in the type and abundance of larval habitat. A key factor in determining malaria transmission is the size of the vector population, which is largely dependent on temperature, rainfall, and

availability of larval habitat. In low-elevation forests, climate is consistently favorable for mosquito and parasite, and larval habitat is sufficient to produce a high abundance of infectious mosquitoes, resulting in an absence of susceptible native species and a high prevalence of chronic infections in the native birds that remain. Under these conditions, susceptible juvenile birds are exposed to infection soon after hatch and adult birds represent the previous years' cohort of malaria survivors.

In midelevation forests, abundance of infectious mosquitoes is lower due to a decrease of larval habitat and cooler temperatures, and here seasonal patterns in malaria transmission are especially evident.[20,73,97] At midelevation, the number of susceptible juvenile birds build up until malaria transmission begins during the late summer and early fall, which coincides with peak mosquito abundance. Transmission continues through the fall and early winter, when temperature and rainfall remain favorable to mosquito survival and parasite development. In temperate regions, seasonal transmission starts in late spring and coincides with emergent populations of mosquitoes and recrudescing infections of immune-suppressed breeding birds.[54] This spring relapse in erythrocytic parasites bridges the gap in seasonal transmission. Transmission in Hawaii, however, occurs well after the breeding season because chronically infected native birds serve as a year-round reservoir of disease. In years or areas with lower transmission, some susceptible juveniles escape exposure and mature to become susceptible adults, allowing for a build-up of susceptibles across years.

At high elevation ($>$1,700 m asl), larval mosquito habitat is typically scarce, and cooler temperatures restrict seasonal abundance of mosquitoes and slow parasite development. These elevations provide disease-free refugia, with only a brief risk of infection during summer. Factors driving year-to-year and site-to-site variation in intensity are less evident.

Annual variation in malaria infection in birds occurs primarily in midelevation forests, likely caused by the abundance of susceptible birds and annual shifts in weather patterns driving vector abundance. These annual disease patterns can vary from full-blown epizootics that infect nearly all of the susceptible population to lower intensity events that infect approximately 50% of the susceptible population.[10,73] This suggests that the severity of epizootics may be related to the build-up of susceptible adult birds, which are dramatically affected when annual rainfall and associated mosquito populations return to normal levels that cause high transmission. Susceptible birds from transmission-free elevations may also descend to a transmission zone while tracking the nectar of ohia lehua (*Metrosideros polymorpha*), the dominate flowering tree on the landscape. Hart *et al.*,[118] however, found little evidence to support this ecological trap hypothesis. Other factors that could produce annual variability in the severity of epizootics include the cycling of malarial variants with different pathogenicity[97] and concomitant avian pox infection.[73,119]

Management and future threats

Control of vectors

Because the parasite depends on vectors for development and transmission, mosquitoes provide a crucial link between infected and susceptible birds. However, many characteristics of the malaria system in Hawaii make vector control challenging. A highly competent vector, favorable climate, lifelong reservoir hosts, and a steady source of susceptible birds all make transmission of avian malaria so efficient that relatively few vectors are required to drive transmission. Therefore, to significantly reduce transmission, control of the vector has to be near complete and applied at large spatial scales across a landscape of natural, agricultural, and residential areas to target relatively small, unevenly dispersed vector populations.[90] General strategies to control mosquito-borne disease have focused on reducing the longevity or abundance of vectors.[90] One of the most effective ways to reduce mosquito abundance is removal of larval habitat. In some forests, significant vector reduction may require a combination of strategies including pig removal, destruction of larval mosquito habitat, and biological control. Other methods, including chemical control of larvae and adults, biological control using predators or microbial pathogens, genetic modification strategies, and sterile males, have also been successfully used to control some vector-borne diseases.[90] Many of these approaches, however, would be unacceptable in Hawaiian forests because of nontarget effects to endemic invertebrates or impractical because of the extreme heterogeneity of island topography.

An important potential opportunity for controlling larval habitat lies in the residential and agricultural communities that encroach on natural areas. Eliminating or controlling artificial larval mosquito habitat in these areas requires considerable cooperation and vigilance from the public, which may require incentives and community involvement.[90] Reduction of tree fern cavities by elimination of feral pigs is also feasible but is expensive and controversial because feral pigs are considered a valuable game species.

Computer modeling of the Hawaiian malaria system indicates that larval habitat reduction in forested areas could provide effective mosquito control.[20,99] In midelevation forests with abundant larval habitat, substantial and sustained reductions of mosquitoes could reduce disease transmission and ultimately increase native bird populations. In midelevation forests with a low abundance of larval habitat and high-elevation forests, mosquitoes and disease transmission are already limited. Thus, further reduction in larval habitat may have minimal impact on native bird populations. In contrast, low-elevation forests typically have ample larval habitat and temperatures favorable for rapid development of larvae, producing a high and continuous abundance of adult mosquitoes. In these low-elevation forests, nearly complete removal of larval habitat may be required to substantially reduce mosquito abundance and subsequently increase native bird populations. Overall, these modeling results suggest that control actions that reduce mosquito larval habitat will be most beneficial to bird populations in midelevation forests where larval habitat is abundant. Unfortunately, these sites may require a permanent reduction of nearly all (>80%) of the larval habitat to produce a significant improvement in native Hawaiian bird abundance. Model simulations indicate that control efforts that substantially reduce larval habitat without reaching critical thresholds may not substantially reduce disease transmission or increase bird populations.

Climate change

Climate change, and global warming in particular, is expected to increase the occurrence, distribution, and intensity of vector-borne disease throughout the world.[116,120] Projected climate warming of 2–3 °C in Hawaii by 2100 will certainly increase the need for conservation measures to reduce malaria transmission but will also increase the challenge in developing successful strategies. Because malaria infection patterns in Hawaii are largely driven by the effects of temperature and rainfall on mosquito dynamics and parasite incubation rates, future climate change will have profound impacts on transmission patterns and undermine current conservation strategies to mitigate malaria transmission and mortality in Hawaiian forest birds.[10,11,20,121] Climate warming will mostly affect mid- to high-elevation bird communities where seasonal disease transmission will increase in both duration and intensity. Cooler, high-elevation forests, which are currently disease-free refugia, are likely to see seasonal transmission. In the long term, climate change poses a significant threat to the viability of Hawaiian forest bird populations because warming temperatures will facilitate upslope spread of mosquitoes and malaria.[121,122]

Recent analyses of climatic data from Hawaii indicate that mean temperatures have shown a significant upward trend over the past 80 years,[123] and recent studies suggest that climate change may already be increasing the risk of malaria infection in some upper elevation native bird communities.[109] Given current land use and a projected 2 °C temperature rise, disease-free, high-elevation forest habitat is predicted to decrease by 60–96% in some parts of Hawaii (Fig. 3).[121,122] Management of current conservation lands in disease-free, high-elevation habitat may not be sufficient for the long-term protection of many Hawaiian forest birds. Securing deforested lands adjacent and above the current refugia and restoring forest cover would provide additional benefit to birds by increasing carrying capacity.[90] On a small scale, disease-free islands of habitat might also be created in *kipuka* if mosquitoes can be eliminated or substantially reduced in these isolated areas.[90]

These imperiled high-elevation refugia exist in a narrow band of native forest that extends to tree line. If climatic changes occur rapidly, the upslope extension of suitable forest habitat could not occur before susceptible bird species are exposed to malaria.[121] These changes would effectively eliminate high-elevation refugia in Hawaii, likely driving remaining populations of threatened and endangered honeycreepers to extinction with severe declines in nonendangered species that exhibit high susceptibility to avian malaria.

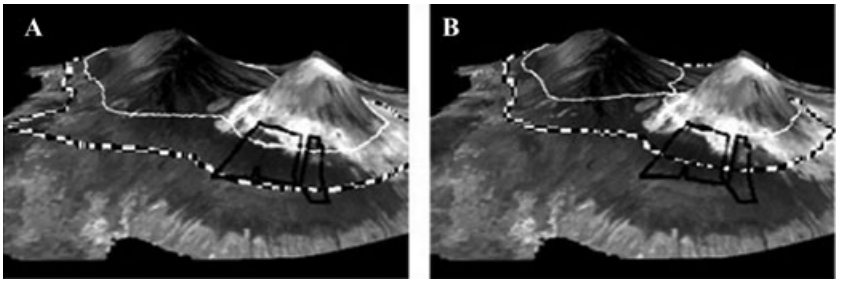

Figure 3. The current (A) and +2 °C projected (B) isotherms for the developmental threshold temperature for *P. relictum* (13 °C, solid while line) and the lower thermal boundary for seasonal transmission (17 °C, hatched line) at the Hakalau Forest National Wildlife Refuge island (black boundary) of Hawaii. The result of this warming scenario is the loss of 96% of disease-free refugia. Modified from Benning *et al.* (2002) with permission from the National Academy of Sciences.

Impacts from warming temperature, however, are only a part of the potential impact of climate change. Rainfall is also an important driver of mosquito population dynamics, and changes in seasonal or annual precipitation could alter transmission patterns across the landscape. The modest, predicted increase in precipitation during the dry season (summer) and decrease in the wet season (winter)[124] could lengthen seasonal availability of larval mosquito habitat and result in longer seasonal transmission.

Will climate change affect avian malaria dynamics and impact birds in continental disease systems? A recent analysis of global avian malaria prevalence and temperature anomalies over the last several decades suggests an increase in avian *Plasmodium* prevalence in Europe and Africa where significant increases in mean temperature have occurred.[125] Though exact mechanisms were not identified, geographical and seasonal expansion in vector populations and changes in parasite extrinsic incubation rate may increase prevalence. In temperate regions, where new infections depend on a spring relapse, higher temperatures may increase transmission by changing the phenology of vectors.[126,127] Climate change is also suspected as a driving factor in the southward expansion of *Cx. quinquefasciatus* in New Zealand.[62]

Protection/treatment of birds

Vaccines and antimicrobial agents have been successfully used to protect humans and domestic animals from infectious disease. Most wildlife vaccines have targeted mammalian reservoirs of zoonotic disease, but vaccines against protozoan pathogens have been difficult to develop. Although birds were some of the first experimental models for development of vaccines against *Plasmodium*, immunizing wild bird populations presents a significant challenge. A variety of experimental vaccines such as ultraviolet light-inactivated; formalin inactive and irradiated sporozoites, merozoites, and gametes; and synthetic vaccines based on parasite surface molecules have been used.[44] DNA vaccines based on the circumsporozoite protein of *P. gallinaceum* and *P. relictum* have been demonstrated to provide protection against *P. relictum* in penguins[128] and canaries,[129] but immunity was short lived and birds were susceptible to infection one year later.[12] Similarly, disease treatment by chemotherapy is typically limited to unique settings such as captive or closely managed flocks. Common antimalarial agents include chloroquine phosphate, primaquine phosphate, pyramethamine–sulfadoxine combinations, mefloquine, and atovaquone/proguanil combinations (Malarone™, GlaxoSmithKline, Research Triangle Park, NC), which have been used in treating canaries, other passerines, penguins, and raptors with avian malaria.[130,131] In general, the effectiveness of antimalarial agents in the treatment of wild birds is limited by the difficulties of delivery and potential development of drug-resistant parasites.[90]

Genetic evolution of resistance/tolerance

On the island of Hawaii, emergent populations of Hawaii amakihi have been found in low-elevation forests.[7,8] In spite of malaria prevalence ranging from 55% to 83%, these populations are expanding in range and densities that exceed those of Hawaii amakihi at elevations above 1500 m asl. Unlike the apparently *Plasmodium*-refractory Oahu amakihi,[110] the lowland Hawaii amakihi appear more tolerant to the pathologic effects of malaria. These

birds have unique nuclear and mitochondrial haplotypes not found in amakihi from high elevation, suggesting their recent resurgence originated from pockets of surviving individuals with some natural disease tolerance, rather than recolonization of the lowlands by high-elevation birds.[9] Low-elevation Hawaii amakihi have also been found on Maui and Molokai where malaria prevalence exceeds 75%, suggesting that selection for disease resistance or tolerance has also occurred on other islands.[89]

Interaction with avian pox virus

The date when avian pox virus was introduced to the Hawaiian Islands remains unknown, but the disease was readily apparent by the late 19th century.[10,132] At that time, it was assumed that domestic poultry were the most likely route for virus introduction to Hawaii. Molecular analysis of isolates circulating in native forest birds, however, has determined that Hawaiian forest bird pox is distinct from fowl pox, has a higher genetic diversity than expected from a single introduction,[133] and is most similar to canarypox.[119] Although pox may be primarily transmitted by *Culex* mosquitoes, we know relatively little about its epizootiology and pathogenesis in Hawaiian forest birds.[134] Other than limited experimental studies,[6,119] information about the potential impacts of pox virus on Hawaiian forest bird populations is based on observations of pox-like lesions on captured wild birds.[10] VanderWerf[135] reported declines in some breeding cohorts of Hawaii elepaio (*Chasiempis sandwichensis*) that were correlated with the occurrence of pox epizootics. In addition, preliminary observations of Oahu elepaio (*C. ibidis*) suggest up to 40% annual mortality of birds with active lesions, while birds with mild infections involving only one or more toes frequently recover.[136] Overall, the prevalence of pox-like lesions in Hawaiian forest birds is substantially less than chronic malaria infections,[73,108,134] and birds with pox-like lesions had concurrent malarial infections more frequently than expected by chance.[7,108] However, we know little about whether this apparent interaction is caused by simultaneous transmission of the pathogens; differential mortality among pox, malaria, or pox-malaria infected birds; or results because older birds are more likely to have both chronic malaria and pox infections. Alternatively, concurrent infections may represent relapses of chronic malaria brought on by immune suppression from pox virus.[119] Whatever the etiology, the high frequency of concurrent pox and malaria infections makes it difficult to separate the demographic impacts of either agent alone. To further confound the issue, at least two variants of avian pox virus, with marked differences in virulence, circulate among passerines in the Hawaiian Islands.[119] How these variants interact with malaria may provide additional insight into the nature and drivers of malarial epizootics in Hawaiian birds. Avian pox virus has been present on the Galapagos Archipelago for over a century, and notable outbreaks and mortality have occurred among Galapagos mockingbirds (*Nesomimus parvulus parvulus*).[137–139] With the recent detection of *Plasmodium* in Galapagos penguins, similar interactions between pathogens may greatly increase the impact of avian disease in the Galapagos Archipelago.[77]

Conclusions, future concerns, and conservation recommendations

With the advent of PCR diagnostics, research in avian malaria has grown exponentially in the last 15 years, but it is unclear if these studies have revealed the dynamics of a rapidly emergent disease on the global scale or merely a leap in our understanding of the ecology of this disease. Certainly the evolving tolerance of once susceptible populations of Hawaii amakihi is a clear indication of the potential dynamics of bird–*Plasmodium* relationships. At the same time, some long-held preconceptions regarding the benign nature of evolutionarily old, host–parasite relationships are slowing giving way as larger population and modeling studies closely examine the survival and reproductive fitness in infected individuals. The growing impact of anthropogenic environmental change has also been documented at both the local and global scales. The effects of climate change, especially increased temperature, may be foretold by the prevalence of avian malaria across altitudinal gradients in the Hawaiian Islands and across the latitudinal gradients of continents. Perhaps the most alarming trend is the establishment and apparent spread of avian malaria in New Zealand and the Galapagos Archipelago, where globalization, deforestation, agriculture, and human encroachment have fostered an emergence of avian disease. Along with that of the Hawaiian Islands, the avifauna of these islands represents some of the most unique adaptive radiations of species in the world.

Whether the Hawaiian avifauna continue to adapt or succumb to avian malaria, globalization and regulatory limitations likely ensure future pathogen (i.e., West Nile virus)[140,141] and vector introductions. International transportation and commerce regulations that do not include improved disinsection and quarantine measures may lead to the further spread of vectors and avian pathogens. Considering the impacts of climate change, planning mitigation now for the loss of disease-free habitat on oceanic islands and fragmented continental landscapes may be necessary to save vulnerable species. Effective control of *Cx. quinquefasciatus*, the cosmopolitan mosquito vector of *P. relictum*, and identification and control of unknown vectors may be the key to disease management. Where traditional approaches to vector control would be difficult to apply across vast natural areas, innovative techniques will be needed to control avian malaria transmission if we want to successfully preserve many of the world's unique island avifaunas for future generations.

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Conflicts of interest

The authors declare no conflicts of interest.

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