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EFFECTS OF CHRONIC AVIAN MALARIA (*PLASMODIUM RELICTUM*) INFECTION ON REPRODUCTIVE SUCCESS OF HAWAII AMAKIHI (*HEMIGNATHUS VIRENS*)

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Abstract.—We studied the effects of chronic avian malaria (*Plasmodium relictum*) infections on the reproductive success of a native Hawaiian honeycreeper, Hawaii Amakihi (*Hemignathus virens*). Chronic malaria infections in male and female parents did not significantly reduce reproductive success as measured by clutch size, hatching success, fledging mass, number of nestlings fledged, nesting success (daily survival rate), and minimum fledgling survival. In fact, nesting success of pairs with chronically infected males was significantly higher than those with uninfected males (76% vs. 38%), and offspring that had at least one parent that had survived the acute phase of malaria infection had a significantly greater chance of being resighted the following year (25% vs. 10%). The reproduction and survival of infected birds were sufficient for a per-capita population growth rate >1, which suggests that chronically infected Hawaii Amakihi could support a growing population. *Received 9 March 2005, accepted 30 September 2005.*

Key words: disease, Drepanidini, evolution, fitness, fledgling survival, Hawaii Amakihi, *Hemignathus virens*, *Plasmodium relictum*.

Efectos de las Infecciones Crónicas de Malaria Aviaria (*Plasmodium relictum*) en el Éxito Reproductivo de *Hemignathus virens*

Resumen.—Estudiamos los efectos de las infecciones crónicas de malaria aviaria (*Plasmodium relictum*) en el éxito reproductivo de un mielero nativo de Hawai (*Hemignathus virens*). Las infecciones crónicas de malaria en los padres de ambos sexos no redujeron de modo significativo el éxito reproductivo medido como el tamaño de la nidada, el éxito de eclosión, el peso de los volantones, el número de pichones que dejaron el nido, el éxito de nidificación (tasa diaria de supervivencia) ni la supervivencia mínima de los volantones. De hecho, el éxito de nidificación de las parejas con machos infectados de modo crónico fue significativamente mayor que el de aquellos con machos no infectados (76% vs. 38%), y los pichones que tuvieron al menos un padre que había sobrevivido la fase pico de la infección de

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malaria presentaron una probabilidad mayor de ser vistos nuevamente el año próximo (25% vs. 10%). La reproducción y la supervivencia de las aves infectadas fueron suficientes como para mantener una tasa de crecimiento poblacional per cápita >1, lo que sugiere que los individuos con infecciones crónicas de *H. virens* pueden mantener una población creciente.

Disease can play an important role in regulating population size (May 1988, Scott 1988, Hudson et al. 1998, Hochachka and Dhondt 2000). High-density populations may be regulated by density-dependent disease effects (May and Anderson 1978, Hudson et al. 1998, Hochachka and Dhondt 2000), and low-density populations may be pushed toward extinction by disease-induced mortality and reproductive failure (May 1986, Jenkins et al. 1989). Over the past two decades, disease has gained recognition as an important aspect in the conservation of wild and captive populations (May 1988, Scott 1988, Cooper 1989, Scott et al. 2001) and as a strong agent of selection (Hamilton and Zuk 1982, May and Anderson 1983).

Numerous studies have examined the effects of chronic disease and parasites on the reproduction and survival of birds and other vertebrates in the field (Schall 1983; Møller et al. 1989; Møller 1990; Korpimäki et al. 1993, 1995; Dale et al. 1996; Dufva 1996; Hudson et al. 1998; Dawson and Bortolotti 2000, 2001). All these studies found a significant negative effect of parasite infection on at least one fitness-related trait that included survival and several aspects of reproduction, including clutch size, time of first breeding, and nesting success. However, several of those studies examined multiple parasites, and only found significant effects for certain taxa. Notably, only Schall's (1983) work on lizards demonstrated significant effects of malaria (*Plasmodium* sp.) on fitness-related traits of animal populations in the field.

In contrast to the species studied in previous research on disease and demography, native Hawaiian birds have a very short evolutionary history with *Plasmodium* (van Riper et al. 1986, Bennett et al. 1988). As a result of the naiveté of Hawaiian birds, we might expect them to incur greater costs from malaria infections than continental birds in terms of reproduction and survival (Atkinson and van Riper III 1991). In support of this, avian malaria (*Plasmodium relictum*) has been shown to cause substantial mortality (65–100%) in several species of Hawaiian honeycreepers in a laboratory setting (Warner 1968; van Riper et al. 1986; Atkinson et al. 1995, 2000; Yorinks and Atkinson 2000). This and other research has provided substantial evidence that the presence of avian malaria and its vector, the southern house mosquito (*Culex quinquefasciatus*), limit the distribution of many native Hawaiian birds (Warner 1968, Scott et al. 1986, van Riper et al. 1986). However, it is unknown whether the effects of malaria on populations of Hawaiian birds are limited to mortality caused by short-term or acute infections or also include reduced reproduction and survival from chronic infections. A previous study found no differences in nesting success of Apapane (*Himatione sanguinea*) across an elevational gradient of malaria transmission (Nielson 2001). However, that study did not explicitly test for the effects of individual chronic malaria infections independently of differences between sites that might be tied to resource availability, predators, and other causes. Chronic infections might lead to reduced reproductive success if infection is accompanied by increased allocation to immune function or by pathology that impairs parents' ability to acquire and defend a suitable nesting site, build adequate nests, feed their young, or defend nests against predators (Hakkarainen et al. 1998, Ardia 2005). By contrast, it is possible that acute malaria infections could act as a selective agent and remove less-fit individuals from the population. If a trait simultaneously makes an individual a better parent and more likely to survive the acute phase of malaria infection, chronically infected individuals could have higher nesting success than uninfected individuals.

The status of populations and the evolution of disease resistance depend strongly on whether birds that are exposed to malaria are able to survive and reproduce. If infections decrease reproductive performance or survival, such that the infected population has a negative population growth rate, the evolution of resistance to

malaria will not occur. Evolution also requires that resistance is a heritable trait. Resistance to malaria and other blood parasites has been linked to a number of genetically heritable traits in humans and other vertebrates (Rosenstreich 1980, Swardson et al. 1997, Miller 1999, Zekarias et al. 2002), but has not yet been demonstrated in a wild bird. If resistance to malaria has a genetic basis and is heritable, we would expect the survival of offspring of infected birds to be higher than the survival of offspring of uninfected birds, because the former are more likely to possess the same resistance genes that allowed parents to survive acute malaria infections (Cichoń and Dubiec 2005). However, the advantage conferred by possessing resistant genes is only as strong as the infection rate, and a low yearly infection rate could be countered by maternal or environmental effects that might favor survival of offspring of uninfected parents. Alternatively, nongenetic maternal or environmental effects could also lead to increased survival of offspring of infected parents if these parents are able to provide greater resources for their offspring (Saino et al. 2003, Hargitai et al. 2005).

Here, we present data on the effect of chronic malaria infections on the reproduction and survival of offspring of a native Hawaiian bird, the Hawaii Amakihi (*Hemignathus virens*; hereafter "amakihi"). This species is one of the few native Hawaiian passerines that exists in lowland areas where mosquitoes and malaria are now prevalent (Woodworth et al. 2005). We compare the reproductive performance of individuals with chronic malaria infections with those that are uninfected to test our hypothesis that chronic infections would lead to reduced reproductive success. We also present data on the minimum survival of offspring of infected and uninfected parents. We predicted, *a priori*, that offspring of infected parents would have higher survival than that of uninfected parents, because we believed that offspring would inherit a genetic basis for resistance and the selective pressure of malaria infection would outweigh other maternal and environmental effects.

METHODS

Study species.—Amakihi are a member of the tribe Drepanidini, a radiation of >70 taxa from a single finch-like ancestor (James and Olson 1991). Their diet consists of a mixture of insects and nectar, primarily from the flowers of ʻohiʻa (*Metrosideros polymorpha*) and mamane (*Sophora chrysophylla*) trees (Baldwin 1953). At the study site, they nest primarily in ʻohiʻa trees, but also use the introduced firetree (*Morella* [previously *Myrica*] *faya*), and sometimes ornamentally planted pine (*Pinus* spp.) trees (A. M. Kilpatrick unpubl. data). The female builds the nest, incubates the eggs, and broods and feeds the young, whereas the male feeds the nestlings and sometimes feeds the female during the incubation and nestling stages (van Riper III 1987, Lindsey et al. 1998, A. M. Kilpatrick pers. obs.). Although this species exhibits exclusive type-A territoriality in some locations (van Riper III 1987), at the study site feeding areas were broadly overlapping and nests often occurred in adjacent trees within 5 m of each other. Few aggressive displays were observed between males of neighboring nests. Nest defense behavior was rarely observed, except during nest checks, even when Common Mynas (*Acridotheres tristis*), believed to be a nest predator in Hawaii, came within a few meters of the nest.

Site description.—The study was performed in Hawaiʻi Volcanoes National Park, in ~1 km^2 area surrounding the former Ainahou Ranch buildings (19°21′N, 155°13′W; elevation, 970 m). From 1990 to 2000, the site received an average annual rainfall of 1,800 mm, but approximately two-thirds of this amount fell each year during the three years of the study, 2001–2003 (Hawaiʻi Volcanoes National Park unpubl. data). During the breeding season (February–June), the daily minimum and maximum temperatures average 10°C and 23°C, respectively. Ainahou Ranch became part of the national park in 1972 after being used for cattle grazing earlier in the 20th century. As a result of grazing and planting of exotic grasses, the vegetation at the field site is an open forest, with some large (>20 m tall) ʻohiʻa trees; smaller (10–15 m) mamane, firetree, olive (*Olea europaea*), and sandalwood (*Santalum* spp.) trees; several native shrubs, including pukiawe (*Styphelia tameiameae*), ʻulei (*Osteomeles anthyllidifolia*), and ʻaʻaliʻi (*Dodonaea viscosa*); and a grassy understory composed of invasive weeds, such as molasses grass (*Melinus minutiflora*), bushy beardgrass (*Andropogon glomeratus*), and kikuyu grass (*Pennisetum clandestinum*).

Blood collection and testing.—Amakihi were caught using mist nets throughout the year from February 2001 to February 2003. Birds were weighed and banded with a federal band and different combinations of three plastic color bands to provide a unique combination for each bird. Upon capture, a 0.1-mL blood sample was taken by jugular venipuncture with a heparinized 28-gauge insulin syringe for malarial diagnostics. A thin blood smear was made immediately, air dried, and fixed with methanol. Plasma from each bird was tested for antibodies to *P. relictum* using an indirect enzyme-linked immunosorbent assay (ELISA) (as described in Graczyk et al. 1993), and red blood cells were frozen for genetic studies. Absorbance values were expressed as a percentage of ELISA value (%EV) of positive and negative Pekin duckling plasma controls that were run on each plate. The %EV was calculated as: (mean absorbance of triplicate samples – the mean absorbance of triplicate negative controls)/(mean absorbance of triplicate positive controls – mean absorbance of triplicate negative controls) × 100. We used a cutoff %EV of 25 to classify birds as antibody positive or negative. Birds testing within a range of 5 points above or below a %EV of 25 were retested by immunoblotting (as described in Atkinson et al. 1995, 2001) to verify ELISA results.

Blood smears were stained with 6% phosphate-buffered Giemsa, pH 7.0, for 1 h, rinsed with tap water, air dried, and examined by microscopy to diagnose intense acute infections (<30 days old) when antibody titers were too low to be detected by ELISA or immunoblotting. One hundred 500× fields (~30,000 erythrocytes) were examined on each slide. On the basis of parasitemia curves and serological analyses (Atkinson et al. 2000, 2001), we identified individuals that were positive for antibodies to *P. relictum* by either ELISA or immunoblotting and that had parasitemias that were <5% of circulating erythrocytes as chronically infected birds. Two females that were negative for antimalarial antibodies were smear positive and were classified as having acute infections (i.e., <30 days old; Atkinson et al. 2000) and excluded from the analysis.

The infection status of some birds at the time of nesting could not always be determined. Birds that were determined to be chronically infected in blood samples taken before a nesting attempt were considered to be infected, because previous work has shown that amakihis exposed to *P. relictum* have both circulating parasites and antibodies to *P. relictum* for at least four years after the initial infection (Atkinson et al. 2001, Jarvi et al. 2002). However, birds that tested negative (by smear, ELISA, and immunoblotting) for malaria in blood samples taken before a nesting attempt could have been exposed in the subsequent months. Of the 49 females scored as uninfected, 13 were bled 8–12 months before the nesting attempt, 4 were bled 1–4 months before the nesting attempt, and 32 were bled after the nesting attempt (and still tested negative). Of the 50 males scored as uninfected, 10 were bled in the previous 9–12 months, 4 were bled 1–4 months before nesting, and 36 were bled afterward.

In addition, we were unable to detect evidence of infection in the first eight days after infection, when parasites were undergoing initial rounds of multiplication in fixed tissues of the bird and when numbers of parasites in the peripheral circulation were extremely low. As a result, it is possible that some birds characterized as uninfected were infected between being bled and the nesting attempt(s) that we observed. However, we believe that few of our birds were incorrectly classified as uninfected, because the chance of being infected each year was relatively low at our site. Recaptures of birds over 244 intervals averaging 6.6 months resulted in only 13 (5.3%) seroconversions, with a monthly infection rate of 0.0084 (95% CI: 0.0046–0.014). It should be noted that this is a minimum estimate of the true transmission, because some fraction of the birds that are infected with malaria likely perish from the acute infection (Atkinson et al. 2000). However, excluding the birds that were sampled more than four months before their nesting attempt did not change the qualitative results of the analysis (see below).

Reproduction.—In 2001 and 2002, we searched for nests of amakihi by observing the behavior of adult birds. Most nests were found in the nest-building (60%) or egg-laying and incubation (20%) stage. When possible, we determined clutch size, hatching success (number of eggs laid that hatched divided by the number of eggs incubated to term), number of nestlings fledged, fledging mass, and nesting success (nests fledging ≥1 young) for each nest. For a subset of the nests, we weighed nestlings (on day 13 after hatching) and placed one federal and three

plastic color bands on their legs for future identification. To avoid causing the nestlings to leave the nest prematurely, we inspected nests on day 15 (rather than day 17, when they normally fledge; van Riper III 1987) and assumed that all young present at that time fledged. We investigated possible determinants of nesting success including parental (male and female) mass and four nest-site characteristics: nest height, distance to trunk, distance to branch tip, and number of branches in nest tree.

Fledgling survival.—In spring and summer 2002, we individually color-banded 71 nestlings (of 30 different pairs) that successfully fledged. In January 2003, we attempted to resight these offspring by searching the entire study area (~1 km^2) and a 500-m-radius circle around each nest where the nestlings were banded. We tested for a difference in the probability of resighting fledglings of pairs that either had at least one parent infected or both parents uninfected. If either parent was chronically infected with malaria (and thus had survived the acute infection), we assumed that the offspring would stand a higher chance of receiving genes for resistance. We used the pair, rather than the fledgling, as the sample unit, because this gave equal weighting to each pair and reduced the possibility that one pair's offspring would bias the results. Because we only performed a single resighting effort, we were unable to estimate the probability of sighting individuals given that they were alive. As a result, our resighting fractions represent minimum survival estimates.

Data analyses.—For several parents, we found multiple nests, either from the same year or in the two years of the study. We averaged the reproductive measures for these nests, so that the unit of measure was the reproductive performance of a set of parents, rather than a single nest. In addition, there were five cases in which a male had a nest with multiple females, either in one year (i.e., simultaneously) or in two different years. There were also three cases in which a female had nests with different male mates in 2002 than in 2001. In the statistical analyses (analysis of variance [ANOVA]), we used a covariance matrix that accommodated the nonindependence of pairs with the same male or female parent. Reproductive measures were compared with two-factor ANOVAs, with the malaria infection status of the mother and father as the two factors (the interaction term was nonsignificant in all analyses [all $P > 0.3$] and removed). Only pairs where the status of both parents was known were included in these analyses.

Nesting success was analyzed using the Mayfield method (Mayfield 1975) to calculate a daily survival rate for nests. Because we had multiple nests for some pairs, the total days of observation were sometimes greater than one nesting cycle (31 days: 2 days for egg laying, 14 days for incubation, and 15 days for the nestling period; van Riper III 1987). Thus, there was the potential to bias the analysis if we included the full number of days of observation. As a result, we used 31 days of observation for pairs when we had >31 days of observation and an appropriately scaled number of nest failures. We used the program CONTRAST (Hines and Sauer 1989) to test for differences in daily survival rates using the infection status of the male or female parent as a factor. Because we used essentially the same data for two statistical tests (male and female infection status separately), a Bonferroni correction was used to interpret the results.

Finally, we performed a power analysis to determine whether the lack of significant differences seen in the comparisons was attributable to insufficient power. We estimated the effect sizes for each of the reproductive measures that we would be able to detect, assuming a power of 0.9 and a type I error rate of 0.05 (Table 1).

RESULTS

Reproduction.—Over the two years, we found a total of 119 nests (14 in 2001 and 105 in 2002) belonging to 80 pairs. The infection status of both members of the pair was known for 60 of those pairs, and analyses were based on data from those pairs. In 38 pairs, both parents were banded and uninfected; in 9 pairs, the male was infected and the female uninfected; in 10 pairs, the female was infected and the male uninfected; and in 3 pairs, both parents were infected. Of the remaining 20 pairs, in 5 the female was uninfected and the male was unbanded; in 2 pairs, the female was infected and the male unbanded; in 9 pairs, the male was infected and the female unbanded; and in 4 pairs the male was uninfected and the female was unbanded. Of the 64 different banded females in these pairs, 15 were chronically infected with malaria and 49 were

TABLE 1. Reproductive measures (mean ± SE) of Hawaii Amakihi pairs categorized according to male or female infection status. Values in parentheses are number of pairs for that cell; these are larger than those used in the two-factor ANOVAs, because they include pairs in which the status of one member was unknown.

Malaria status	Clutch size (eggs)	Hatching success (%)	Fledglings per successful nest	Fledglings per nest	Fledging mass (g)
Uninfected females	2.68 ± 0.083 (44)	0.80 ± 0.040 (39)	2.32 ± 0.108 (33)	1.30 ± 0.167 (49)	12.68 ± 0.213 (21)
Infected females	2.54 ± 0.226 (12)	0.74 ± 0.102 (6)	2.50 ± 0.224 (6)	1.00 ± 0.350 (14)	13.13 ± 0.417 (5)
Comparison	$F = 0.97$, df = 1 and 46, $P = 0.33$	$F = 0.04$, df = 1 and 33, $P = 0.85$ [a]	$F = 0.30$, df = 1 and 29, $P = 0.59$	$F = 0.84$, df = 1 and 49, $P = 0.37$	$F = 0.01$, df = 1 and 16, $P = 0.92$ [b]
Difference for power = 0.9	0.18	0.13	0.18	0.33	0.42
Uninfected males	2.64 ± 0.102 (45)	0.80 ± 0.042 (33)	2.34 ± 0.11 (28)	1.07 ± 0.16 (50)	12.75 ± 0.21 (19)
Infected males	2.69 ± 0.133 (13)	0.82 ± 0.069 (12)	2.31 ± 0.21 (13)	1.75 ± 0.29 (16)	13.14 ± 0.41 (6)
Comparison	$F = 0.94$, df = 1 and 46, $P = 0.34$	$F = 0.88$, df = 1 and 33, $P = 0.35$ [a]	$F = 0.03$, df = 1 and 29, $P = 0.87$	$F = 5.89$, df = 1 and 49, $P = 0.019$	$F = 0.05$, df = 1 and 16, $P = 0.83$ [b]
Difference for power = 0.9	0.17	0.17	0.22	0.33	0.42

[a] Hatching success was arcsine-square root transformed before analysis.

[b] The ANOVA for fledging mass also included the number of fledglings per nest in the model. This effect was nonsignificant ($F = 2.56$, df = 2 and 16, $P = 0.11$), but significant in a one-way ANOVA ($F = 4.23$, df = 2 and 29, $P = 0.025$) that included additional data that were excluded in the analysis with both parents' infection status.

uninfected. Of the 66 different banded males, 16 were chronically infected with malaria and 50 were uninfected. There was no evidence that pairs mated assortatively with respect to infection status (female: $\chi^2 = 0.07$, df = 1, $P = 0.55$; male: $\chi^2 = 0.36$, df = 1, $P = 0.79$).

For all comparisons of reproductive success, the effect sizes that could be detected with a power of 0.9 were relatively small (Table 1). Nonetheless, there were no significant effects of female infection status on clutch size, hatching success, number of nestlings fledged per successful nest, number of fledglings including all nests, daily nest survival, and fledging mass (all $P > 0.15$; Tables 1 and 2). Fledging mass varied significantly with number of fledglings (fledging mass: one fledgling: 12.75 ± 1.98 g; two fledglings: 13.21 ± 0.99 g; three fledglings: 12.261 ± 0.54 g; ANOVA: $F = 4.23$, df = 2 and 29, $P = 0.025$). However, fledging mass was still unrelated to parental infection status after taking the number of fledglings into account (both $P > 0.7$). There was also no significant effect of male infection status on clutch size, hatching success, number of nestlings fledged per successful nest, or fledging mass (all $P > 0.3$; Table 1). However, pairs with infected males had higher daily survival rates of their nests ($P = 0.002$; Table 2), and this translated into higher numbers of nestlings fledged ($P = 0.019$; Table 1).

Excluding the parents that were scored as uninfected and bled more than four months before the nesting attempt did not change the significance of the results; daily nest survival was still higher for infected males ($\chi^2 = 6.027$, df = 1, $P = 0.014$; significant after Bonferroni correction at $P = 0.05$).

There was no difference in mass of infected (13.16 ± 0.19 g) and uninfected parents (13.09 ± 0.10 g; two-factor ANOVA, sex: $F = 1.74$, df = 2 and 124, $P = 0.19$; infection status: $F = 0.10$, df = 2 and 124, $P = 0.75$; the interaction term was nonsignificant [$P = 0.46$] and removed). Neither male nor female mass was correlated with nesting success using a repeated-measures logistic regression that accounted for multiple nests

TABLE 2. Comparison of daily nest survival and nesting success analyzed once on the basis of the father's status and once on the basis of the mother's status. Nesting success is the overall survival rate of nests over one nesting cycle, assuming a duration of 31 days.

Parental status	Daily survival rate (DSR) (mean ± SE)	(days observed)	Nesting success	Comparison of DSR
Male infected	0.991 ± 0.0050	372	0.758	$\chi^2 = 9.64$
Male uninfected	0.970 ± 0.0048	1,287	0.384	$P = 0.002$ [a]
Female infected	0.959 ± 0.0042	370	0.272	$\chi^2 = 1.95$
Female uninfected	0.977 ± 0.0124	1,261	0.486	$P = 0.326$

[a] Significant at $P < 0.005$ with Bonferroni correction.

for single pairs (both $P > 0.2$). By contrast, nest height was positively correlated with nest success (logistic regression including nests found during nest-building or egg-laying stage only: $z = 1.92$, $P = 0.05$, $n = 57$). However, nest height of pairs with infected males (mean ± SD, 9.1 ± 4.9 m) was no different than that of pairs with uninfected males (10.4 ± 4.5 m; $F = 0.79$, df = 1 and 53, $P = 0.38$). Similarly, nest height was unrelated to female infection status (uninfected females: 9.9 ± 4.5 m; infected females: 10.1 ± 4.5 m; $F = 0.02$, df = 1 and 48, $P = 0.91$). Finally, nesting success was unrelated to distance to trunk, distance to branch tip, and number of branches (all $P > 0.15$).

Nestling survival.—We resighted 11 of 71 nestlings from 10 of 30 pairs, representing 6 of 24 offspring (25% minimum survival over 8 months) from 5 of 9 (56%) pairs that had at least one parent infected and 5 of 48 offspring (10.4% minimum survival over 8 months) from 5 of 21 (24%) pairs that did not have an infected parent. The fraction of broods that were resighted are significantly different ($\chi^2 = 2.857$, one-tailed, $P = 0.05$).

DISCUSSION

Our study did not find significant negative effects of chronic malaria infections on amakihi reproduction, despite adequate power to do so (Table 1). Surprisingly, we found higher nesting success and number of nestlings fledged of infected males compared with uninfected males. The observational nature of the study made it difficult to separate factors that lead to infection from those that affect reproductive success (Norris et al. 1994). However, it is unlikely that the reproductive effort of parents influenced their infection status, as has been found in several clutch-manipulation experiments (Norris et al. 1994, Oppliger et al. 1996, Deerenberg et al. 1997), because our study used antibody tests rather than blood smears to detect infections and because infected individuals were already infected before the nesting attempt we observed. As a result, the infection status of individuals was not influenced by the reproductive effort that we measured.

However, it is possible that some males have traits that simultaneously make them better parents and more likely to be infected (e.g., age; Dale et al. 1996). Unfortunately, we found it difficult to identify a mechanism for the higher nesting success of infected males. The main cause of nest failure at the site appeared to be predation; most nests failed during the nestling period, and we found only two cases in which nestlings were found dead in the nest and one of those followed an intense storm. The primary nest predators at the site are most likely 200- to 300-g rats (*Rattus* spp.), which makes it difficult to understand how a 13- to 14-g male parent could play a large role in altering predation rates. Nonetheless, we attempted to test the hypothesis that the selective pressure of mortality because of malaria resulted in infected males being a superior subset of the sampled population. However, we found no difference in the mass of infected and uninfected males, which is in agreement with a larger analysis of North American birds (Bennett et al. 1988). We also found no correlation between male mass and nesting success, which was contrary to other studies (Blomqvist et al. 1997, Sæther et al. 1997). Finally, we tested the hypothesis that infected males were associated with different nest-site selection, which in turn influenced nest survival, but male and female infection status were unrelated to nest height. We were unable to test

whether age played a role, because birds could be aged only in their first two years and only one of the male parents in our study was a second-year bird. As a result of these inconclusive results, we are unable to provide a mechanism for the higher nesting success of infected males and suggest that it could be attributable to an unmeasured factor, such as age. Nonetheless, our results suggest that chronic malaria infections do not have significant negative effects on amakihi reproduction.

Our nestling resighting data suggested that offspring of infected parents had higher survival than offspring of uninfected parents. However, the result was based on small sample sizes, and it is possible that the higher resighting fraction was attributable to lower dispersal of the fledglings from the study area, rather than differential survival. Because infected adult birds had lower survival than uninfected birds (Kilpatrick 2003), their offspring may have had less pressure from their parents to disperse. We did not find a difference in fledging mass between offspring of uninfected and infected parents, which suggests that maternal or environmental effects (Saino et al. 2003, Hargitai et al. 2005) did not result in nestlings of infected parents having a size advantage at fledging. As a result, the data are consistent with both the differential dispersal hypothesis and the hypothesis that offspring inherited genes for malaria resistance from their infected parents that led to increased survival.

Whether resistance to disease will evolve in a population is determined in part by the population growth rate of the resistant population (including chronically infected birds). If chronically infected individuals can survive and reproduce at a rate that can support a growing population and resistance to acute malaria infections is heritable, this suggests that the population has the capacity to evolve toward a completely resistant state. We used mark–recapture techniques to estimate the yearly survival of infected adult birds in this population to be 0.62, and enumeration to estimate the minimum survival of juvenile birds to be 0.35 (Kilpatrick 2003). Combining these survival data with the daily nest survival rate, the breeding season length, and number of nestlings per successful nest generates an estimate of the population growth rate of the infected population of 1.17, or a 17% growth rate per year. However, whether the Ainahou Ranch population will evolve toward a resistant state depends on the genetic basis of resistance, and the selective pressure exerted by malaria transmission (Kilpatrick 2003, 2006). Chronically infected adults had 17% lower yearly survival than uninfected birds over the two years of the present study (Kilpatrick 2003). As a result, if malaria transmission is low, resistant genes may be swamped by genes from uninfected susceptible birds.

Measuring the effect of disease on populations has value in both conservation and basic science. Many endangered species are affected by disease (e.g., May 1986, Jacobi and Atkinson 1995, Biggins and Kosoy 2001), and prioritizing management efforts depends on measuring the effect of disease in relation to other threats. In addition, studies of the effects of disease on populations, like this one, can provide information on which individuals to use for captive breeding or translocations. If chronically infected individuals can survive and reproduce sufficiently to support a growing population, in lieu of genetic markers for resistance in uninfected individuals, they represent the best hope in creating a population that is able to sustain high levels of disease transmission (though care must be taken not to introduce a new pathogen or strain of pathogen through translocations). The effect of diseases on populations is also important in understanding the processes that govern populations (Hudson et al. 1998, Hochachka and Dhondt 2000). Only comprehensive studies of the influences of disease on survival and reproduction can determine the importance of disease in relation to predation, food limitation, competition, and other factors that regulate populations.

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