Increasing avian pox prevalence varies by species, and with immune function, in Galápagos finches

Maxine Zylberberg^{a,*}, Kelly A. Lee^a, Kirk C. Klasing^a, Martin Wikelski^b

^a University of California, Davis, One Shields Avenue, Davis, CA 95616, USA

^b Max Planck Institute of Ornithology, Eberhard-Gwinner-Str., 82319 Seewiesen, Germany

A B S T R A C T

Avian pox virus (APV), a pathogen implicated as a major factor in avian declines and extinctions in Hawaii, was introduced to the Galápagos in the late 1890s. While APV is thought to have increased in prevalence in recent years, no study has carefully evaluated the threat this pathogen poses to the Galápagos avifauna. In this paper, we examine the course of the APV epidemic in seven species of Galápagos finch on Santa Cruz Island (*Geospiza fuliginosa*, *G. fortis*, *G. magnirostris*, *G. scandens*, *Camarhynchus parvulus*, *Cactospiza pallida*, and *Certhidea olivacea*). We describe temporal changes in the prevalence of the avian pox disease (AP) caused by APV and the proportion of individuals that have recovered from AP from 2000 to 2009. Then we examine species differences in susceptibility to AP and how this variation correlates with differences in innate immune function. We show that AP prevalence has increased dramatically from 2000 to 2009. However, this increase in prevalence varied by species; specifically, we found that prevalence increased rapidly in *G. fuliginosa*, *C. parvulus*, *G. scandens*, and *C. olivacea*, but not at all in *G. fortis*. Furthermore, innate immune function varies between years and species, and this variation correlates with increased prevalence and species variation in susceptibility to APV. To our knowledge, this is the first study to demonstrate significant interannual variation in innate immune function in wild birds, and to show that this immune variation correlates with susceptibility to an introduced disease.

Keywords:

Ecoimmunology
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1. Introduction

The spread of infectious pathogens to new ecosystems and host species is a threat to conservation and public health worldwide. Avian pox virus (APV), a pathogen implicated as a major factor in avian declines and extinctions in Hawaii, was introduced to the Galápagos in the 1890s (Parker et al., 2011; van Riper et al., 2002) (for a thorough examination of the natural history of avian pox, see van Riper and Forrester (2007)). APV, which causes the avian pox disease (AP) characterized by distinctive cutaneous lesions, can be transmitted directly between host individuals, environmentally, or mechanically by biting arthropods. While mosquitoes are often a major mode of transmission, the potential mosquito vectors in the Galápagos are restricted to areas of human habitation due to

their need for fresh water, and it remains unknown what the primary mode of APV transmission is in the Galápagos. Nonetheless, APV has spread through the Galápagos avifauna and, along with other introduced pathogens, may be driving population declines and range contractions (Parker et al., 2006; Wikelski et al., 2004). Furthermore, the proportion of Galápagos finches affected by AP (either currently infected or recovered) increased from 2000 to 2004 (Kleindorfer and Dudaniec, 2006). This indicates that APV has spread, but obscures whether it is the proportion of infected or recovered individuals that is increasing, scenarios pointing to opposite conclusions regarding population health.

Strength of innate (non-specific) immunity at an individual and group level helps to determine whether and how a novel disease spreads through a population by determining the susceptibility of individuals to a pathogen and, in turn, the transmission rate of the pathogen. Individuals exhibit intra-annual variation in immune function, linked to seasonal variation in environmental conditions, pathogen prevalence, and investment in events such as migration, molt, or reproduction (Martin et al., 2008; Nelson, 2004). Likewise, disease threats, environmental conditions, and physiological requirements vary between years and, therefore, can also be expected to underlie inter-annual variation in immune function. Galápagos finches exhibit striking phenotypic plasticity in life-history traits (Grant, 1986), providing a natural experiment

Abbreviations: AP, avian pox disease; APV, avian pox virus; SGF, small ground finch (*Geospiza fuliginosa*); MGF, medium ground finch (*Geospiza fortis*); LGF, large ground finch (*Geospiza magnirostris*); CF, cactus finch (*Geospiza scandens*); STF, small tree finch (*Camarhynchus parvulus*); WPF, woodpecker finches (*Cactospiza pallida*); WAF, warbler finches (*Certhidea olivacea*).

* Corresponding author. Address: 32 Dearborn Str., San Francisco, CA 94110, USA. Tel.: +1 530 219 7446; fax: +1 530 752 8391.

E-mail addresses: mzylberberg@ucdavis.edu (M. Zylberberg), kallee@ucdavis.edu (K.A. Lee), kcklasing@ucdavis.edu (K.C. Klasing), martin.wikelski@uni-konstanz.de (M. Wikelski).

for determining whether such plasticity can result in inter-annual variation in immune function.

In this paper, we examine the course of the AP epidemic in seven species of finch on Santa Cruz Island: small, medium, and large ground finches (*Geospiza fuliginosa*, SGF; *G. fortis*, MGF; *G. magnirostris*, LGF), cactus finches (*G. scandens*, CF), small tree finches (*Camarhynchus parvulus*, STF), woodpecker finches (*Cactospiza pallida*, WPF), and warbler finches (*Certhidea olivacea*, WAF). We model the change in the proportion of currently infected and recovered individuals from 2000 to 2009 to determine whether AP prevalence (the proportion of a population that is actively infected) and the proportion of recovered individuals in the population increased. We then evaluate whether AP appears to pose a serious mortality risk to Galápagos finches. If individuals are successfully recovering from infection then, as the proportion of infected individuals increases, the proportion of recovered individuals will also increase (and should increase more rapidly than the proportion of infected individuals as individuals remain infected for a limited period of time, but recovered individuals remain so for life). On the other hand, if the proportion of recovered individuals does not keep pace with increases in prevalence, this indicates that APV is causing particularly high levels of mortality.

We use this same relationship to examine whether the threat posed by AP varies by species. Previous data on pox prevalence in the Galápagos finches show very low proportions of individuals both actively infected with and recovered from AP (Kleindorfer and Dudaniec, 2006; Parker et al., 2011); therefore we treat this as an emerging disease newly spreading through the population, rather than as one that has reached equilibrium in the population. In addition, while it is unknown whether Galápagos finches develop long-standing immunity to APV after recovery from infection, other species are known to acquire immunity to this pathogen; therefore we assume that the Galápagos finches also develop acquired immunity. One hypothesis is that the observed increase in AP prevalence is a result of an increased number of infected individuals leading to a more rapid spread of the pathogen through the population (Fig. 1). This hypothesis predicts a concurrent increase in both the proportion of individuals infected with and recovered from APV in all species. Under this scenario, the proportion of individuals infected with and recovered from APV will both increase until a large proportion of the population has developed

acquired immunity, at which time the prevalence will decrease as the proportion of recovered individuals increases; however, given the short time-frame of this study and low recent levels of disease in the population, we do not expect disease spread to have reached this equilibrium stage during the study period. A second hypothesis is that a modest increase in pathogen virulence has increased the spread of APV and prevalence of AP. This hypothesis predicts an increase in the proportion of individuals infected with APV accompanied by a decrease in the proportion of individuals recovered from APV across all species. A third hypothesis is that susceptibility varies by species, and it is an increase in prevalence in a subset of species that is driving the overall observed increase in prevalence from 2000 to 2009. This hypothesis predicts variation between species in the proportion of individuals infected with and recovered from APV.

We find evidence of species-specific variation in susceptibility to pox; therefore, we go on to examine what factors could contribute to this variation. One hypothesis is that species-specific factors (for example, nutrition, exposure to stressors, or exposure to other pathogens) result in variation in body condition, which in turn alters ability to recover from disease. For the purposes of this paper, we define body condition as the ratio of mass to structural body size, which reflects the resource reserves that an individual can draw upon in combating and surviving APV; for example, an individual with greater fat reserves or muscle mass will have more energetic resources to sustain them during periods when they are too weak to forage. This hypothesis predicts that body condition will vary indirectly with prevalence (i.e., as body condition decreases, prevalence will increase) and directly with the proportion of individuals recovered from AP across species (i.e., as body condition increases, recovery rate will increase, resulting in an increase in the proportion of recovered individuals in the population.). An alternative hypothesis is that these species-specific factors do not alter body condition, but instead result in species-specific changes in constitutive immune function of healthy (not yet infected) individuals, which in turn account for changes in prevalence and the proportion of individuals recovered from AP. This hypothesis predicts that we will observe a decrease in innate immune function of *healthy* individuals in those species that show an increase in AP prevalence from 2008 to 2009, but not in those species that do not. These hypotheses are not mutually exclusive.

Hypothesis 1: An overall increase in the number of infected individuals increases the proportion of infected and recovered individuals
Assumptions: Each infected individual will infect X susceptible individuals

Hypothesis 2: An increase in pathogen virulence increases prevalence, and decreases the proportion of recovered individuals
Assumptions: Infected individuals live at least long enough to transmit the pathogen to susceptible individuals.
each infected individual will infect X+Y susceptible individuals (as a result of increased virulence)

Hypothesis 3: Species variation exists in susceptibility to APV
Assumptions: Species specific factors underlie variation in susceptibility to APV

Fig. 1. Three hypotheses explaining increased APV prevalence.

Indeed, body condition and immune function may be, but are not necessarily, linked; therefore, we chose to treat them separately for the purposes of this study.

2. Material and methods

We sampled birds at six sites on Santa Cruz Island, Galápagos, (00°38'S 90°22'W), spanning a range of habitats from the lowland arid zone to moist highland areas. Birds were caught in mist nets during the breeding season (January–February) in 2008 and 2009. We measured mass and tarsus, banded each bird, and collected blood samples using heparinized microcapillary tubes within 25 min of capture. Each sample was kept on ice until it was centrifuged, and the plasma collected and frozen. The ratio mass:tarsus³ was used as a measure of body condition as tarsus provides a good estimate of structural body size in Galápagos finches (Freeman and Jackson, 1990; Grant and Grant, 2002; Keller et al., 2001). Only a handful of banded birds were recaptured, and only data from the first instance of capture was included in this study.

We examined each individual for evidence of current or past infection with APV; lesion scorers received instruction on pox lesion identification from Gustavo Jiménez-Uzcátegui, DVM. Individuals with active swollen lesions were classified as having AP (Fig. 2), while otherwise healthy birds with lesion-induced scarring and missing digits were classified as recovered (hereafter referred to as “infected” or “recovered”). Our scoring system was identical to that described by Kleindorfer and Dudaniec (2006); in addition, we spent several days working in the field with Kleindorfer's team in 2008 and field-compared lesion identification methods with comparable results. We, therefore, use Kleindorfer and Dudaniec's data (2006) in combination with our own to look at long term trends in AP prevalence. While confirmation of APV as the cause of cutaneous lesions can be done via histology, this step was considered unnecessary in this case due to the well-documented presence of APV in the Galápagos, and lack of other pathogens resulting in similar symptoms (Lindström et al., 2004). To examine

Fig. 2. Typical active APV lesion. The lesion is indicated with an arrow; note that the tip of the toe, including the claw, is already gone.

whether there was a change in severity of APV infections from 2008 to 2009, we scored APV infections for number of lesions and lost or deformed digits, which result from severe infections. Birds were processed with the approval of UC Davis IACUC protocol 13171.

2.1. Immune function

The innate immune system provides a first line of defense against novel pathogens, including viruses such as APV. For this study, we measured three aspects of innate immune function: (1) complement; (2) natural antibodies; and (3) PIT54 acute phase protein. While immune parameters change with infection status, when measured in healthy individuals these measures provide information on investment in both innate and adaptive immunity. Therefore, we limited our immune analysis to healthy individuals (showing no signs of APV infection or indications of other diseases; i.e., abnormal crusts, growths, or lethargy).

The hemolysis–hemagglutination assay measures levels of complement activity and natural antibody activity, and was carried out as previously described (Matson et al., 2005). We modified the original protocol to use 10 μ l of plasma, rather than 25 μ l, because of the small size of our samples; we scaled the quantity of all reagents accordingly. PIT54 is the avian analog of mammalian haptoglobin acute phase protein and plasma levels were measured using a commercial kit (from Tri-delta Diagnostics Inc., Morris plains, NJ) (Millet et al., 2007); we modified the protocol to use 5 μ l of plasma rather than the 10 μ l that the kit protocol suggests and also halved the amount of each reagent added to samples.

2.2. Analysis

To determine whether there was a long term trend in proportion of individuals infected with, or recovered from AP from 2000 to 2009, we developed two linear mixed effects models (hierarchical linear model) based on data newly collected in 2008 and 2009 (as described above) combined with data for 2000, 2002, and 2004 taken from Kleindorfer and Dudaniec (2006). In each case species was used as a random grouping factor to create a random intercept linear model, thereby allowing each species to have its own offset term to account for variation in prevalence or proportion recovered between species. Analyses of data from 2000 to 2009 include small, medium and large ground finches, small tree finches, woodpecker finches, and warbler finches.

Of the individuals sampled from 2000 to 2004, one infected MGF was sampled in 2004, three additional infected MGFs and one infected SGF were sampled in unspecified years, and all other affected individuals had recovered from infection (Kleindorfer and Dudaniec, 2006). In order to determine whether there was a change in infected individuals over time, we developed a model of change in prevalence over time (using JMP 7.0.1). A scatter plot of infected individuals by year showed that the relationship between the two variables was not linear. We tested numerous transformations of the year variable to linearize the model and selected e^{year} based on adjusted R^2 values. The final model included e^{year} as the fixed effect (with year ranging from 0 to 9) and species as a random effect to control for repeated measures of each species having been taken in each year. We assigned infected individuals sampled in unspecified years (in the four cases where Kleindorfer and Dudaniec (2006) did not report sample year of an infected individual) to the most conservative year, namely that resulting in the model with the lowest estimate of growth in prevalence over time and lowest significance. However, we also report the range of possible outcomes that we obtained when these individuals were assigned to other possible years. We inspected this model to determine if there was a

significant increase in the proportion of infected individuals over time. We repeated the same process to examine whether the proportion of recovered individuals increased with time.

We then use our data from 2008 and 2009 to examine the change in proportion of infecteds and recovered from 1 year to the next by species, and to relate these changes to variation in immune function. We limited this analysis to species for which we had at least eight samples each year (SGF, MGF, STF, WAF, and CF). We used likelihood ratio (LR) chi-square tests to test whether the proportion of infecteds or recovered increased from 2008 to 2009 overall and by species.

As we found differences between species in change in proportion of infecteds and recovered over time, we used one-way ANOVAs to test for differences in body condition (mass/tarsus³) or immune function between 2008 and 2009 (with year as the explanatory variable and immune parameters or body condition as the response variables); these tests included only healthy individuals. We used one-tailed *t*-tests to test whether APV infection severity decreased from 2008 to 2009. We used a LR chi-square test to determine if proportion of severely infected individuals, those that had at least one missing or deformed digit, decreased from 2008 to 2009.

To further elucidate the relationship between body condition, immune function, and AP prevalence, we conducted path analyses for the three species for which we had the largest data sets (MGF, SGF, and STF). Individuals were grouped by year and capture site, with path analysis conducted at the group level. For these models, elevation and precipitation, which can impact both host physiology (e.g., via dietary nutrition) and vector abundance, were the exogenous variables. For precipitation, we used the cumulative precipitation for the three months prior to sampling, the time period during which individuals that were infected with APV when sampled are likely to have become infected (van Riper and Forrester 2007); precipitation data was obtained from the Charles Darwin Foundation online climate database (Charles Darwin Foundation 2012). We conducted a pair-wise correlation to determine the relationship between elevation and rainfall, and used fit model standardized betas to determine all other relationships between variables. Statistical analyses were conducted using JMP 7.0.1.

3. Results

We sampled 675 individuals in January and February of 2008 and 2009 (322 SGF, 111 MGF, 12 LGF, 178 STF, 26 WAF, and 26 CF). Of these, we obtained and analyzed blood samples from 403 individuals (185 SGF, 118 MGF, 84 STF, 16 CF); when there was not a large enough sample to run all immune tests, samples were assigned randomly to a test type. Data on the infection status of an additional 636 birds sampled January–March of 2000, 2002 and 2004 (351 SGF, 58 MGF, 21 LGF, 142 STF, and 64 WAF) was obtained from the literature (Kleindorfer and Dudaniec, 2006).

3.1. Temporal change in avian pox infection

We found that AP prevalence increased over time. The most conservative model, the one with the least change in prevalence over time, showed a significant increase in AP prevalence over time ($R^2_{adj} = 0.31$, $F = 11.73$, $p = 0.0012$) (Fig. 3); while there is uncertainty as to capture year for four infected individuals, all possible models gave a similar result, with slight variations in explanatory power and significance ($0.25 \leq R^2_{adj} \leq 0.31$, and $0.0003 \leq p \leq 0.0012$). The model that best fits the long term data for infected individuals (2000–2009) is one of exponential growth in AP prevalence. We found a significant change in proportion of recovered individuals with year from 2000 to 2009, but the best model, that with year²

Fig. 3. Change in AP prevalence across all finches (2000–2009). Diamonds indicate annual mean, with black lines showing the 95% confidence interval around the mean; the gray trend line shows the most conservative model fit ($R^2_{adj} = 0.31$, $F = 11.73$, $p = 0.0012$).

as the explanatory variable, could not explain the observed variation ($R^2_{adj} = 0$ in all cases, $7.07 \leq F \leq 7.57$, $0.0081 \leq p \leq 0.010$).

From 2008 to 2009 there was a significant increase in AP prevalence overall from 4% in 2008 to 9% in 2009 ($X^2 = 10.76$, $N = 933$, $DF = 931$, $p = 0.0010$, LR chi-square test). With the exception of MGF, which had the same prevalence in 2008 and 2009, there was a tendency for each species' prevalence to increase (Fig. 4). This increase was only significant in SGF ($X^2 = 15.72$, $N = 322$, $DF = 320$, $p < 0.0001$, LR chi-square test). However, the consistent change coupled with the substantially smaller sample sizes for CF, WAF, and STF suggests that changes in these species would have been significant given a larger sample size ($X^2 = 0.75$, $N = 26$, $DF = 24$, $p = 0.39$ (CF), $X^2 = 1.13$, $N = 26$, $DF = 24$, $p = 0.29$ (WAF), $X^2 = 2.20$, $N = 111$, $DF = 109$, $p = 0.14$ (STF); LR chi-square test). In addition, there was a non-significant tendency for the proportion of recovered birds to decrease in every species, once again with the exception of MGF, which showed a significant increase in recovered birds ($X^2 = 5.87$, $N = 178$, $DF = 176$, $p = 0.015$ (MGF), $X^2 = 0.12$, $N = 322$, $DF = 320$, $p = 0.73$ (SGF), $X^2 = 0.77$, $N = 26$, $DF = 24$, $p = 0.38$ (CF), $X^2 = 0.79$, $N = 26$, $DF = 24$, $p = 0.38$ (WAF), $X^2 = 0.11$, $N = 111$, $DF = 109$, $p = 0.74$ (STF); LR chi-square test) (Fig. 4).

We see neither concurrent changes in prevalence and the proportion of individuals recovered from AP, nor a consistent decrease in the proportion of recovered individuals across all spe-

Fig. 4. Change in proportion of infected and recovered birds (2008–2009). Numbers on left indicate proportion of population infected (solid line) and recovered (dashed line) by species in 2008, numbers on right indicate proportions for 2009.

cies, suggesting that neither a simple spread of disease nor an increase in APV virulence is responsible for the observed changes in prevalence and the proportion of recovered individuals. Nor was there any change in body condition from 2008 to 2009 overall, or within species ((F ratio = -1.12 , $N = 488$, $DF = 486$, $p = 0.29$ (overall), F ratio = 0.17 , $N = 19$, $DF = 17$, $p = 0.68$ (CF), F ratio = 0.015 , $N = 19$, $DF = 17$, $p = 0.90$ (WAF), F ratio = 0.35 , $N = 220$, $DF = 218$, $p = 0.55$ (SGF), F ratio = 1.51 , $N = 140$, $DF = 138$, $p = 0.22$ (MGF), F ratio = 0.0069 , $N = 90$, $DF = 88$, $p = 0.93$ (STF); two-tailed t -tests); thus, change in body condition can not explain the observed variation in prevalence or the proportion of recovered individuals between years. Immune function, on the other hand, did vary both between years and between species.

3.2. Interannual variation in immune function (2008–2009)

Across all healthy finches, levels of the acute phase protein PIT54 decreased significantly from 2008 to 2009, while natural antibody levels increased significantly, after Holm–Bonferroni correction for multiple comparisons (Table 1). Looking at individual species, SGF and STF each showed a highly significant decrease in PIT54 levels (Fig. 5); interestingly, this was balanced by a non-significant trend (after Holm–Bonferroni adjustment) to increase natural antibody levels, as well as a significant increase in complement levels in STF. In contrast, MGF showed no significant change in immune function from 2008 to 2009, after correcting for multiple tests, though they did show a non-significant trend to decrease PIT54 levels. This agreed with our expectation that, if a change in immune function underlies change in AP prevalence, then we would observe a change in immune function of healthy individuals in those species that exhibited a change in prevalence, but not in those that did not (specifically, MGF).

Path analysis further supported a relationship between innate immune function and prevalence of APV infections, though the importance of the different measures of innate immune function varied by species; it also revealed a consistent relationship between body condition and APV prevalence. Specifically, there was a significant inverse relationship between PIT54 levels, body condition and prevalence in SGF, such that as PIT54 and body condition of healthy individuals decreased, prevalence increased at the group level (Fig. 6). In STF, prevalence increased at the group level as PIT54, natural antibody levels and body condition decreased, but none of these relationships were significant; however, there was a significant relationship between both precipitation and elevation and PIT54 levels, such that as elevation increased PIT54 levels increased and as precipitation increased, PIT54 levels decreased

Fig. 5. Change in PIT54 acute phase protein levels from 2008 to 2009 in MGF ($N = 62$), SGF ($N = 122$), and STF ($N = 56$). Gray lines connect the mean PIT54 levels in 2008 and 2009 within species.

at the group level. In MGF, there were significant relationships between PIT54 and natural antibody levels, body condition, elevation and prevalence; specifically, as PIT54 and natural antibody levels increased, prevalence increased, while as body condition and elevation decreased, prevalence increased.

3.3. Avian pox infection severity (2008–2009)

There was no decrease between years in the number of pox lesions in currently infected individuals, which ranged from 1 to 6 in both 2008 and 2009 (t ratio = -1.04 , $N = 49$, $DF = 7.23$, $p = 0.17$; one-tailed t -test). Nor was there a decrease in the number of missing or deformed digits per individual (ranging from 0 to 1 in 2008; 0 to 3 in 2009) (t ratio = 0.54 , $N = 49$, $DF = 13.04$, $p = 0.70$; one-tailed t -test). For recovered individuals, there was no decrease in the number of healed lesions (1–4 in 2008; 1–8 in 2009), or the number of missing or deformed digits (1–3 in 2008; 1–8 in 2009) (t ratio = 0.20 , $N = 95$, $DF = 83.79$, $p = 0.58$, and t ratio = 1.05 , $N = 95$, $DF = 92.58$, $p = 0.85$, respectively; one-tailed t -tests). Finally, there was no decrease in the proportion of severe infections between years (14.3% of infected individuals had severe infections in each year) ($X^2 = 0.0$, $N = 49$, $DF = 47$, $p = 1.0$; LR chi-square test).

Table 1
Change in immune parameters in healthy individuals from 2008 to 2009. Table shows the p -value associated with the change (two-way ANOVA), sample size (N) and degrees of freedom (DF). p -Values with an asterisk remain significant at the $\alpha = 0.05$ level after Holm–Bonferroni correction for multiple comparisons. Arrows in “change” column indicate the directionality of statistically significant changes in immune function from 2008 to 2009 (two-way ANOVA, $p < .05$); dashes indicate no change. The “ Δ AP” column shows change in AP prevalence (see text for significance).

Immune parameter	Species	F ratio	N	DF	p -Value	Change	Δ AP
PIT54 acute	All finches	28.55	253	251	<0.0001*	↓	↑
Phase protein	SGF	16.36	122	120	<0.0001*	↓	↑
	STF	12.54	56	54	0.0008*	↓	↑
	MGF	4.88	62	60	0.031	↓	–
Natural	All finches	11.01	286	284	0.0010*	↑	↑
Antibodies	SGF	5.74	128	126	0.018	↑	↑
	STF	5.46	52	50	0.024	↑	↑
	MGF	2.26	85	83	0.14	–	–
Complement	All finches	2.39	293	291	0.12	–	↑
	SGF	0.12	132	130	0.73	–	↑
	STF	8.02	54	52	0.0066*	↑	↑
	MGF	0.016	86	84	0.9	–	–

Fig. 6. Path analysis of the relationship between precipitation, elevation, innate immune function, body condition and APV prevalence in SGF, STF, and MGF. For each path, the top number indicates the standard beta (a reflection of correlation such that a negative number indicates an inverse relationship and a positive number indicates a direct correlation) in SGF ($N = 12$, average group size = 20), the middle number indicates the correlation for STF ($N = 8$, average group size = 12), and the bottom number indicates the correlation for MGF ($N = 9$, average group size = 16). A single asterisk indicates a p -value < 0.1 , a double asterisk indicates a p -value < 0.05 .

Taken together, the lack of difference in infection severity or the proportion of severe infections between years, and the trend for higher numbers of lesions and missing digits in 2009 compared to 2008, strongly suggest that pox severity did not decrease from 2008 to 2009.

4. Discussion

APV is generally self-limiting in mainland avian populations; however, it has proven to be a disease of concern in remote island systems where species have had little or no prior exposure to APV (van Riper et al., 2002; van Riper and Forrester 2007). Most notoriously, APV has been implicated in population declines and species extinctions in Hawaii (Atkinson et al., 2005; van Riper et al., 2002). In the Galápagos, APV has resulted in decreased recruitment of juvenile mockingbirds into the adult population (Vargas, 1987). APV is a disease of concern not only because of the high mortality rates it can cause in naïve populations, but also because of its tendency for rapid spread after introduction into a population. For example, APV was first observed in the Canary Islands in 2000, and had spread to infect 50% of short-toed larks (*Calandrella rufescens*) within just a few years (Smits et al., 2005). In the Falkland Islands, the first reports of an outbreak of APV in early February in two colonies of gentoo penguins was followed by rapid spread of the disease, with 27% of one colony dead from the disease just one month later (Munro, 2006). While AP is widely considered a disease of concern in island systems, relatively little is known about the threat that this disease poses to endemic island species outside of Hawaii.

4.1. Temporal change in avian pox infection

In Galápagos finches, AP prevalence increased dramatically from 2000 to 2009; this is consistent with Kleindorfer and Dudaniec's (2006) findings. However, this increase in prevalence was not coupled with an increase in the proportion of recovered individuals. This suggests that, while APV continues to spread rapidly, the recovery rate of individuals with AP is low (van Riper et al., 2002). Alternatively, it is possible that a decrease in infection severity would lower our detection of recovered individuals; such

a change should be reflected in a decrease in the average severity of current and past detectable infections. However, we found no decrease in severity of current or past infections. Instead, we saw an increase in maximum number of lost digits from 2008 to 2009 in currently infected individuals, along with an increase in maximum number of lesions and lost digits in recovered individuals, strongly suggesting that infection severity did not decrease.

While AP prevalence increased overall from 2000 to 2009, the magnitude of increase in prevalence varied by species for the 2008–2009 period. The increase in AP prevalence was large for SGF, STF, CF, and WAF, with 6–13% of the population displaying active infections in 2009. AP prevalence in these species approached that observed in the native Hawaiian fauna, which ranged from 9% in Elepaio (*Chasiempis sandwichensis*) to 20% in Omao (*Myadestes obscurus*) from 1977 to 1980 (van Riper et al., 2002). This rapid increase in prevalence is reason for concern. Furthermore, the proportion of recovered birds from 2008 to 2009 decreased for all these species, again suggesting, in the absence of a decrease in infection severity, a decrease in resistance to APV and decreased survival of APV infected individuals over time (van Riper et al., 2002). Of particular concern is the possibility that this apparent AP-induced adult mortality could combine synergistically with depressed rates of reproduction as a result of *P. downsi* nest parasitism (Fessler et al., 2006) to push Galápagos finch populations into decline, much as APV and malaria together have decimated Hawaiian bird populations (Atkinson et al., 2005; van Riper et al., 2002).

In contrast, MGF, while more affected by AP early in the decade (Kleindorfer and Dudaniec, 2006), are showing signs of reduced disease spread and improved recovery. AP prevalence in MGF remained constant from 2008 to 2009 while the proportion of recovered individuals increased, suggesting that the species may be adjusting to the AP epidemic. This could occur through genetic selection for an immune response better able to cope with APV infection. Alternatively, phenotypic plasticity (due to factors such as prior pathogen exposure or environmental conditions) could result in changes in immune responses that provide a more effective defense against APV. The observed difference in the relationship between innate immune function and APV prevalence between MGF and the other species lends some support to these possibilities. While it does not seem likely that increased levels of PIT54 acute phase proteins and natural

antibodies in MGF would themselves lead to an increase in prevalence, as indicated by the path analysis, this pattern could be the result of immune trade-offs such that other unmeasured aspects of immunity (i.e., other acute phase proteins or white blood cell mediated immunity) are upregulated in MGF; this, in turn, could allow them to cope more successfully with exposure to and infection with APV despite lower levels of PIT54 and natural antibodies.

In the best case scenario, the AP epidemic in the other species is following a similar pattern to MGF, but MGF is simply adjusting sooner as they were hit by the AP epidemic earlier on (Kleindorfer and Dudaniec, 2006). On the other hand, if MGF are more capable of adapting to pox due to increased immune variation on which selection can act, or increased immunological phenotypic plasticity, then other species may not adapt as readily to APV and we may expect to see population declines as APV continues to spread through species with high mortality rates. Indeed, in this scenario, the resistance we see in MGF could allow infected MGFs with improved survival to act as a source of APV spread in other increasingly rare species (Woodruffe, 1999). Further data will be needed to determine what is underlying MGF resistance to APV, whether we can expect to see similar resistance develop in other species, and whether the slowing in APV spread in MGFs is a long-term trend or simply a downward swing in an ongoing multi-year disease cycle.

Future studies should examine not only interspecies variation in susceptibility, but also intra-species variation in susceptibility, which can have important impacts on disease spread through populations and overall population viability in the face of a rapidly spreading and virulent pathogen. It will also be important to determine the key transmission routes for APV in the Galápagos avifauna; APV can be transmitted directly, environmentally, and mechanically by biting arthropods. However, it remains unknown what the primary transmission route is in the Galápagos avifauna. Precipitation and elevation are both known to affect vector abundance (Craig et al., 1999; van Riper et al., 2002; Wegbreit and Reisen, 2000); however, precipitation did not have a significant relationship with AP prevalence, and elevation only had a significant relationship with prevalence in MGF. This suggests that changes in vector populations are not driving the changes we observed in AP prevalence. However, this is by no means conclusive as these measures are only proxies for vector abundance. A long-term study of the relationship between vector density, host densities and prevalence could shed light on the importance of vector transmission for APV dynamics in the Galápagos. Captive studies where individuals of different species are exposed to either non-infectious agents that elicit an immune response (such as Complete Freund's Adjuvant) or to APV itself would shed light on species variation in ability to cope with infection. All this information could then be used to develop predictive, field testable models of disease spread that would further elucidate the dynamics of APV in the Galápagos.

4.2. Interannual variation in immune function (2008–2009)

We saw neither concurrent changes in prevalence and the proportion of recovered individuals, a consistent decrease in the proportion of recovered individuals across all species, nor any variation in body condition within or between species. Path analysis revealed that, across all species, APV prevalence increased as body condition decreased. However, we did not see a significant change in body condition from 2008 to 2009 in any species, so this relationship is unlikely to underlie observed changes in AP prevalence. Nonetheless, it does suggest that in years of particularly low rainfall, when food availability decreases and can lead to poor body condition (Grant, 1986), that we can expect to see a spike in pox prevalence.

We were left with the hypothesis that variation in immune function is underlying observed variation in disease prevalence

and the proportion of recovered individuals. Thus, we anticipated observing a decrease in innate immune function of healthy individuals in those species that showed an increase in AP prevalence, but not in those species that did not (specifically, MGF). Indeed, we saw a significant decrease in PIT54 in those species in which we saw an increase in AP prevalence from 2008 to 2009, but not in those species that showed no change in prevalence. Contrary to expectation, we saw a significant increase in complement in STF; it is possible that this increase to some extent compensated for the decrease in PIT54 and this may explain why the increase in AP prevalence in STF was less extreme than in SGF. The consistent trend of decreasing PIT54 coupled with a non-significant increase in natural antibody levels may point to a trade-off in investment between these two aspects of immunity. It is unclear why innate immune function would exhibit this pattern of change from 2008 to 2009. One explanation is that food resources varied between years; given that food supply is an important limiting resource that varies widely from year to year, and between species, annual variation in resource availability could lead to observed variation in immune function (Grant, 1986).

To our knowledge, this is the first study to demonstrate significant interannual variation in innate immune function in wild birds. Future studies should address to what extent interannual variation in immune function occurs in other species and the factors underlying the variation. Some of these factors will be similar to those underlying intra-annual variation in immune function; for example, we suggest that variation in climate, resource availability, density, and social structure may play an important role in inter-annual immune variation in addition to the role that they are known to play in intra-annual variation (Hawley, 2006; Nelson, 2004; Saino et al., 2000). In addition, while environmental factors occurring along consistent seasonal rhythms, such as photoperiod, are unlikely to be important driving factors in interannual immune variation, those occurring in multi-annual rhythms, such as El Niño events or the Pacific decadal oscillation may well play a role in interannual immune function fluctuations. Similarly, long term changes, such as alterations in land use patterns, vegetation cover, species introductions, climate change, or factors causing allostatic overload may be expected to play an important role in interannual immune variation. Ultimately, understanding and anticipating interannual immune variation will improve our understanding of multi-year disease dynamics in host populations and our ability to make predictive models regarding the outcome of disease introduction and emergence.

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