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COMPARISON OF BLOOD VALUES AND HEALTH STATUS OF FLOREANA MOCKINGBIRDS (*MIMUS TRIFASCIATUS*) ON THE ISLANDS OF CHAMPION AND GARDNER-BY-FLOREANA, GALÁPAGOS ISLANDS

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ABSTRACT: The Floreana Mockingbird (*Mimus trifasciatus*) is one of the rarest bird species in the world, with an estimated 550 individuals remaining on two rocky islets off the coast of Floreana, Galápagos, Ecuador, from which the main population was extirpated more than 100 yr ago. Because they have been listed in critical danger of extinction, a plan to reintroduce this species to Floreana has been initiated. Determining the health status of the source mockingbird populations is a top priority within the reintroduction plan. We report the health status, over the course of 4 yr, of 75 Floreana Mockingbirds on Champion Island and 160 Floreana Mockingbirds on Gardner-by-Floreana, based on physical examinations, hematology, hemolysis–hemagglutination assay, exposure to selected infectious disease agents, and ecto- and endoparasite counts. Birds on Gardner-by-Floreana had higher body condition index scores, packed cell volumes, total solids, and lymphocyte counts. Additionally, Gardner-by-Floreana birds had lower heterophil counts, eosinophil counts, and heterophil:lymphocyte ratios. No *Chlamydophila psittaci* DNA or antibodies to paramyxovirus-I, adenovirus-II, or *Mycoplasma gallisepticum* were found in any of the mockingbirds tested. Ectoparasites were present on birds from both islands, although species varied between islands. A coccidian species was found in eight of the 45 fecal samples from birds on Gardner-by-Floreana, but none of 33 birds examined from Champion. Birds on Gardner-by-Floreana were classified as healthier than those on Champion based on clinical and laboratory findings. These health data will be analyzed in conjunction with genetics, population structure, and disease presence on Floreana for developing recommendations for the Floreana Mockingbird reintroduction plan.

Key words: Floreana Mockingbird, hematology, *Mimus trifasciatus*, parasites, reintroduction plan, serology.

INTRODUCTION

The Floreana mockingbird, upgraded in 2008 to critically endangered by the International Union for the Conservation of Nature (IUCN), is among the rarest bird species in the world, with an estimated 550 individuals (IUCN, 2010). Extirpated from Floreana Island over 125 yr ago, it now resides on two small satellite islands, Champion (n=20–53; Grant et al., 2000) and Gardner-by-Floreana (n=200–500; P. E. A. Hoeck and L. F. Keller 2009, unpubl. census data). Because of the limited geographic range and small number of birds, these two fragmented populations are in critical danger of extinction. Immediate management actions believed necessary to avert extinction of this iconic Galápagos bird resulted in the initiation of the Floreana Mockingbird Reintroduction Plan (Charles Darwin Foundation, 2008). Determining the health status of the source mockingbird populations was indicated as a top priority within this plan.

There are few health data available for Floreana mockingbirds. Annual censuses, focused on population size estimates, include observations for obvious gross lesions associated with avian poxvirus and *Philornis downsi* in mockingbirds and other bird species on the islands (e.g., Jiménez-Uzcátegui, 2008). The objective of this study was to determine baseline health parameters for the Floreana mockingbirds on Champion and Gardner-by-Floreana, and to compare these populations for their suitability as source populations for the reintroduction.

METHODS

Study area and field sampling

The field work was conducted February 2006–May 2009, including four trips to Champion and five trips to Gardner-by-Floreana (Table 1 and Fig. 1). Champion Island (90°21′47″W, 1°13′55″S) is 9.4 ha and less than 1 km from Floreana. It is an arid and littoral island. Gardner-by-Floreana Island (90°17′44″W, 1°20′48″S) is 81 ha, with both arid and semiarid land, approximately 8 km from Floreana. These two islands are separated by ocean (14 km), and genetic data indicate that mockingbirds do not migrate between them (Hoeck et al., 2010).

Mockingbirds were captured with the use of Potter traps (Reinhard Vohwinkel, Velbert, Germany) baited with banana. Birds were removed from the traps immediately after capture and handled for <20 min. Each mockingbird was banded (if not previously banded), categorized as juvenile (born in the same year) or adult, and physical examinations were conducted, with careful inspection to detect avian pox-like lesions and evidence of *P. downsi* infestation. With the use of digital calipers, beak and tarsus lengths were measured to the nearest 0.01 mm and the eighth primary feather and the unflattened longest primary feather (wing chord) lengths were measured with a ruler and recorded to the nearest 1 mm. Body weight to the nearest 1.0 g was obtained by spring balance scale (Pesola A. G., Baar, Switzerland).

A cloacal swab was collected (Fisherbrand® Sterile Swabs, Fisher Scientific, Pittsburgh, Pennsylvania, USA), individually placed in cryotubes (Nalge Nunc

TABLE 1. Field trips and sample collection of Floreana Mockingbirds (*Mimus trifasciatus*) from Champion and Gardner-by-Floreana Islands, Galápagos.

	Champion					Gardner-by-Floreana					
Diagnostic test[a]	Total tested	February–March 2006	December 2006	January 2008	February 2009	Total tested	March 2006	November 2006	January 2008	January–February 2009	May 2009
PE	75	15	17	28	15	160	23	32	21	37	47
Morphometrics	61	×	×	×	×	79	×	×	×	×	
Dust ruffling	12		×	×		25	×				
Fecal parasites	33			×	×	45			×	×	×
Sexing	61	×	×	×		78	×	×	×		
Hematology	16				×	47					×
A and L	23	×	×			32	×	×			
Hemoparasites	0					46					×
Serology	38	×	×			50		×			×
Chlamydiophilia psittaci	21			×		29			×		×

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[a] PE = physical examination; Sexing = molecular sex determination; Hematology = hematology and blood parasite determination using microscopy; A and L = agglutination and lysis tests; Hemoparasites = hemoparasite detection with the use of molecular techniques.

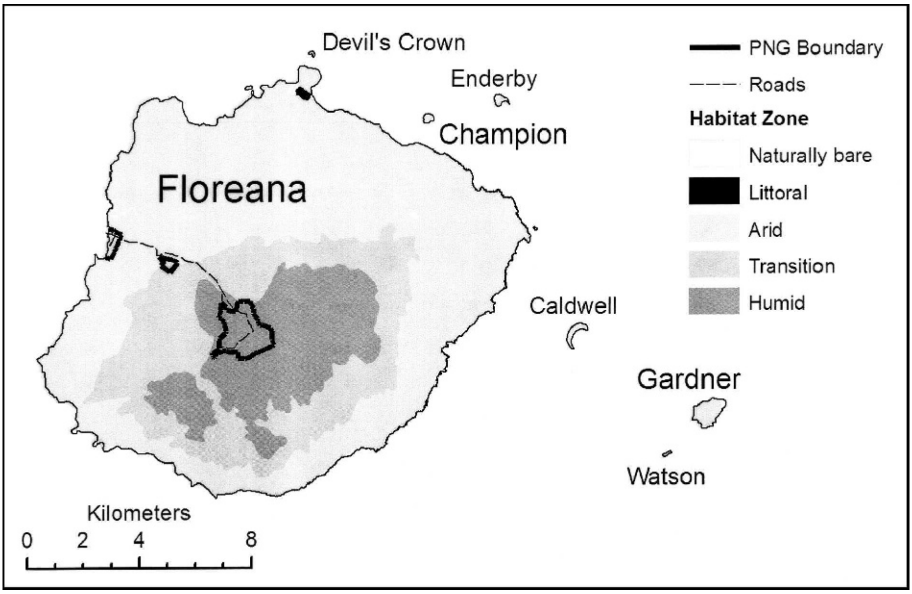

FIGURE 1. Floreana Island and its two satellite islands, Champion and Gardner-by-Floreana, within the Galápagos Islands, Ecuador.

International, Rochester, New York, USA), and frozen at −20 C while in the field and −80 C in the laboratory. Blood samples (<1% of body weight) were collected from the ulnar vein with the use of a 25- or 26-g needle by pricking the vein and then filling 1–4 heparinized capillary tubes (Fisherbrand®, Fisher Scientific). One tube was used to prepare fresh blood smears, which were then fixed in 99% methanol in the field, and blood from one tube was stored in a lysis buffer preservative solution (Longmire et al., 1988) for future genetic analyses (e.g., hemoparasite identification). Packed cell volumes (PCV) were determined with the use of a portable 12-V centrifuge (Mobilespin, Vulcan Technologies, Grandview, Missouri, USA), and plasma total solids (TS) were measured with the use of a hand-held refractometer (Schulco, Toledo, Ohio, USA) calibrated on site. The remaining capillary tubes (if available) were sealed with clay and kept cool while in the field. Later that day at the field camp, capillary tubes were centrifuged for 10 min and plasma decanted, placed in cryotubes, and frozen at −20 C while in the field and −80 C in the laboratory.

To quantify ectoparasite load, we dust-ruffled a subset of mockingbirds following the method described in Walther and Clayton (1997). We used pyrethron powder (0.3% natural flower-extract pyrethrum and 1% piperonyl butoxid; Vetyl-Chemie GmbH, Saarland, Germany) and applied 0.7 g of insecticide to the plumage of the birds, including all feather tracts except the head. Dusting was performed for 2.5 min, followed by 1 min of incubation and 2.5 min ruffling over a clean plastic tray to extract ectoparasites. Ectoparasites were stored in 97% ethanol until they were counted and identified in the lab. Fecal samples, collected opportunistically from some birds that defecated during handling, were preserved in 10% buffered formalin.

Molecular sexing

To distinguish between males and females, molecular sexing was performed for mockingbirds evaluated in all years except 2009. We amplified the CHD-W and CHD-Z genes (Griffiths et al., 1998) with the use of redesigned primers as described by Hoeck et al. (2009).

Hematology

Blood smears were stained with the use of a Wright-Giemsa stain (EK Industries, Joliet, Illinois, USA) and evaluated for estimated leukocyte counts, differentials, and hemoparasites at the Clinical Pathology Laboratory, Saint Louis Zoo. All slides were read by one of the authors (J.M.). The leukocyte-estimate-from-smear technique was used to determine total white-blood-cell (WBC) counts (Fudge, 2000). Differential WBC counts were performed by counting 100 leukocytes under oil immersion. Heterophil:lymphocyte ratio was determined from the differential. Blood smears were evaluated for hemoparasites by searching at 100× magnification for 5 min recording presence or absence of hemoparasites. Additionally, 200 fields were reviewed at 1,000× oil immersion to look for smaller hemoparasites (e.g., *Plasmodium* and *Haemoproteus* spp.).

Assays for immunocompetence

Agglutination and lysis titers were assessed with a hemolysis–hemagglutination assay, as described by Matson et al. (2005). We selected these tests because agglutination titers indicate levels of natural antibodies, which are known to facilitate initial pathogen recognition and initiate acquired immune responses, and lysis titers are indicative of complement and other circulating lytic enzymes. Plasma was serially diluted twofold with saline in a 96-well assay plate and incubated with rabbit red blood cells (Harlan Laboratories UK Ltd., Leicestershire, UK) for 90 min at 37 C. Samples were placed on plates haphazardly and, as a control, a chicken plasma sample (Harlan Laboratories) was added onto each batch. After completion of the test, we determined the dilution step at which either the agglutination or lysis reaction stopped (titer score) and took digital images. As a control, titer scores were confirmed a few days later with the digital images only. All scoring was carried out blindly with respect to bird identity and always performed by the same person (P.E.A.H.). To account for differences between plates processed at different times, all mockingbird lysis and agglutination scores were corrected for the chicken control that was run at the same time by subtracting the score of the chicken sample from the mockingbird sample score.

Infectious and parasitic agents

Cloacal swab samples were tested for *Chlamydophila psittaci* by polymerase chain reaction (PCR) at the Infectious Diseases Laboratory, College of Veterinary Medicine, University of Georgia, Athens, Georgia, USA (Sayada et al., 1995). Serologic tests were performed at the Veterinary Medical Diagnostic Laboratory, University of Missouri—Columbia, Columbia, Missouri, USA. Antibody titers to avian paramyxovirus I/ Newcastle disease (cutoff >396), *M. gallisepticum* (>1,076; FlockChek™, IDEXX laboratory Inc., Westbrook, Maine, USA), and adenovirus-II (cutoff >2,000; ProFLOK®, Synbiotics Corporation, San Diego, California, USA) were determined with the use of enzyme-linked immunosorbent assays (ELISA).

To determine the presence of any Haemosporidian parasites, in addition to direct microscopic evaluations, molecular tests were conducted at the laboratory of one of the authors (P.G.P.) at the University of Missouri—Saint Louis, Saint Louis, Missouri, USA. DNA was extracted from blood with the use of a standard phenol-chloroform extraction protocol (Sambrook and Russell, 1989), and PCR was used to amplify a region of the parasite mitochondrial cytochrome b gene (Waldenström et al., 2004). Amplification was detected by gel electrophoresis. Positive and negative (distilled water) controls were used in each test. Positive controls were from a Gálápa-

TABLE 2. Morphometric data for Floreana mockingbirds (*Mimus trifasciatus*) evaluated February 2006 to May 2009 on Champion and Gardner-by-Floreana Islands, Galápagos.

Parameter	N	Champion mean (SD)	Range	*N*	Gardner-by-Floreana mean (SD)	Range
Beak length (mm; Male+female)	61	20.21 (0.58)[a]	19.14–21.38	79	20.5 (0.91)[a]	17.82–23.27
Male	26	20.48 (0.58)[a]	19.2–21.38	46	20.9 (0.84)[a]	19.31–23.27
Female	35	20.01 (0.5)	19.14–21.15	33	19.9 (0.64)	17.82–20.76
Tarsus length (mm; Male+female)	61	39.67 (1.24)	36.19–42.15	78	39.98 (1.21)	37.76–42.36
Male	26	40.57 (1.05)	38.34–42.15	45	40.67 (0.98)	37.76–42.36
Female	35	39.00 (0.91)	36.19–42.15	33	39.03 (0.13)	37.88–41.22
Feather length (mm; Male+female)	61	83 (3.67)	77–91	71	84 (4.35)	77–93
Male	26	86 (2.86)	80–91	44	87 (3.5)	78–93
Female	35	81 (2.18)	77–87	32	80 (1.87)	77–85
Wing length (cm; Male+female)	61	12.0 (0.48)[b]	11.2–12.9	77	12.2 (0.57)[b]	11.2–13.1
Male	26	12.4 (0.24)[b]	11.9–12.9	44	12.6 (0.32)[b]	11.6–13.1
Female	35	11.6 (0.26)	11.2–12.4	33	11.6 (0.31)	11.2–12.5
Body weight (g; Male+female)	63	58 (3.68)[a]	50–64	77	63 (5.52)[a]	52–76
Male	26	61 (2.41)[a]	55–64	44	66 (4.04)[a]	57–76
Female	37	56 (3.31)[a]	50–64	33	58 (2.57)[a]	52–64

[a] Statistically significant difference between islands; *t*-test ($P<0.05$).

[b] Mann-Whitney *U*-test ($P<0.05$).

gos dove (*Zenaida galapagoensis*) infected with *Haemoproteus multipigmentatus* (Valkiunas et al., 2010) and a Galápagos penguin (*Spheniscus mendiculus*) infected with *Plasmodium* sp. (Levin et al., 2009).

Because lice were the most prevalent and abundant ectoparasites, we only quantified and identified these species. Identification of louse genera was done by Vincent Smith, Natural History Museum, United Kingdom. Fecal samples were analyzed by fecal floatation, with the use of a sugar-saturated solution, and a McMaster chamber for semiquantification at the Laboratory of Epidemiology, Genetics, and Pathology, Puerto Ayora, Galápagos.

Statistical analysis

Sample sizes are provided for each health parameter because not all data points were collected for all birds (Tables 2 and 3). A condition index was calculated for each individual as the residual of the regression of body mass against wing length for both populations combined (Green, 2001). Analysis of variance was used to compare condition index scores of populations by sex and island (Petrie and Watson, 2006). All other numerical data were inspected for normality, and *t*-tests were performed on normally distributed data and Mann-Whitney *U*-tests used where normality was rejected to compare the two island populations (Petrie and Watson, 2006). Chi-square was used to compare sex ratios, and Fisher's exact test was used to compare ectoparasite infestation and endoparasite loads on mockingbirds between the two islands (Petrie and Watson, 2006). Statistical significance was set as $P<0.05$. Data were analyzed with the use of a commercial statistical software package (NCSS, Kaysville, Utah; SPSS, version 13.0, Chicago, Illinois, USA).

RESULTS

We evaluated 75 adult birds on Champion during four field trips and 160 birds (157 adults and three juveniles) during five field trips to Gardner-by-Floreana (Table 1).

TABLE 3. Hematology values for Floreana mockingbirds (*Mimus trifasciatus*) evaluated in February 2009 on Champion and in May 2009 on Gardner-by-Floreana Islands, Galápagos.

Parameter	Mean	Standard deviation	Range	Sample size
Packed cell volume[a] (%)				
Champion	43	1.74	39–46	16
Gardner-by-Floreana	46	1.97	42–51	47
Total solids[b] (g/dl)				
Champion	4.0	0.1	3.8–4.1	16
Gardner-by-Floreana	4.4	0.5	3.8–6.1	47
Leukocyte count ($\times 10^3$/ml)				
Champion	18.3	6.1	8.1–28.8	16
Gardner-by-Floreana	19.3	7.2	7.1–37.1	46
Heterophil[a] (%)				
Champion	22.3	8.3	8–38	16
Gardner-by-Floreana	11.6	6.1	0–25	45
Lymphocytes[a] (%)				
Champion	64.9	11.3	37–84	16
Gardner-by-Floreana	79.3	8.3	64–98	45
Monocytes (%)				
Champion	2.88	1.93	1–8	16
Gardner-by-Floreana	2.18	1.85	0–7	45
Basophils (%)				
Champion	0.19	0.40	0	16
Gardner-by-Floreana	0.16	0.37	0–1	45
Eosinophils[a] (%)				
Champion	10.8	6.9	0–23	16
Gardner-by-Floreana	6.9	4.6	0–20	45
Heterophil:lymphocyte[b]				
Champion	0.37	0.22	0.10–0.95	16
Gardner-by-Floreana	0.15	0.09	0–0.38	45

[a] Statistically significant difference between islands, *t*-test ($P<0.05$).

[b] Mann-Whitney *U*-test ($P<0.05$).

Physical examinations and molecular sexing data

Morphometric data are presented in Table 2. Males were significantly larger in beak length (*t*-test; $P=0.02$), wing length (Mann-Whitney *U*; $P<0.001$), and body weight (*t*-test; $P<0.001$) and females were significantly heavier (*t*-test; $P=0.03$) on Gardner-by-Floreana than on Champion. Significant differences (ANOVA; $P<0.001$) were found between the condition indices for mockingbirds based on island and sex; after adjusting for overall body size, males were heavier (better condition) than females regardless of island, and males and females on Gardner-by-Floreana were heavier than males and females on Champion (Fig. 2). Of the 61 mockingbirds on Champion that were sexed by molecular techniques, 25 (41%) were male and 36 (59%) were female. On Gardner-by-Floreana we determined the sex of 78 mockingbirds with 46 males (59%) and 32 females (41%). There was a significant difference between the sex ratio of mockingbirds evaluated on the two islands (chi-square; $P<0.05$).

The general health of the Floreana mockingbirds was rated high in all but one of the 235 birds (0.4%) we handled on both islands. This bird, examined on

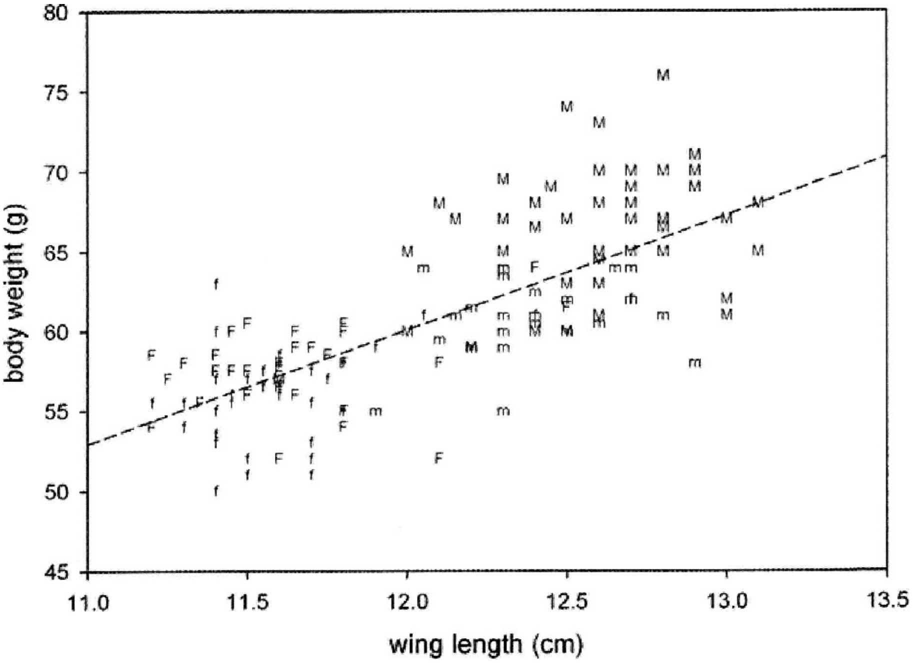

FIGURE 2. Condition index scores, by sex and island, for Floreana mockingbirds (*Mimus trifasciatus*) on Champion and Gardner-by-Floreana Islands, Galápagos. F = females on Gardner-by-Floreana; M = males on Gardner-by-Floreana; f = females on Champion; m = males on Champion. $R^2=0.53$ and $y=-26.1+7.2x$.

Champion in February 2009, was in very poor body condition with an abnormally shaped beak. No pox-like or *P. downsi* lesions were seen in any of the mockingbirds or in any other passerines observed on the islands.

Hematology

Hematology results are presented in Table 3. The bird in poor condition examined on Champion had the lowest PCV (39%) and TS (3.8 g/dl) values of all mockingbirds. Mockingbirds on Gardner-by-Floreana had significantly higher PCV (*t*-test; $P<0.001$) and TS (Mann-Whitney *U*-test; $P<0.001$) values than those on Champion. Although the estimated leukocyte count did not differ between the two island populations, we found significant differences in heterophil (greater on Champion) and lymphocyte (greater on Gardner-by-Floreana) counts (*t*-tests; $P<0.001$), heterophil:lymphocyte ratio (Mann-Whitney *U*-test; $P<0.001$) and eosinophil counts (*t*-test; $P=0.007$) were significantly higher on Champion than on Gardner-by-Floreana (Table 2).

Assays for immunocompetence

There were no significant differences in lysis and agglutination scores between the mockingbirds on the two islands (*t*-test; $P>0.05$). The 23 birds on Champion tested for lysis had a score of 3.3±1.6 (mean±SD), range of 0.5–7. The 32 Gardner-by-Floreana birds had a score of 3.5±1.4, range of 0.5–6. Agglutination scores were 9.35±0.70, range 8–11 in the Champion birds and 9.03±0.83, range 7.5–11 in the Gardner-by-Floreana birds.

Infectious and parasitic agents

No test results were positive for any of the four infectious disease agents (Table 4). Lice, identified on all birds sampled, were from two genera, *Brueelia galapagensis* (Ischnocera) and *Myrsidea* sp. (Amblycera). Although we found no statistical differences between the number of birds with louse infestations (Fisher's

TABLE 4. Pathogen, diagnostic tests performed, antibody titer defined as positive and number of positive mockingbirds of select infectious agent exposure in Floreana mockingbirds (*Mimus trifasciatus*) on Champion and Gardner-by-Floreana Islands, Galápagos.

Pathogen	Test method[a]	Positive titer	Champion	Gardner-by-Floreana
Chlamydophila psittaci	PCR	n/a[b]	0/21	0/22
Adenovirus-II	ELISA	>200	0/34	0/47
Mycoplasma gallisepticum	ELISA	>1,076	0/38	0/50
Paramyxovirus-1	ELISA	>396	0/36	0/50
Haemosporidian parasites	PCR	n/a	n/a	0/46

[a] PCR = polymerase chain reaction (detects DNA); ELISA = enzyme-linked immunosorbent assay (detects antibodies to the agent).

[b] n/a = not applicable.

exact tests; $P>0.05$), or between intensities of infestations (Mann-Whitney U-Test; $P>0.05$), between the two island populations, no adults or nymphs of *B. galapagensis* were detected from any of the 12 mockingbirds sampled on Champion. On Gardner-by-Floreana, six of the 25 (24%) dust-ruffled birds had *B. galapagensis*. All dust-ruffled birds had *Myrsidea* sp.

Of the 33 fecal samples collected from mockingbirds on Champion, one (3%) sample had an unidentified nematode egg. Eight of the 45 (18%) fecal samples from birds on Gardner-by-Floreana were positive for a coccidian parasite. Based on morphology of oocysts and previous reports from Galápagos birds, we identified this protozoan as an *Isospora* sp. or *Polysporella* sp. (McQuistion and Wilson, 1988, 1989; McQuistion, 1990). Quantification of coccidian oocysts varied from 2 to 750 eggs per bird in these eight fecal samples. There was a significant difference in the prevalence of coccidian oocysts in mockingbirds on the two islands (Fisher's exact test; $P=0.02$). No blood parasites were found in any of the Champion ($n=16$) or Gardner-by-Floreana ($n=47$) birds evaluated by microscopy or in Gardner-by-Floreana ($n=46$) mockingbirds evaluated by PCR.

DISCUSSION

Our findings suggest that the mockingbirds on Champion are clinically less healthy than mockingbirds on Gardner-by-Floreana, based on lower condition index scores, PCV, TS, and higher heterophil:lymphocyte ratio. No mockingbird had evidence of infection with the avian poxvirus or infestations with *P. downsi*, two disease-causing agents of high conservation concern for mockingbirds and other Galápagos passerines (Vargas, 1987; Fessl et al., 2006; O'Connor et al., 2010). One bird on Champion was in extremely poor condition. None of the 160 mockingbirds evaluated on Gardner-by-Floreana had clinical lesions. Additionally, the significantly higher condition index score for mockingbirds on Gardner-by-Floreana (Fig. 1) may confer an advantage to mockingbirds from this island during reintroduction, as the heavier body mass may allow birds longer intervals without food while they acclimatize to their surroundings on Floreana. However, these health data must be interpreted in conjunction with recent genetic data demonstrating that each island population contains unique alleles from the original Floreana population, suggesting both populations should be considered for reintroduction (Hoeck et al., 2010).

The higher PCV and TS in the Gardner-by-Floreana birds may have been associated with a higher plane of nutrition associated with their island habitat, which is larger and wetter than Champion (Grant et al., 2000). Leukocyte counts, which did not differ between the two island populations, were estimated from fixed blood smears. This technique may be more variable than those of hemacytometer-based techniques when performed under ideal conditions (Russo et al., 1986). However, hemacytometer-based techniques are often impractical in remote field situations. In the field, where the decline in condition of cell quality in unpreserved whole blood may create artifacts, more reliable results are likely from estimated total WBC counts from freshly made and fixed blood smears (Fudge, 2000).

The data for heterophil:lymphocyte ratios were inverse to the estimates of genetic variability. The population of Floreana mockingbirds on Champion have low genetic variability (Hoeck et al., 2010) and high heterophil:lymphocyte ratio (this study), whereas the birds on Gardner-by-Floreana have higher genetic variability (Hoeck et al., 2010) and lower heterophil:lymphocyte ratio (this study). The lower heterophil:lymphocyte ratio in the Gardner-by-Floreana mockingbirds suggests that this population may be under less physiologic stress than the birds on Champion, although genetics or some combination of genetics and stressors is possible. Stressors such as food and water deprivation, temperature extremes, and changing social situations will elevate heterophils and lower lymphocytes in birds (Gross and Siegel, 1983). These stressors are likely to be present on the small, arid island of Champion, in which the mockingbird carrying capacity may be reached during years of good climatic conditions (Grant et al., 2000); such conditions occurred in 2009. Additionally, more tourist boats are present near Champion and, therefore, it is possible that the difference in human presence results in physiologic differences between the two island populations of Floreana Mockingbirds, as shown for other animals in Galápagos (Romero and Wikelski, 2002).

The reason for the higher eosinophil count in the Champion birds is unknown; the function of the avian eosinophil is still unclear, although there is some suggestion that parasites may increase eosinophils in birds, as in mammals (Latimer and Prasse, 2003). However, the Champion birds had lower ecto- and endoparasite loads than the Gardner-by-Floreana birds for the parasites we measured.

Scores of the lysis and agglutination tests were not different between the two island populations, but both were high in comparison to those found in other species (Matson et al., 2005; Matson, 2006; Mendes et al., 2006). These tests measure natural antibodies (agglutination) and complement action (lysis). As natural antibodies play an important role in the initial recognition of foreign particles and support subsequent defense by the complement cascade and the acquired humoral response (Ochsenbein and Zinkernagel, 2000), the high natural antibodies and relatively high complement enzyme titers found here could indicate that both populations of Floreana mockingbirds are equipped with a strong first line of defense. Increased innate defenses of insular birds in comparison to their continental relatives have previously been reported and interpreted as a shift in the immune defense strategy toward innate as opposed to acquired immune responses (Matson, 2006). If natural antibody response does also predict the strength of the adaptive humoral immune response (Kohler et al., 2003; Lammers et al., 2004), this would further suggest that both populations of Floreana mockingbirds could have a potent adaptive immune system. This finding may confer natural resistance if exposed to novel pathogens on Floreana following reintroduction.

No mockingbirds were positive for *C. psittaci* or antibodies to adenovirus, *M. gallicepticum*, and PMV-I. These four pathogenic agents were selected based on their known presence in domestic chickens and wild birds in Galápagos and their pathogenicity in wild bird populations (Padilla et al., 2003, 2004; Gottdenker et al., 2005; Travis et al., 2006a, b; Soos et al., 2008). However, due to a technical error, the mockingbirds were tested for adenovirus-II and not adenovirus-I, the agent to which antibodies in Galápagos birds have previously been found (Padilla et al., 2003; Travis et al., 2006a). The negative findings in mockingbirds from both islands suggest that neither population has been exposed to these pathogens, although none of the tests we used have been validated in this species. Additionally, small sample sizes preclude stating with certainty whether an agent was present if not detected. However, to detect disease presence with 95% confidence of finding at least one positive and based on 15% prevalence, we needed to test 12–16 mockingbirds on Champion (estimated population size of 20–53) and 18–19 mockingbirds on Gardner-by-Floreana (estimated population size of 200–500) if using a diagnostic test with 100% sensitivity and specificity (Cannon and Roe, 1982). Although the sensitivity and specificity of these tests are unknown for the Floreana Mockingbird, we tested 21–38 mockingbirds on Champion and 29–50 mockingbirds on Gardner-by-Floreana. In future studies it would be beneficial to increase sample sizes and to include other pathogens known to be present in Galápagos birds (e.g., *Toxoplasma gondii*) or of concern in passerines globally, such as bacterial infections caused by *Salmonella* (Pennycott et al., 2002; Hall and Saito, 2008; Deem et al., 2010).

Amblyceran lice (*Myrsidea*) were much more abundant than ischnocerans (*Brueelia*), which were totally absent from Champion mockingbirds. This pattern is generally observed when these suborders co-occur on the same host (Whiteman and Parker, 2004). *Myrsidea* spp. lice may cause an immune reaction through their tissue- and blood-feeding behavior (Moller and Rozsa, 2005). In contrast, *Brueelia* spp. belongs to the feather-chewing lice and is unlikely to have a direct interaction with the birds' immune system (Moller and Rozsa, 2005). However, because feather-chewing lice are also known to have negative effects on host fitness by damaging feathers, which compromises thermoregulatory ability (Booth et al., 1993) or reduces survivorship (Clayton et al., 1999), one might conclude that birds on Gardner-by-Floreana had increased physiologic stress related to ectoparasite infestation. Our data do not support this interpretation, as mockingbirds on Gardner-by-Floreana were classified as healthier based on clinical and laboratory results.

No hemoparasites were identified in any of the mockingbirds by direct microscopy and PCR. We found a significant difference in prevalence of coccidian oocysts between the island populations, with oocysts found only in Gardner-by-Floreana mockingbirds. These oocysts were most likely within the *Isospora* or *Polysporella* genera based on studies of other passerine species in Galápagos (McQuistion and Wilson, 1988, 1989; McQuistion, 1990). The finding of an *Isospora* sp. in a Darwin's finch on Floreana (Dudaniec et al., 2005) suggests that the reintroduction of mockingbirds from Gardner-by-Floreana may not introduce a novel coccidian genus to the island. However, we recommend the coccidian in the mockingbirds be identified to species, based on sporulated oocysts, prior to bird reintroductions.

Limitations to this study include the possibility that differences in some of our clinical and laboratory findings between the two island populations were associated with seasonal differences. For example, the hematology analyses compared birds on Champion sampled in February 2009 to Gardner-by-Floreana birds sampled in May 2009. However, seasonal effects would predict that more favorable food availability and environmental conditions during the rainy season (December to April) would lead to higher condition indices for Champion birds than for

Gardener-by-Floreana. Our data suggest the opposite. Another limitation was the sample size for a number of the parameters, although we sampled a large percentage of the population on both islands. For example, the hematology data included 16 of the 47 mockingbirds (34%) alive on Champion in February 2009 and 47 of 480 mockingbirds (9.8%) estimated during the February 2009 census on Gardner-by-Floreana. Lastly, serum samples were tested for antibodies to adenovirus-II, when our intention was testing for adenovirus-I based on past studies in Galápagos (Padilla et al., 2003; Travis et al., 2006a).

The long-term conservation of the Floreana mockingbird is challenging because of its limited geographic distribution, fragmented and isolated populations, and small number of individuals remaining. However, this species is arguably the most important in the history of science because of its pivotal role in triggering Darwin's theory on the evolution of species by natural selection (Darwin, 1859), and extinction of this iconic bird should be averted. The initiation of a reintroduction plan to expand its geographic distribution may minimize the risks of extinction in the short term. The baseline health parameters presented here are an important component within the reintroduction plan. However, these health data must now be integrated with other findings, including genetic evaluations, population biology, disease presence on Floreana, and understanding and eliminating the cause(s) that led to the extirpation of this mockingbird species from Floreana (Grant et al., 2000; Charles Darwin Foundation, 2008; Hoeck et al., 2010).

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