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Galápagos mockingbirds tolerate introduced parasites that affect Darwin's finches

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Abstract. Introduced parasites threaten native host species that lack effective defenses. Such parasites increase the risk of extinction, particularly in small host populations like those on islands. If some host species are tolerant to introduced parasites, this could amplify the risk of the parasite to vulnerable host species. Recently, the introduced parasitic nest fly *Philornis downsi* has been implicated in the decline of Darwin's finch populations in the Galápagos Islands. In some years, 100% of finch nests fail due to *P. downsi*; however, other common host species nesting near Darwin's finches, such as the endemic Galápagos mockingbird (*Mimus parvulus*), appear to be less affected by *P. downsi*. We compared effects of *P. downsi* on mockingbirds and medium ground finches (*Geospiza fortis*) on Santa Cruz Island in the Galápagos. We experimentally manipulated the abundance of *P. downsi* in nests of mockingbirds and finches to measure the direct effect of the parasite on the reproductive success of each species of host. We also compared immunological and behavioral responses by each species of host to the fly. Although nests of the two host species had similar parasite densities, flies decreased the fitness of finches but not mockingbirds. Neither host species had a significant antibody-mediated immune response to *P. downsi*. Moreover, finches showed no significant increase in begging, parental provisioning, or plasma glucose levels in response to the flies. In contrast, parasitized mockingbird nestlings begged more than nonparasitized mockingbird nestlings. Greater begging was correlated with increased parental provisioning behavior, which appeared to compensate for parasite damage. The results of our study suggest that finches are negatively affected by *P. downsi* because they do not have such behavioral mechanisms for energy compensation. In contrast, mockingbirds are capable of compensation, making them tolerant hosts, and a possible indirect threat to Darwin's finches.

Key words: *Galápagos Islands;* Geospiza fortis; *host defense;* Mimus parvulus; *nest parasite;* Philornis downsi; *tolerance.*

Introduction

Introduced parasites can threaten native host populations that lack effective defenses (Daszak et al. 2000, Keesing et al. 2010). Not all hosts are vulnerable to introduced parasites, however. The fitness of some host species is clearly reduced, while the fitness of other hosts is relatively unaffected. "Unaffected" hosts may alleviate parasite damage with defense mechanisms that can include both resistance and tolerance. These two forms of defense are important to distinguish, because resistant hosts lower parasite populations, whereas tolerant hosts do not negatively affect parasite populations. Therefore, tolerant hosts provide a more stable resource for the introduced parasite (Schmid-Hempel 2011). By supporting the parasite population, tolerant hosts sustain, or increase, the "force of infection" for vulnerable host populations, defined as the fraction of the susceptible host population that the infected hosts can infect per unit of time (Anderson and May 1991, Hudson et al. 2002). For example, the introduction of parapoxvirus to Great Britain by tolerant, nonnative grey squirrel hosts has been implicated in the decline of native red squirrels (Tompkins et al. 2003). Tolerant hosts, such as the grey squirrel, maintain high levels of the parasite in the environment, while more vulnerable host species decline (Nokes 1992). For this reason, tolerant hosts can represent an indirect threat to populations of more vulnerable host species (Daszak et al. 2001, McCallum 2012).

Small island populations are particularly vulnerable to the effects of introduced parasites (Wikelski et al. 2004, Atkinson and Lapointe 2009). A classic example involves the historical introduction of avian malarial parasites and their mosquito vectors to the Hawaiian Islands. This

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introduction is thought to be partly responsible for the extinction of 17 endemic honeycreeper species (Atkinson and Lapointe 2009). Some species of honeycreepers have been relatively unaffected by introduced malarial parasites, however. Experiments with captive birds suggest that the amakihi honeycreeper (*Hemignathus virens virens*) is tolerant of the malarial parasite, and that this species of honeycreeper may therefore be a reservoir host that helps maintain the parasite in the local environment (Atkinson et al. 2000). In other words, the amakihi may be a host that essentially amplifies the negative effect of the malarial parasite on more vulnerable and declining honeycreeper species (Atkinson and Lapointe 2009).

The concept of host tolerance has seldom been tested directly under natural conditions (Read et al. 2008, Råberg et al. 2009, Svensson and Råberg 2010, Medzhitov et al. 2012). Testing for tolerant hosts requires comparing the fitness of different host genotypes or species under similar environmental conditions. Such studies are challenging because the most rigorous method for assessing the relative effects of parasites is to experimentally manipulate parasite abundance, which can be very difficult under natural conditions (McCallum and Dobson 1995).

Introduced parasites have colonized the Galápagos Islands of Ecuador in recent decades, threatening endemic birds as well as other groups of animals and plants (Wikelski et al. 2004). A notorious example of an introduced parasite is the nest fly *Philornis downsi,* which has been implicated in the decline of critically endangered species of Darwin's finches, such as the mangrove finch (*Camarhynchus heliobates*) (O'Connor et al. 2009, Fessl et al. 2010). Adult flies, which are not parasitic, lay their eggs in the nests of finches and other land birds in the Galápagos. Once the eggs hatch, fly larvae feed on the blood of nestlings and adult females as they brood the nestlings. Several studies have shown that *P. downsi* reduces the reproductive success of the medium ground finch (*Geospiza fortis*) and other species of Darwin's finches (reviewed in Koop et al. 2011). In some years, 100% of finch nests fail to produce fledglings due to *P. downsi* (Koop et al. 2011, 2013*a*, O'Connor et al. 2013). Moreover, Kleindorfer et al. (2014) recently suggested that *Philornis*-related mortality in finches has increased over the past decade, with nestling age at mortality decreasing due to *P. downsi* infestations earlier in the nestling developmental period.

Other host species nesting near Darwin's finches, such as the endemic Galápagos mockingbird (*Mimus parvulus*), may be less affected by *P. downsi* infestation. Anecdotal observations suggest that mockingbird nestlings often do not die when parasitized by *P. downsi* (personal observation). If so, then mockingbirds could be tolerant hosts that effectively amplify the force of infection for vulnerable hosts, such as Darwin's finches. The goal of the current study was to test this hypothesis by comparing the effects of *P. downsi* on the fitness of mockingbirds and medium ground finches at the same time and location. We measured the effects of parasites on nestling mockingbirds and finches over two field seasons, then compared the reaction norms of host nestling survival and parasite density between the two host species.

During the first season, we compared the effect of *P. downsi* on the size and fledging success of nestling mockingbirds and finches. We predicted a significant negative effect of *P. downsi* on the size and fledging success of finches, but not mockingbirds. We also tested for evidence of nestling immune responses that combat *P. downsi* in mockingbirds and finches.

During the second field season, we repeated these comparisons, and also explored possible mechanisms of tolerance, such as the rapid replacement of blood lost to the parasite. To test this possibility, we compared the effect of *P. downsi* on the hemoglobin of finch and mockingbird nestlings. Another possible mechanism of tolerance is increased parental care of parasitized nestlings (Tripet and Richner 1997, Hurtrez-Bousses et al. 1998, Tripet et al. 2002). Parents of such nestlings might increase their feeding rates to compensate for energy lost to parasites. One cue known to lead to increased feeding rates is increased begging by parasitized nestlings (Bengtsson and Rydén 1983, Christe et al. 1996). In some cases, however, parasitized nestlings appear to be too weak to solicit more food by begging. We compared parental and nestling behavior, as well as energy levels (via glucose), of finches and mockingbirds with and without parasites in the nest. We predicted that mockingbird nestlings in parasitized nests would beg more than nestlings in unparastized nests, and that parents in parasitized nests would provision nestlings more than parents in nonparasitized nests. We further predicted that increased begging would lead to increased glucose levels in parasitized nestlings. In contrast, finch parental provisioning does not differ in response to parasitism (Koop et al. 2013*a*). Thus, we predicted that parasitized and nonparasitized finch nestling begging and glucose levels would decrease or not differ because parasitized nestlings are too weak to increase begging.

Methods

Study system

The study was conducted January–April in both 2012 and 2013 on the island of Santa Cruz in the Galápagos Archipelago. Our 3 × 4 km field site, known as El Garrapatero, is located in the arid coastal zone; it is located approximately 10 km east of the main town of Puerto Ayora. Galápagos mockingbirds and medium ground finches are abundant at the site. Mockingbirds build open, cup-shaped nests, which are made of *Acacia* thorns (bottom layer), moss (middle layer), and coarse grasses (top layer/nest liner) and primarily found in giant prickly pear cacti (*Opuntia echios gigantea*) or *Acacia* trees. Mockingbird clutch size ranges from 1 to 5 eggs, and females incubate the eggs for about 15 d. Nestlings

spend an average of 15 d in the nest, and both the adult females and males feed them. Mockingbirds feed their nestlings by placing food items in the nestling's open mouth rather than by regurgitating food, as in the case of finches (see below). Mockingbirds usually lay a single clutch of eggs per breeding season; however, they have been reported to re-nest in a new location when the first clutch fails, sometimes due to delayed rains. Mockingbirds normally do not reuse the same nest.

Finches build dome-shaped nests, which are made of coarse grasses (exterior layer) and fine grasses (nest liner that contacts the nestlings in the cup of the nest) and primarily found in giant prickly pear cacti or *Acacia* trees (Grant 1999). Their clutch sizes range from 2 to 5 eggs, with females incubating the eggs for 10–14 d. Nestlings spend an average of 12 d in the nest, and adult females and males both feed the nestlings by regurgitating food into the nestling's throat. In years of favorable weather and food resources, medium ground finches may lay additional clutches of eggs over the course of a single breeding season; however, like mockingbirds, they do not reuse the same nest (Grant 1999).

Experimental manipulation of parasites

To quantify the effect of *P. downsi* on host fitness, experimental nests were fumigated with a 1% aqueous permethrin solution (Permectrin™ II). Control nests were sham-fumigated with water. Permethrin, which has been used in previous studies (Fessl et al. 2006, Koop et al. 2013*a,b*, O'Connor et al. 2013), is harmless to birds, including newly hatched nestlings. Nests were sprayed soon after the first nestling hatched, then again 4–6 d later. Nest contents (nestlings, unhatched eggs, and the nest liner) were removed during the spraying process. Nest contents were then returned to the nest once it had dried (<10 min). Parents quickly returned to the nest following treatment, with no cases of nest abandonment due to treatment observed for either bird species.

Nestling size and fledging success

In 2012, each nestling was weighed twice: within 24 h of hatching, then again at 9–10 d of age. In 2013, each nestling was weighed three times: within 24 h of hatching, then at one-third and again at two-thirds of the nestling developmental period. Thus, the second weighing occurred when finch nestlings were 4–5 d old, and mockingbird nestlings were 5–6 d old. The third weighing occurred when finch nestlings were 8–9 d old, and mockingbird nestlings were 10–11 d old.

Each nestling was banded with a unique darvic color band combination. Successful fledging was confirmed by identifying birds once they left the nest, as in previous studies (Koop et al. 2011, 2013*a,b*). After the birds in a nest had all fledged or died, the nest was collected and placed in a sealed plastic bag. The number of *P. downsi* in the nest was then quantified, as described below. Fledging success for finches from 2013 was first reported in Knutie et al. (2014).

Quantifying P. downsi

Each nest was carefully dissected within 8 h of collection and *P. downsi* larvae, pupae, and eclosed pupal cases were counted (Koop et al. 2011, 2013*a,b*). First instar larvae can live subcutaneously in nestlings, making them impossible to quantify reliably. Therefore, parasite abundance was the sum of counts of second and third instar larvae, pupae, and eclosed pupal cases (for both infested and uninfested nests). Parasite abundance was used to calculate parasite density, which is the number of parasites per unit of host (Bush et al. 1997). For mockingbirds and finches, density was calculated by dividing the number of parasites per nest by the total mass of nestlings for a given nest at 2/3 of the mean nestling developmental period.

Larvae and pupae were reared to the adult stage to confirm that they were *P. downsi* (Dodge and Aitken 1968). Most larvae were third instars when the nests were collected; these larvae usually pupated within 24 h. Younger larvae, which require a blood meal, died soon after they were collected from the nest and were therefore not reared to adulthood. The length and width of pupae were measured with digital calipers in mm. These measurements were then used to estimate pupal volume as a measure of individual parasite size, which is related to lifetime fitness in other Muscid flies (Schmidt and Blume 1973, Moon 1980).

Nestling hemoglobin

In 2012, blood was sampled from 9 to 10 d old nestlings. In 2013, blood was sampled from nestlings when they were at one-third and two-thirds of the nestling period. A small blood sample (<30 μL) was collected in a microcapillary tube via brachial venipuncture. Using a portion of this blood, hemoglobin concentration was quantified immediately in the field (2013 only). Hemoglobin concentration can provide an accurate estimate of ectoparasite-induced anemia (O'Brien et al. 2001, Dudaniec et al. 2006, Carleton 2008). Hemoglobin was measured with a HemoCue® HB 201+ portable analyzer, using ten microliters of whole blood per disposable microcuvette. Hemoglobin was measured in g/dL.

The remainder of each blood sample was stored on wet ice in the field. Within 6 h of collection, samples were spun at 8000 rpm for 10 min in a centrifuge. Plasma and red blood cells were stored separately in 0.5 mL vials in a −20°C freezer at the Charles Darwin Research Station. Samples were later frozen at −80°C after being transported in liquid nitrogen to the University of Utah. The samples were ultimately used for the immunological and glucose assays described below.

Nestling immunology

Enzyme-linked immunosorbent assays (ELISA) were used to detect the presence of *P. downsi*-binding antibodies in the plasma of finches and mockingbirds, with a modification of the protocol in Koop et al. (2013*a*). Ninety-six well plates were coated with 100 μL/well of *P. downsi* protein extract (capture antigen) diluted in carbonate coating buffer (0.05 mol/L, pH 9.6). Plates were incubated overnight at 4°C, then washed and coated with 200 μL/well of bovine serum albumin (BSA) blocking buffer and incubated for 30 min at room temperature on an orbital table. Between each of the following steps, plates were washed five times with a Tris-buffered saline wash solution, loaded as described, and incubated for 1 h on an orbital table at room temperature. Triplicate wells were loaded with 100 μL/well of individual host plasma (diluted 1:100 in sample buffer). Plates were then loaded with 100 μL/well of Goat-αBird-IgG (diluted 1:50,000) (Antibodies Online, Atlanta, GA, USA; ABIN351982). Finally, plates were loaded with 100 μL/well of peroxidase substrate (tetramethylbenzidine, TMB; Bethyl Laboratories, Montgomery, TX, USA) and incubated for exactly 30 min. The reaction was halted using 100 μL/well of stop solution (Bethyl Laboratories). Optical density (OD) was measured with a spectrophotometer (BioTek, Winooski, VT, USA; PowerWave HT, 450-nanometer filter).

On each plate, a positive control of pooled plasma from naturally *P. downsi* parasitized adult female finches from the 2013 field season was used in triplicate to correct for inter-plate variation (Koop et al. 2013*a*). In addition, each plate contained a non-specific binding (NSB) sample in which capture antigen and detection antibody were added, but plasma was excluded. Finally, each plate included a blank sample in which only the detection antibody was added, but plasma and capture antigen were excluded. NSB absorbance values were subtracted from the mean OD value of each sample to account for background binding of the detection antibody to the capture antigen.

Nestling glucose

Plasma glucose was measured using blood samples taken from mockingbird and finch nestlings at about the same time their behavior was quantified; see below. An Endpoint Autokit (Wako, Diagnostics, Mountain View, CA, USA) was used to measure plasma glucose for mockingbirds and finches with a modified protocol based on Guglielmo et al. (2013). The kit provided 500 mg/dL and 200 mg/dL standards. Following the manufacturer's protocol, the buffer solution and color reagent were mixed together, then refrigerated at 4°C until they were used in the assay. Three microliters of sample or standard were run primarily in duplicate, assuming sufficient sample was available, on Nunc® MicroPlate™ 96-well polystyrene plates (Sigma-Aldrich, St. Louis, MO, USA). The buffer solution was pre-warmed to 37°C, then 300 μL were added to each well. The plate was incubated at 37°C on a microplate incubator shaker (Stat Fax® 2200) for 10 min, then shaken for 10 s on low speed. Optical density (OD) was measured using a spectrophotometer (BioTek; PowerWave HT, 505-nanometer filter). Samples were corrected for intraplate variation based on the 500 mg/dL standard. From the standards, a standard curve was created ranging from 50 to 500 mg/dL. Glucose concentration (mg/dL) for each sample was calculated using the OD value of the sample (x), and the slope and intercept of the line from the standard curve ($y = 0.003x + 0.0352$).

Nestling and adult behavior

Mockingbird behavior was recorded during the 2013 field season. Because we had a limited number of nest cameras and recording devices, and because we collected behavioral data from fumigated and sham-fumigated finch nests from the same field site in 2010, we chose to concentrate on recording mockingbird data in 2013; see Koop et al. (2013*a*) for details on finch behavior. Behavior was monitored with battery-powered Sony® video camera systems. Small nest cameras (31 mm in diameter, 36 mm in length) were suspended above nests; seven-meter long cables connected the cameras to small recording devices (PV700 Hi-res DVR; StuntCams, Grand Rapids, MI, USA) hidden near the base of the tree supporting the nest. Behavior was recorded between 0600 and 1000 using haphazard subsamples of fumigated and sham-fumigated nests.

Mockingbird behavior was quantified from videos by one of the authors (M.T.) who was blind to nest identity or treatment. A similar "blind" approach was used for finch behavior (Koop et al. 2013*a*). Videos were analyzed with the software VLC media player (VideoLAN, Paris, France), except in the case of begging, which was analyzed using CowLog v.2.1 (Hänninen and Pastell 2009). A single day of video from each of two nests was paired between treatments to control for hatch date, brood size, and nestling age. There was no significant difference in brood size or nestling age between treatments.

Nestling begging was defined as one or more nestlings tilting their head back, with the neck extended and the open mouth showing (Christe et al. 1996). Begging time was calculated as the proportion of total video time. The proportion of video time with nestling agitation behavior, defined as shaking, repositioning, or jumping in the nest, was also quantified.

Adult behaviors included the proportion of time each adult spent at the nest. We were unable to distinguish female and male mockingbirds because they are not sexually dimorphic. The following adult behaviors were quantified: brooding nestlings, standing erect in the nest, standing motionless on the rim of the nest, nest sanitation, self-preening, allopreening nestlings, and provisioning (feeding) nestlings. Brooding was defined as the adult sitting on the nest in direct contact with nestlings. Nest sanitation was defined as the adult contacting or

manipulating nest material with its bill. Provisioning of nestlings was defined as the insertion of the bill into the mouths of nestlings by adults; note, however, that we were unable to determine how much food was actually delivered. Because adults often preen themselves while brooding nestlings, self-preening was analyzed separately from the other behaviors. All other behaviors were analyzed as the proportion of total time that adults were observed.

Mockingbird behaviors were quantified from a total of 41 h of video, with an average of 2.5 h of video for each of the 16 mockingbird nests (eight fumigated, eight sham-fumigated). Mockingbird nestlings in the videos ranged in age from 3 to 6 d, and brood size ranged from 1 to 5 nestlings. Finch behaviors were quantified from a total of 54 h of video, with an average of 3 h of video for each of the 18 finch nests (nine fumigated, nine sham-fumigated; Koop et al. 2013*a*). Finch nestlings in the videos ranged in age from 2 to 6 d, and brood size ranged from 1 to 5 nestlings. The data for adult finch behavior were reported separately by sex in Koop et al. (2013*a*). For our analyses these data were pooled.

Statistical analyses

Parasite abundance, density, and volume were analyzed using generalized linear models (GLM) with a negative binomial family and logit link function for abundance and a Gaussian family and identity link function for density and volume; year (2012 or 2013) and host species (mockingbird or finch) were fixed effects for all three variables and treatment (fumigated or sham-fumigated) was a fixed effect for parasite abundance.

Data for individual nestlings were analyzed with generalized linear mixed models (GLMM) using nest as a random effect and year, host species, age, and treatment as fixed effects. Fledging success was modeled with a binomial family and logit link function; year, host species, and treatment were fixed effects. Mass, immune response, hemoglobin, and glucose were modeled with a Gaussian family and identity link function; year, host species, age, and treatment were fixed effects for mass and immune response, host species, age, and treatment were fixed effects for hemoglobin, and host species and treatment were fixed effects for glucose.

For each of the GLM and GLMM analyses, we developed a set of *a priori* models that included single, additive, and interactive effects of variables that we hypothesized had biologically meaningful effects of the response variables of interest. For example, year, host species, and treatment were predicted to affect parasite abundance; therefore, we analyzed the effect of year, species, and treatment alone, and all two and three-way interactions (Appendix S1). We ranked models using Akaike's Information Criterion with adjustment for small sample size (AICc). We report AICc differences (ΔAICc) and Akaike weights (ω) to determine the strength of evidence for each model, relative to the set of candidate models (Burnham and Anderson 2002). To account for model selection uncertainty, we averaged across all models to calculate model-averaged parameter estimates ($\tilde{\beta}$) with shrinkage, as well as *z*-values and *P*-values, for each variable and their interaction(s).

Host nestling and adult behavior were compared between treatments using a chi-square test to match previously reported analyses of finch behavior from Koop et al. (2013*a*); specific behaviors were compared between treatments using Fisher's exact tests. GLMM and GLM analyses were performed in the program RStudio, version 3.1.1 (R Core Team 2014) using the lme4, MuMln, nlme, and MASS packages. Prism® v.5.0b (GraphPad Software, Inc., La Jolla, CA, USA) was used for all other analyses and to create figures.

Results

Quantifying P. downsi

Top ranked models included the effect of treatment and host species on parasite abundance and both variables were in every model with an Akaike weight of >0.10, indicating their importance (Appendix S1: Table S2). The experimental treatment of nests with permethrin was effective at reducing parasite abundance, compared to sham-fumigated control nests for both host species in both years of the study (GLM, Treatment, $\tilde{\beta} \pm SE = -5.06 \pm 0.57$, $P < 0.0001$; Appendix S1: Table S3). Parasite abundance was significantly higher in mockingbird nests than finch nests in both years (Species, $\tilde{\beta} \pm SE = 1.11 \pm 0.39$, $P < 0.001$; Appendix S1: Table S3). However, variation in parasite density (parasites per gram of nestling) and parasite size (pupal volume) was not explained by any of the predictors that we measured in our study (Density: Species, $\tilde{\beta} \pm SE = -0.06 \pm 0.21$, $P = 0.80$; Table 1; Appendix S2: Tables S2 and S3 Size: Species, $\tilde{\beta} \pm SE = 0.01 \pm 0.04$, $P = 0.67$; Table 1; Appendix S3: Tables S2 and S3).

Nestling mass

For nestling mass, all top models included the effect of age ($\omega > 0.10$; Appendix S4: Table S3). Nestling mass increased significantly with increasing age in both species (GLMM, Age, $\tilde{\beta} \pm SE = 1.43 \pm 0.05$, $P < 0.0001$; Appendix S4: Table S4). Top models ($\omega > 0.10$) also included the interaction between age and species and age and treatment. As expected, mockingbirds weighed significantly more than finches (Age × Species, $\tilde{\beta} \pm SE = 1.96 \pm 0.05$, $P < 0.0001$; Appendix S4: Table S4). *P. downsi* had a significant effect on the mass of both mockingbird and finch nestlings, but only in older nestlings (Age × Treatment, $\tilde{\beta} \pm SE = 0.13 \pm 0.05$, $P = 0.01$; Appendix S4: Table S4). The body mass of older nestlings in fumigated nests was significantly greater than the body mass of older nestlings in sham fumigated nests (Appendix S4: Table S1).

TABLE 1. Comparison of *Philornis downsi* number and size, and host fledging success in mockingbirds and finches in fumigated (F) and sham-fumigated (SF) nests. Parasite density is the number of parasites per gram of host.

	Galápagos mockingbird				Medium ground finch			
	2012		2013		2012		2013	
	F	SF	F	SF	F	SF	F	SF
Mean ± SE parasite density (Number of nests)	—	1.00 ± 0.29 (14)	—	1.06 ± 0.47 (13)	—	1.49 ± 0.52 (8)	—	1.06 ± 0.42 (12)
Mean ± SE pupal volume, mm^3 (Number of nests)	—	115.20 ± 6.57 (13)	—	120.10 ± 8.52 (14)	—	108.30 ± 6.93 (9)	—	117.50 ± 5.70 (9)
Fledglings, %	76.5	77.8	70.0	66.7	86.0	34.2	83.3	53.7
(Number of nestlings)	(51)	(54)	(47)	(54)	(43)	(38)	(60)	(54)
Nests with at least one fledgling, %	87.5	87.5	76.5	76.5	91.7	50.0	95.0	64.7
(Number of nests)	(16)	(16)	(17)	(17)	(12)	(12)	(20)	(17)

Fledging success

For fledging success, all top models ($\omega > 0.10$) included the effect of an interaction between species and treatment (Appendix S5: Table S1). Treatment had a significant effect on the fledging success of finches, but not mockingbirds (GLMM, Species × Treatment, $\tilde{\beta} \pm SE = -4.33 \pm 1.14$, $P < 0.001$; Tables 1; Fig. 1; Appendix S5: Table S2). That is, *P. downsi* reduced fledging success of finches but had no effect on mockingbirds. Parasite density was a significant predictor of fledging success in finches, but not mockingbirds (GLM, Density × Species, $\chi^2 = 16.24$, df = 1, $P < 0.0001$; Fig. 1).

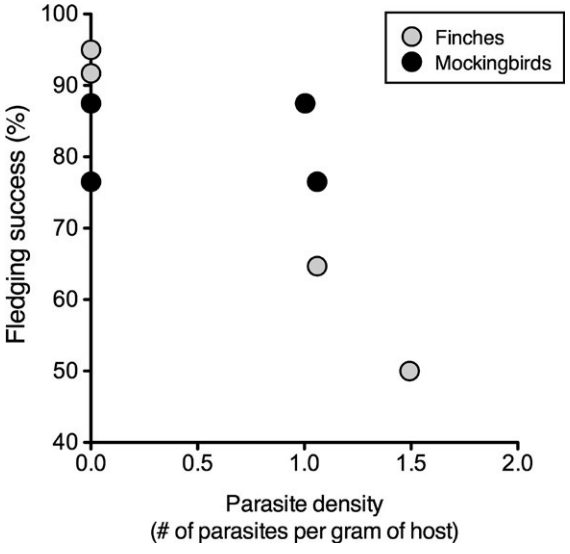

FIG. 1. Reaction norms for fledging success in finches and mockingbirds across different *Philornis downsi* densities. Each point represents percentage of fledging success, or the percentage of hatchlings that successfully left the nest, plotted against mean parasite density within a treatment and year. Mockingbirds are more tolerant to *P. downsi*; parasite density is not a significant predictor of fledging success. In contrast, parasite density is a significant predictor of fledging success in finches.

Nestling immunology

Philornis downsi was not a significant predictor of antibody levels because treatment was not included in any of the top models ($\omega > 0.10$; Appendix S6: Table S3). Antibody levels within each species were low (Appendix S6: Table S1). However, the top models included an effect of year, species, and their interaction, on antibody levels (Appendix S6: Table S3). For finches, antibody levels were significantly higher in 2012 than 2013 (GLMM, Species × Year, $\tilde{\beta} \pm SE = 0.16 \pm 0.04$, $P < 0.001$; Appendix S6: Table S4). Finches also had significantly higher antibody levels than mockingbirds (Species, $\tilde{\beta} \pm SE = -320.70 \pm 88.40$, $P < 0.001$; Appendix S6: Table S4).

Nestling hemoglobin

Top models included the effect of age and an age × treatment interaction on nestling hemoglobin levels ($\omega > 0.10$; Appendix S7: Table S1). Mockingbird and finch nestling hemoglobin increased significantly with age (GLMM, Age, $\tilde{\beta} \pm SE = 0.33 \pm 0.04$, $P < 0.0001$; Appendix S7: Table S3). There was a significant effect of *P. downsi* on the hemoglobin of older nestlings in both species of hosts (Age × Treatment, $\tilde{\beta} \pm SE = 0.30 \pm 0.06$, $P < 0.0001$; Appendix S7: Table S3). Older mockingbird nestlings in fumigated nests had 40% more hemoglobin than similar aged nestlings in sham-fumigated nests (Fig. 2; Appendix S7: Table S1). Older finch nestlings in fumigated nests had 14% more hemoglobin than similar aged nestlings in sham-fumigated nests (Fig. 2; Appendix S7: Table S1).

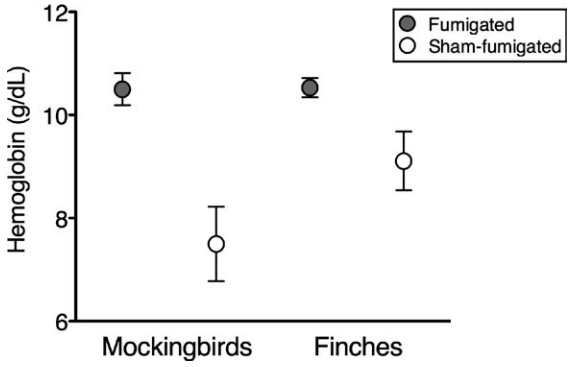

FIG. 2. Mean (±SE) hemoglobin in nestlings from fumigated and sham-fumigated nests. Nestlings in fumigated nests had significantly higher hemoglobin levels than nestlings in sham-fumigated nests for both species of birds.

Nestling glucose

The top model included the effect of a species by treatment interaction on nestling glucose levels and carried nearly all of the Akaike weight ($\omega = 0.99$; Appendix S8: Table S2). Mockingbird nestlings in fumigated nests had significantly lower plasma glucose levels than mockingbird nestlings in sham-fumigated nests (GLMM, Species × Treatment, $\tilde{\beta} \pm SE = -31.10 \pm 15.79$, $P = 0.05$; Fig. 3; Appendix S8: Table S3). In contrast, parasite abundance was not a significant predictor of glucose concentration in finch nestlings (Fig. 3).

Nestling and adult behavior

Agitation behavior did not differ significantly between mockingbird nestlings from fumigated and sham-fumigated nests (Table 2). However, mockingbird nestlings from sham-fumigated nests spent significantly more time begging than nestlings from fumigated nests (Table 2; Fig. 4A).

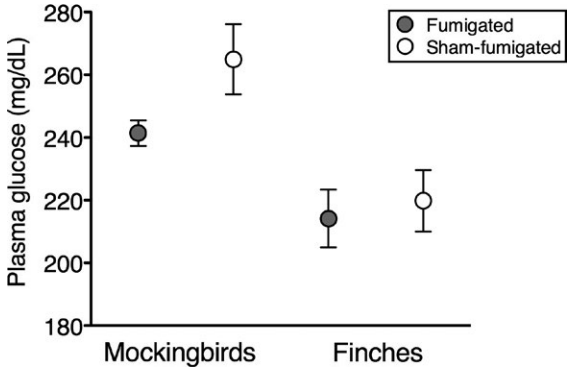

FIG. 3. Mean (±SE) plasma glucose levels in mockingbird and finch nestlings from fumigated and sham-fumigated nests. Mockingbird nestlings in sham-fumigated nests had higher glucose levels than nestlings in fumigated nests. In contrast, glucose levels did not differ significantly between treatments for finches.

The amount of time adult mockingbirds spent at fumigated and sham-fumigated nests did not differ significantly (Table 2). There was no significant effect of treatment on self-preening by adult mockingbirds ($W = -3.00$, $P = 0.81$); note, however, that the birds spent <0.01% of their time engaged in self-preening at the nest. We did not collect data on self-preening or other behaviors in birds away from the nest.

Adult mockingbirds differed significantly in the time they devoted to other (mutually exclusive) behaviors at fumigated vs. sham-fumigated nests (Chi-square test: $\chi^2 = 18.90$, df = 5, $P < 0.001$). The largest difference was in the time adult mockingbirds spent brooding nestlings, with adults at fumigated nests spending significantly more time brooding than adults at sham-fumigated nests (Table 2). When mockingbirds from sham-fumigated nests were not brooding, they were either standing erect in the nest, or standing erect on the rim of the nest (Table 2); however, these behaviors did not differ significantly between treatments. Adults on the rim of nests occasionally probed nest material (nest sanitation behavior), allopreened nestlings, or provisioned nestlings.

Adult mockingbirds spent very little time in nest sanitation behavior, and there was no significant effect of treatment on this behavior (Table 2). When adult mockingbirds from sham-fumigated nests were not brooding, but were still present at the nest, they spent most of their time allopreening nestlings while standing on the rim of the nest; however, there was no significant difference in allopreening between treatments (Table 2).

Adults from fumigated nests spent significantly less time in provisioning behavior, compared to adults from sham-fumigated nests (Table 2; Fig. 4A). The amount of time parents spent in provisioning behavior was positively correlated with the amount of time nestlings spent begging (Spearman rank correlation: $r_S = 0.52$, $P = 0.04$).

In contrast to mockingbirds, nestlings in fumigated finch nests did not beg more than nestlings in sham-fumigated finch nests (Table 2; Fig. 4B). The time adult finches spent at fumigated and sham-fumigated nests did not differ significantly (Table 2). The time parents spent in provisioning behavior was correlated with nestling begging time (Spearman rank correlation: $r_S = 0.81$, $P < 0.0001$). However, adult finches did not differ significantly in the amount of time they spent in provisioning behavior at fumigated and sham-fumigated nests (Table 2; Fig. 4B). See Koop et al. (2013*a*) for further details of finch behavior.

DISCUSSION

The effect of *P. downsi* varied considerably between finches and mockingbirds within the same years and location. *P. downsi* reduced the fledging success of Darwin's finch nestlings; however, despite a similar density of flies in mockingbird nests, *P. downsi* had no significant effect on mockingbird fledging success. Thus,

TABLE 2. Behavior of nestling and adult mockingbirds and finches in fumigated and sham-fumigated nests. For mockingbirds, each treatment contained eight nests; for finches, each treatment contained nine nests. For nestlings, values are the mean ± SE percent time out of total video time. For adults, most values are the mean ± SE percent time out of total attendance time at nest. Attendance time is the total time at the nest out of total video time. Wilcoxon signed rank tests were used to compare treatments within each behavior. See Koop et al. (2013*a*) further details of finch behavior.

	Fumigated (%)	Sham-fumigated (%)	*W* statistic	*P*-value
Galápagos mockingbird				
Nestlings				
Begging	3.12 ± 0.74	5.78 ± 0.98	−32.00	0.02
Agitation	10.38 ± 2.16	12.86 ± 3.07	−6.00	0.74
Adults				
Attendance at nest	54.59 ± 5.00	50.45 ± 6.78	10.00	0.55
Brooding	70.35 ± 5.05	41.12 ± 8.11	32.00	0.02
Standing erect in nest	2.27 ± 0.57	9.03 ± 7.46	8.00	0.64
Standing on rim of nest	7.77 ± 1.85	11.87 ± 2.65	−18.00	0.25
Nest sanitation	0.92 ± 0.43	1.24 ± 0.32	−16.00	0.31
Allopreening	15.18 ± 3.85	30.77 ± 7.36	−24.00	0.11
Provisioning behavior	3.50 ± 0.56	5.98 ± 1.04	−32.00	0.02
Medium ground finch				
Nestlings				
Begging	6.85 ± 0.90	5.53 ± 0.86	25.00	0.16
Adults				
Attendance at nest	47.29 ± 6.10	57.72 ± 9.14	−23.00	0.20
Provisioning behavior	11.05 ± 2.19	10.45 ± 4.90	27.00	0.13

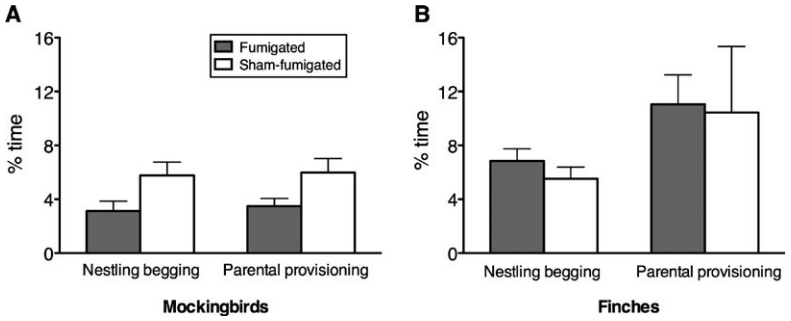

FIG. 4. Nestling and parental behavior (mean ±SE) in fumigated and sham-fumigated nests for (A) mockingbirds and (B) finches. Time allocated to nestling begging and parental provisioning behavior was significantly higher in sham-fumigated mockingbird nests than in fumigated nests. In contrast, the amount of time spent on these behaviors did not differ significantly between treatments in finch nests.

we suggest that this provides evidence that mockingbirds are tolerant hosts, whereas finches are highly vulnerable to the parasite. We then explored potential tolerance mechanisms used by mockingbirds to deal with *P. downsi*. We found that mockingbird nestlings from sham-fumigated nests begged significantly more than nestlings from fumigated nests. Greater begging was correlated with increased parental provisioning, which may have been responsible for the higher glucose concentration in nestlings in sham-fumigated nests. In contrast to mockingbirds, finch nestling begging and parental provisioning did not change in response to *P. downsi*, nor was there a difference in the plasma glucose levels of nestlings in fumigated and sham-fumigated nests. We suggest that these behavioral differences indicate adaptive tolerance of *P. downsi* by mockingbirds. The difference in the effect of *P. downsi* on tolerant vs. non-tolerant hosts motivates the question: How can hosts vary in their susceptibility to the same parasite at the same time in the same place?

Neither mockingbird nor finch nestlings produced a significant antibody-mediated immune response to *P. downsi* in our study. In fact, antibody levels in nestling finches (and mockingbirds) were nearly undetectable, compared to those measured in adult finches in an earlier study (Koop et al. 2013*a*). Captive house sparrows (*Passer domesticus*) are capable of producing independent antibody-mediated immune responses at 3 d of age when challenged with non-specific antigens (King et al. 2010). However, we found no evidence of such responses by finch or mockingbird nestlings parasitized by *P. downsi*.

It is possible that our assay was not sensitive enough to detect low concentrations of antibodies. Antibody levels did increase with nestling age, but this increase did not differ significantly between experimental treatments. One other possibility is that these hosts are exposed to relatively few native parasites in the Galápagos, meaning that non-specific antibody-mediated immune responses are not primed as much as they would be on the mainland. On the other hand, the antibodies we detected may not be specific to *P. downsi*. Instead, the antibodies may have also been influenced by other biting insects, such as mosquitoes, which have antigens in their saliva that induce similar responses to those induced by *P. downsi* (e.g., IgG) (Peng et al. 1996). Nevertheless, our results suggest that nestling immune responses do not ameliorate the effects of *P. downsi* on mockingbirds or finches.

Mockingbird parents from sham-fumigated nests brooded their nestlings less than parents from fumigated nests. Mockingbird parents were still present at the nest, but they may have been avoiding the parasites by standing on the rim of the nest. Koop et al. (2013*a*) found that adult finches in sham-fumigated nests also brood their nestlings less, and stand erect more, compared to adult finches in fumigated nests. Mockingbird parents also allopreen their nestlings; however, it was not clear from our video analyses whether allopreening removes or damages *P. downsi* (cf. Clayton et al. 2010). It is possible that allopreening does provide at least some defense against *P. downsi*. Further tests are needed to determine the extent to which mockingbirds can reduce *P. downsi* on their nestlings by allopreening them.

Mockingbirds may tolerate the effects of *P. downsi* by increasing parental provisioning of nestlings to compensate for energy lost to parasites. In other systems, parasitic flies are known to increase host metabolic rate, which depletes host energy resources (Careau et al. 2010). Several studies of other systems have also shown that parents in parasitized nests feed their nestlings more than parents in nonparasitized nests, leading to increased fledging success (Tripet and Richner 1997, Hurtrez-Bousses et al. 1998, Tripet et al. 2002). Our study suggests that increased begging by mockingbird nestlings in sham-fumigated nests led to increased provisioning by parents, which likely contributed to the improved survival of nestlings in these nests. A more definitive test of this hypothesis would involve comparison of the quality and quantity of food being delivered to nestlings between experimental treatments and species. It would also be interesting to test the begging-provisioning hypothesis by using recordings to simulate increased begging in nests to see if parents respond with the delivery of more food to the nestlings (Bengtsson and Rydén 1983, Ottosson et al. 1997).

Why do finch nestlings not beg more in sham-fumigated nests, the way that mockingbird nestlings do? The answer may lie in the smaller body size of finch nestlings, which are only half the size of mockingbird nestlings. Smaller birds require more energy per gram of body mass because they have a higher surface-to-volume ratio than larger birds (Schmidt-Nielsen 1984). Thus, small-bodied species tend to beg more than large-bodied species (Price and Ydenberg 1995, Christe et al. 1996, Leech and Leonard 1996, Kitaysky et al. 2001, Saino et al. 2001, Simon et al. 2005). As a result, they may also be fed more by their parents (Christe et al. 1996). Interestingly, nestlings in fumigated finch nests spent more than twice as much time begging as nestlings in fumigated mockingbird nests. Because begging by small birds is more energetically costly (per gram) than begging by large birds (Jurisevic et al. 1999), finch nestlings may experience an energetic ceiling beyond which they are simply incapable of additional begging. On the other hand, some small-bodied species of birds are known to increase begging in response to native parasitic flies (Christe et al. 1996). However, O'Connor et al. (2013) similarly found that *P. downsi* does not have a significant effect on another small-bodied species of Darwin's finch. This topic clearly requires further exploration.

Similar to finches, older parasitized mockingbird nestlings had lower hemoglobin and mass compared to nonparasitized nestlings. Dudaniec et al. (2006) similarly found that hemoglobin decreases as *P. downsi* abundance increases in Darwin's finches. Because hemoglobin and mass are indicators of body condition, we cannot discount the possibility that the post-fledging survival of parasitized mockingbirds was less, compared to nonparasitized mockingbirds. For example, Streby et al. (2009) found that, despite similar fledging success, parasitized ovenbirds (*Seiurus aurocapilla*) had lower post-fledging survival than nonparasitized fledglings. Alternatively, fledglings from parasitized nests may recover body mass and hemoglobin once they have left the nest, especially given that mockingbird parents continue to feed fledglings for at least a month after they leave the nest (S.A.K. personal observation). A future study could track post-fledgling survival of parasitized and nonparasitized mockingbird and finch nestlings to determine if *P. downsi* has a delayed effect on survival.

Our study is one of the first to show differential effects of an introduced parasite on different host species under natural conditions, including evidence for possible tolerance mechanisms. Only recently has the idea of animal host tolerance to parasitism become widely recognized as an important defense strategy (Read et al. 2008, Råberg et al. 2009, Baucom and de Roode 2011, Medzhitov et al. 2012, Sorci 2013). Tolerant hosts may be important ecological mediators of the effects of parasites on vulnerable hosts. Tolerant hosts may serve as parasite reservoirs, amplifying the effects of parasites on nontolerant hosts. Identifying reservoir hosts can have important conservation implications if the vulnerable host population is declining, or if the reservoir host population is increasing. However, rigorous studies of reservoir hosts are difficult because they ideally would require experimental manipulations at the population level. For example, suspected reservoir hosts could be

removed from the community, and the consequences of removal assessed for more vulnerable host species (Haydon et al. 2002, Laurenson et al. 2003). This approach is typically not feasible, particularly if the reservoir host is a protected species. In the mockingbird-finch-fly system, a future study could control or eliminate parasites from mockingbird nests at some sites, then compare the population dynamics of finches (and flies) across all sites.

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Supporting Information

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