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ECOSPHERE

Tri-trophic ecology of native parasitic nest flies of birds in Tobago

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Citation: Knutie, S. A., J. M. Herman, J. P. Owen, and D. H. Clayton. 2017. Tri-trophic ecology of native parasitic nest flies of birds in Tobago. Ecosphere 8(1):e01670. 10.1002/ecs2.1670

Abstract. Introduced parasites threaten host populations around the world. For example, introduced parasitic nest flies (*Philornis downsi*) have contributed to the decline of several species of Darwin's finches in the Galápagos Islands. Introduced parasites are thought to have severe effects on native hosts because the hosts do not have effective defenses against such parasites and/or because introduced parasites have escaped the native enemies that keep their own populations in check. Studying effects of parasites on native hosts is an essential step in testing these causal hypotheses. We conducted a field experiment to assess the virulence of a native species of *Philornis* (*Philornis trinitensis*), which parasitizes birds on the island of Tobago. We manipulated flies in nests of black-faced grassquits (*Tiaris bicolor*), a close relative of Darwin's finches, as well as tropical mockingbirds (*Mimus gilvus*), a congener of the Galápagos mockingbird (*Mimus parvulus*). We predicted that *P. trinitensis* would be relatively avirulent because its native hosts in Tobago have had time to evolve effective defenses against it. We also noted the presence of parasitoids and other enemies of *Philornis* in Tobago nests. Surprisingly, effects of native *P. trinitensis* on Tobago birds were similar to the effects of introduced *P. downsi* on birds in the Galápagos. Flies reduced the reproductive success of grassquits, but not mockingbirds, which are also relatively unaffected by *Philornis* in the Galapagos. Thus, native *Philornis* flies are not less virulent than introduced flies. The prevalence of *Philornis* in Tobago was lower than the prevalence of *Philornis* in the Galápagos. Presumed enemies of *Philornis* (parasitoid wasps and ants) were relatively common in nests of birds in Tobago, but largely absent from nests in the Galápagos. We suggest that introduced *P. downsi* in the Galápagos is widespread, not because hosts lack defenses, but because it has left its enemies behind.

Key words: ecoimmunology; enemy release hypothesis; host defense hypothesis; invasive species; tolerance.

Received 28 May 2016; revised 7 December 2016; accepted 7 December 2016. Corresponding Editor: Christopher Lepczyk.

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INTRODUCTION

Introduced parasites are often more virulent than native parasites (Daszak et al. 2000, Keesing et al. 2010). One hypothesis to explain this observation is that hosts lack effective defenses against introduced parasites ("host defense" hypothesis). A classic example is the historical introduction of avian malarial parasites and their mosquito vectors to the Hawaiian Islands. This introduction is thought to have been partly responsible for the extinction of many endemic honeycreeper species, which presumably had few defenses against the parasites (Atkinson and Lapointe 2009). In contrast, native hosts of malarial parasites do not usually suffer the same detrimental effects, presumably because their long-standing interaction with the parasites has selected for effective host defenses (Lachish et al. 2011). Generally speaking, birds have many effective behavioral and

immunological defenses against native parasites (Loye and Zuk 1991, Clayton and Moore 1997), including ectoparasites (Moller et al. 1990, Lehmann 1993, Clayton et al. 2010, Owen et al. 2010). This battery of defenses by native birds against native parasites is consistent with the host defense hypothesis.

Another hypothesis to explain the detrimental effects of introduced parasites on hosts, which focuses on the parasite, is the "enemy release" hypothesis (Keane and Crawley 2002, Liu and Stiling 2006). This hypothesis suggests that introduced parasites spread rapidly because they have escaped native enemies that keep their populations in check. The enemy release hypothesis is one of the most cited explanations for the success of introduced plant species, but only recently has this hypothesis been empirically tested (reviewed in Liu and Stiling 2006). Enemy release may also be important in animal host–parasite systems, but most research has focused on introduced hosts escaping parasites in new locations, rather than parasites escaping native enemies (Torchin et al. 2003, Torchin and Mitchell 2004).

Tests of the host defense and enemy release hypotheses, which are not mutually exclusive, are important for understanding the effect of introduced parasites on hosts. Unfortunately, relatively few comparative data are available regarding the effects of introduced and native parasites on hosts under similar ecological conditions (reviewed in Colautti et al. 2004, Wikelski et al. 2004). Because introduced parasites are increasingly common worldwide, it is important to understand their effect on native communities.

An introduced parasite that has received a great deal of attention is the nest fly *Philornis downsi* (Diptera: Muscidae), which parasitizes land birds in the Galápagos Islands. It was first documented in nests of Galápagos birds in 1997 (Fessl et al. 2001). The source of colonization was probably mainland Ecuador, 1000 km east of the Galápagos (Bulgarella et al. 2015). Adult *Philornis* flies are not parasitic, but feed on decaying matter. The females deposit their eggs in bird nests where, after the eggs hatch, the larval flies feed on the blood of nestlings and their mothers (Fessl et al. 2006, Koop et al. 2013). The flies pupate in the nest, from which they eclose as adults about ten days later (Fessl et al. 2006). Several studies show that *P. downsi* reduces the reproductive success of Darwin's finches by as much as 100% (Koop et al. 2011, 2013*b*, 2016, Knutie et al. 2014, Kleindorfer and Dudaniec 2016). For this reason, *P. downsi* has been implicated in the decline of several threatened and endangered species of Darwin's finches (O'Connor et al. 2009, Fessl et al. 2010).

The genus *Philornis* includes approximately 50 species of flies that are found in tropical and subtropical habitats of the Americas (De Carvalho et al. 2005). Little is known about the ecology of most of these species, including those living under ecological conditions similar to the Galápagos (Dudaniec and Kleindorfer 2006). The main goal of our study was to explore the effects of a native species of *Philornis* on island birds living under conditions similar to those in lowland scrub habitat in the Galápagos. To this end, we chose *Philornis trinitensis* on the island of Tobago, which is of volcanic origin, like the Galápagos, but very close (<50 km) to the South American mainland. *Philornis trinitensis* was originally described from adjacent Trinidad (Dodge and Aitken 1968), and it is the only known species of *Philornis* on Tobago.

In our study, we explored the host defense and enemy release hypotheses using native *Philornis* flies and their hosts. We quantified the effect of *P. trinitensis* on growth and survival of black-faced grassquit (*Tiaris bicolor*) nestlings, which is a close relative of Darwin's finches (Burns et al. 2002). We also measured the effect of *P. trinitensis* on the tropical mockingbird (*Mimus gilvus*), which is a congener of the Galápagos mockingbird (*Mimus parvulus*). We quantified the antibody-mediated immune response (i.e., resistance) to *P. trinitensis* in grassquits and mockingbirds and sampled the nests of both species for enemies of *Philornis*, such as parasitoids and ants. We predicted that Tobago birds would have effective defenses (e.g., *Philornis*-binding immune responses) because of their long association with *P. trinitensis*. Concomitantly, we predicted that *P. trinitensis* would have a relatively small effect on the growth and survival of Tobago hosts.

Methods

Study species and site

Our study was conducted in western Tobago from May to July 2012. Tobago is located in the southern Caribbean Sea (11°15′ N, 60°40′ W).

Fig. 1. Twelve-day-old tropical mockingbird nestling with approximately 70 subcutaneous *Philornis trinitensis* larvae [Photo by Jordan Herman].

Black-faced grassquits, tropical mockingbirds, and *P. trinitensis* are all abundant on the island. Black-faced grassquits build dome-shaped nests primarily in ornamental shrubs. Clutch size ranges from 1 to 5 eggs, and adult females incubate the eggs for approximately 12 days (Restall 2003). After the eggs hatch, nestlings spend 9–12 days in the nest, prior to fledging. Tropical mockingbirds build open nests, primarily in ornamental shrubs and palm trees. Clutch size ranges from 1 to 4 eggs and females incubate for 13–15 days. After the eggs hatch, nestlings spend about 15 days in the nest prior to fledging (Ffrench 1991). Adult *P. trinitensis* flies, which are not parasitic, lay their eggs in the nests of both grassquits and mockingbirds. Once the fly eggs hatch, the larvae burrow beneath the skin of the nestlings, where they feed on blood and other fluids (Fig. 1).

Experimental manipulation of Philornis trinitensis

The same experimental approach was used for both host species. To quantify the effect of *P. trinitensis* on host fitness, we searched for active nests and assigned alternate nests (within species) to experimental and control groups. Experimental nests were sprayed with a 1% permethrin solution (Permectrin™ II, KMG-Bernuth, Inc., Houston, Texas, USA) soon after the first nestling hatched, then again 4–6 days later. Control nests were sham-fumigated with water. Nest contents (nestlings, unhatched eggs, and the nest liner) were removed during the spraying process and then returned to the nest after it had dried (<10 min). Thus, nestlings had little, if any, direct contact with permethrin. Parents quickly returned to the nest following treatment, with no cases of nest abandonment due to treatment observed for either bird species.

Newly hatched nestlings were marked individually by coloring one of their toenails with a permanent marker. When they were 9–10 days of age, they were given a unique color band combination and weighed (g). Their first primary feather and right tarsus were also measured (mm), and we took a blood sample (<30 μL) from each nestling via brachial venipuncture to test for antibody-mediated immune responses. Blood samples were collected in heparinized microcapillary tubes and stored on wet ice in the field. Within 6 h of collection, the samples were spun for 10 min in a hand-crank centrifuge to separate plasma from red blood cells. We did not observe any hemolysis in our plasma samples. Samples were stored in a freezer at −20°C until we returned to our home institution, where they were stored at −80°C.

Successful fledging was confirmed by identifying individual birds after they left the nest, as in

previous studies (Koop et al. 2013*b*, Knutie et al. 2016). Once the birds in a nest had fledged or died, the nest was collected and placed in a sealed plastic bag for transport back to the lab. Each nest was dissected within 4 h of collection. *Philornis trinitensis* abundance was the sum of counts of second- and third-instar larvae, pupae, and eclosed pupal cases (Koop et al. 2011, 2013*a*, *b*). *Philornis trinitensis* prevalence was calculated as the percent of sham-fumigated nests with at least one parasite out of all sham-fumigated nests.

Larvae and pupae were reared to the adult stage in netted butterfly enclosures (Live Monarch Castles, Boca Raton, Florida, USA) to confirm their identification as *P. trinitensis* (Dodge and Aitken 1968). Larvae generally pupated within 24 h (most were third instars when nests were collected and dissected). The length (mm) and width (mm) of pupae were measured with digital calipers in order to calculate pupal volume as an estimate of individual parasite size, which is related to lifetime fitness in muscid flies (Schmidt and Blume 1973). After the first three weeks of nest dissections, we observed wasps and ants in some sham-fumigated nests. We measured the prevalence of parasitoid wasps emerging from *P. trinitensis* pupae, and we counted ants found in (sham-fumigated) dissected nests. All parasitoid wasps and ants were collected for identification.

Philornis trinitensis*-binding antibody response*

Ninety-six-well plates were coated with 100 μL/well of *P. trinitensis* 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 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 anti-bird IgG (diluted 1:50,000; Antibodies Online, Atlanta, Georgia, USA; ABIN351982). Finally, plates were loaded with 100 μL/well of peroxidase substrate (tetramethylbenzidine, TMB: Bethyl Laboratories, Montgomery, Texas, USA) and incubated for exactly 30 min. The reaction was stopped using 100 μL/well of stop solution (Bethyl Laboratories). Optical density (OD) was measured using a spectrophotometer (BioTek, PowerWave HT, Winooski, Vermont, USA, 450-nm filter).

On each plate, a positive control was used in triplicate to correct for interplate variation. In addition, each plate contained a nonspecific-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.

Statistical analyses

We used general linear models (GLMs) or general linear mixed models (GLMMs) to analyze parasite and nestling data in RStudio, version 0.98.1062 (R Core Team 2014). Parasite prevalence was analyzed using a GLM with binomial errors, and parasite abundance, density, and volume were analyzed using GLMs with Gaussian errors. Host species (grassquit or mockingbird) was a fixed effect for all parasite response variables, and treatment (fumigated or sham-fumigated) was a fixed effect for parasite abundance only. Response variables for individual nestlings (i.e., fledging success, antibody levels, growth metrics) were analyzed with GLMMs using nest as a random effect. Fledging success was modeled with binomial errors and logit link function; host species and treatment were fixed effects. Antibody levels and growth metrics were analyzed with Gaussian errors and identity link function for each host; treatment was a fixed effect. GLM and GLMM analyses were conducted using glm and lmer functions, respectively, within

Table 1. Comparison of *Philornis trinitensis* prevalence, abundance, density, and size in the sham-fumigated nests of black-faced grassquits and tropical mockingbirds.

Parasite parameters	Grassquits	Mockingbirds
Parasite prevalence	52.6% (10/19 nests)	76.5% (13/17 nests)
Parasite abundance	12.42 ± 4.01 (19)	36.12 ± 8.80 (17)
Parasite density	5.89 ± 1.43 (10)	3.36 ± 0.92 (13)
Pupal volume, mm^3	78.14 ± 13.40 (7)	107.80 ± 8.16 (14)

Notes: Numbers are mean ± SE, except for parasite prevalence. Numbers in parentheses are the number of nests.

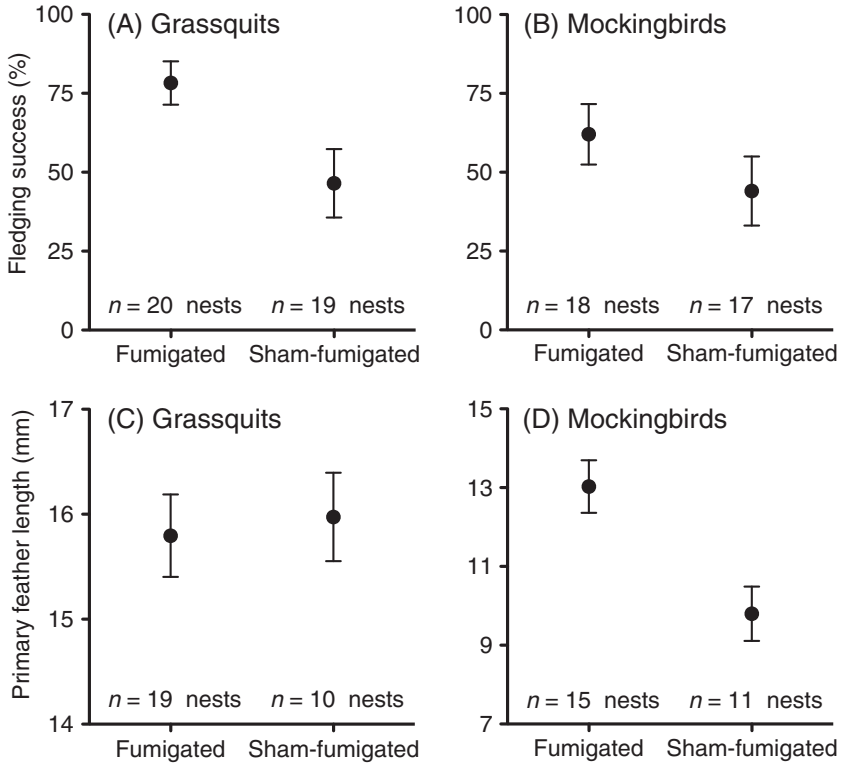

Fig. 2. Grand mean (±SE) for fledging success (%) and feather growth for black-faced grassquit and tropical mockingbird nestlings from fumigated and sham-fumigated nests. *Philornis trinitensis* significantly decreased fledging success of grassquit nestlings (A), but not mockingbird (B) nestlings. In contrast, *P. trinitensis* significantly decreased feather growth of mockingbird nestlings (D), but not grassquit (C) nestlings (see *Results* for statistical analyses).

the lme4 package. Probability values were calculated using log-likelihood ratio tests using the Anova function in the car package. Results are summarized as means ± standard errors (SE). We considered $P \leq 0.05$ as significant. Prism v.5.0b (GraphPad Software, Inc., La Jolla, California, USA) was used to create figures.

Results

Fumigated nests had fewer parasites than sham-fumigated nests for both grassquits and mockingbirds ($\chi^2 = 27.22$, df = 1, $P < 0.0001$). Indeed, the permethrin treatment completely eliminated *P. trinitensis* from fumigated grassquit nests (0 parasites; $n = 20$ nests) and from fumigated mockingbird nests (0 parasites; $n = 17$ nests). Parasite abundance in sham-fumigated nests differed significantly between host species (Treatment × Species, $\chi^2 = 6.83$, df = 1, $P = 0.009$), with mockingbirds having more parasites than grassquits. In contrast, parasite density (parasites per gram of host) did not differ significantly between the two bird species (Table 1; $\chi^2 = 2.38$, df = 1, $P = 0.12$). Parasite size, measured as pupal volume, was significantly smaller in grassquit nests than in mockingbird nests (Table 1; $\chi^2 = 3.97$, df = 1, $P = 0.05$). The prevalence of flies in sham-fumigated mockingbird nests was significantly greater than that of flies in sham-fumigated grassquit nests (Table 1; $\chi^2 = 4.49$, df = 1, $P = 0.03$).

Philornis trinitensis significantly reduced the fledging success of grassquits (Fig. 2A; $\chi^2 = 3.85$, df = 1, $P = 0.049$), but not mockingbirds (Fig. 2B; $\chi^2 = 0.34$, df = 1, $P = 0.56$). Moreover, as parasite density increased, the fledging success of grassquits decreased significantly (Fig. 3; $\chi^2 = 4.14$, df = 1,

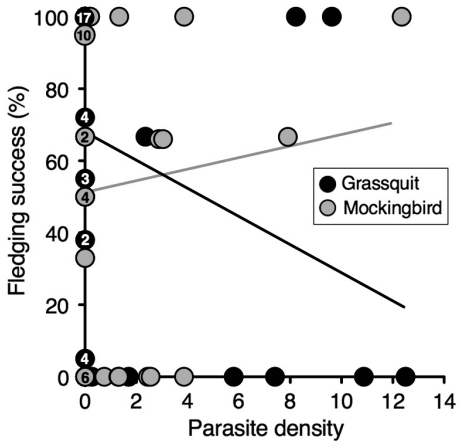

Fig. 3. Reaction norms for fledging success of sham-fumigated and fumigated grassquit and mockingbird nests across different *Philornis trinitensis* densities. Each point represents % fledging success for one nest plotted against parasite density. Parasite density was not a significant predictor of the fledging success of mockingbirds (gray points and line), which were tolerant hosts. In contrast, parasite density was a significant predictor of the fledging success of (non-tolerant) grassquits (black points and line; see *Results* for statistical analyses). When applicable, the number of overlapping points is represented within the points, which are offset on the *y*-axis for each species.

$P = 0.04$). In contrast, mockingbirds appeared to be tolerant to *P. trinitensis*, as there was no significant relationship between parasite density and fledging success (Fig. 3; $\chi^2 = 0.29$, df = 1, $P = 0.59$).

Philornis trinitensis did not have a significant effect on nestling grassquits' mass ($\chi^2 = 0.48$, df = 1, $P = 0.49$), first primary feather length (Fig. 2C; $\chi^2 = 0.52$, df = 1, $P = 0.47$), nor tarsus length ($\chi^2 = 1.33$, df = 1, $P = 0.25$). In contrast, for mockingbird nestlings, *P. trinitensis* significantly reduced first primary feather length (Fig. 2D; $\chi^2 = 11.37$, df = 1, $P = 0.0007$) and tarsus length ($\chi^2 = 6.89$, df = 1, $P = 0.009$), but not body mass ($\chi^2 = 1.61$, df = 1, $P = 0.20$).

Antibody levels (OD values) were very low in the nestlings of both host species. Antibody levels did not differ significantly between fumigated and sham-fumigated grassquits ($\chi^2 = 0.35$, df = 1, $P = 0.55$). Antibody levels in nestlings from fumigated grassquit nests were a mean (±SE) of 0.06 ± 0.02 ($n = 18$ nests), compared to 0.10 ± 0.04 ($n = 9$ nests) in nestlings from sham-fumigated nests. Similarly, antibody levels did not differ significantly between treatments for mockingbirds ($\chi^2 = 0.20$, df = 1, $P = 0.65$). Antibody levels in nestlings from fumigated mockingbird nests were 0.16 ± 0.02 ($n = 13$ nests), compared to 0.11 ± 0.04 ($n = 10$ nests) in nestlings from sham-fumigated nests.

One of seven (14%) sham-fumigated grassquit nests had *Brachymeria philornisae* parasitoid wasps emerging from *P. trinitensis* pupae in the nest (Delvare et al., *in press*). Four of twelve (33%) sham-fumigated mockingbird nests had the same wasp species emerging from *P. trinitensis* pupae. Six species of ants were also found in grassquit and mockingbird nests. Five of ten (50%) sham-fumigated grassquit nests contained one or more of the following species of ants: *Crematogaster rochai, Monomorium floricola, Crematogaster limata,* and *Solenopsis* sp. no. 1. Only one of the five grassquit nests examined had more than one species of ant (*C. rochai* and *Solenopsis* sp. no. 1). *Philornis trinitensis* was found in three of five grassquit nests with ants (14.20 ± 9.98 parasites), compared to two of five nests without ants (21.00 ± 9.00 parasites). Four of eight (50%) tropical mockingbird nests contained one or more of the following species of ants: *C. rochai, M. floricola, Crematogaster curvispinosa,* and *Solenopsis* sp. no. 2. Two of four mockingbird nests had two species of ants each (nest no. 1: *M. floricola* and *C. rochai*; nest no. 2: *C. curvispinosa* and *Solenopsis* sp. no. 2). *Philornis trinitensis* was found in two of four mockingbird nests with ants (50.00 ± 30.11 parasites), compared to two of four nests without ants (30.00 ± 21.94 parasites).

Discussion

The results of our study are more consistent with the enemy release hypothesis than the host defense hypothesis. Native *P. trinitensis* decreased fledging success of grassquits, but not mockingbirds, which appeared to be tolerant (Fig. 3). These results are similar to another study we conducted showing that introduced *P. downsi* reduces the reproductive success of Darwin's finches, but not Galápagos mockingbirds (Knutie et al. 2016). In short, effects of *Philornis* on native hosts are similar to effects of *Philornis* on novel hosts, suggesting that native hosts are not better defended against *Philornis*. In contrast, we found potential

enemies of *P. trinitensis* (wasps and ants) in nests of birds in Tobago, but not in nests of birds in the Galápagos (Knutie 2014). Wasps and ants may be responsible for the lower prevalence of native flies in the nests of birds in Tobago, compared to the Galápagos, where *P. downsi* occurs in nearly all finch and mockingbird nests.

Neither grassquits nor mockingbirds in Tobago had detectable antibody-mediated immune responses to *P. trinitensis*. King et al. (2010) reported that 3-day-old house sparrow (*Passer domestics*) nestlings are capable of mounting a detectable antibody response. Thus, it is at least possible for young nestlings of some species to mount antibody-mediated immune responses, but we did not observe such responses in our study. Antibody levels may be below the sensitivity of the assay, or the hosts may not up-regulate antibodies in response to *Philornis* and rely on innate defenses (e.g., complement proteins). Future studies could experimentally test whether other immune measures, such as total IgY antibody level, white blood cell abundance, or complement proteins, are effective against *P. trinitensis*.

Our results suggest that tropical mockingbirds defend themselves through tolerance of *Philornis* flies. In the Galápagos, mockingbirds tolerate the effects of introduced *P. downsi* by changing their behavior (Knutie et al. 2016): Parents from parasitized nests feed their nestlings more than parents from non-parasitized nests, thus compensating for energy lost to the parasite. Several studies of other host–parasite systems have also shown that parents in parasitized nests feed nestlings more than parents in fumigated nests, leading to increased fledging success (Tripet and Richner 1997, Hurtrez-Bousses et al. 1998, Tripet et al. 2002). Because mockingbirds from Tobago are also relatively unaffected by *P. trinitensis*, they may have similar behavioral mechanisms for offsetting the costs of parasitism. However, because we were unable to quantify the behavior of birds in Tobago, we cannot test this hypothesis.

Philornis trinitensis significantly decreased primary feather length and tarsus length in mockingbird nestlings in Tobago, suggesting that mockingbirds do experience sublethal effects of the parasite. It is conceivable that the sublethal effects of the parasites could affect longer-term post-fledging survival. For example, Streby et al. (2009) found that, despite similar fledging success, parasitized ovenbirds (*Seiurus aurocapilla*) have lower post-fledging survival than non-parasitized fledglings. Alternatively, it is possible that nestlings remained in the nests for a longer period of time, allowing more time for them to grow, as has been found in other studies (Johnson and Albrecht 1993, Young 1993). However, we did not measure nestlings after they were ten days old (i.e., closer to fledging), nor did we quantify the precise age at fledging because visiting nests late in the nesting cycle can lead to premature fledging. In contrast, *P. trinitensis* decreased the survival of grassquit nestlings, despite showing no effect on more proximal growth parameters. However, nestlings in nine of 19 sham-fumigated grassquit nests died before we could measure them, which made it difficult to test for sublethal effects of parasites on growth with adequate statistical power.

Our results indicate that native *P. trinitensis* parasites negatively affect host survival, contrary to the assumption that hosts are better defended against native than introduced parasites. Several correlational studies have reported that nestlings parasitized by native *Philornis* flies have lower survival than nestlings in non-parasitized nests (Arendt 2000, Rabuffetti and Reboreda 2007, Segura and Reboreda 2011, Quiroga and Reboreda 2012, Olah et al. 2013). However, the prevalence of flies in these studies was less than 50% of nests, which has also been shown for other native host–parasite systems (Whitworth and Bennett 1992). In contrast, *P. downsi* is found in at least 80% of Galápagos finch and mockingbird nests and, in most years, it is found in 100% of nests (Koop et al. 2011, 2013*b*, Kleindorfer et al. 2014, Knutie et al. 2014, Kleindorfer and Dudaniec 2016). These earlier studies did not explore why *Philornis* is found in lower prevalence in the native range.

Our results suggest that native *P. trinitensis* occurs in lower prevalence than introduced *P. downsi* because *Philornis* enemies are common in the native range, yet rare in the introduced range. In Tobago, we found *Brachymeria* parasitoid wasps in 26% of the nests we examined for wasps, and ants in 50% of the nests we examined. In contrast, parasitoids and ants are not common in Galápagos nests; for example, we did not find ants or parasitoid wasps in sham-fumigated finch or mockingbird nests in 2012–13 (Knutie 2014). Another Galápagos study reported that 2–5% of

Darwin's finch nests have two species of parasitoid wasps (*Spalangia endius* and *Brachymeria podagrica*; Lincago and Causton 2008); however, such low prevalence is unlikely to have much effect on *P. downsi* populations.

We documented six species of ants in grassquit and mockingbird nests in Tobago. Some species of birds preferentially nest near ants because they may protect birds from predators (Hindwood 1959, Young et al. 1990). To date, however, there is little or no evidence in the literature that ants forage on nest parasites. The ants found in Tobago bird nests could conceivably be foraging on *Philornis*. We did not find that *P. trinitensis* abundance was lower in bird nests with ants, suggesting that if ants were feeding on *Philornis* larvae, this did not lead to a decrease in larval abundance. However, it is possible that ants could have been eating the contents of *P. trinitensis* pupae, rather than empty pupal cases reflecting successful eclosion of adult flies (J. Longino, *personal communication*). These data, combined with our data on parasitoid wasps described above, are consistent with the enemy release hypothesis (Keane and Crawley 2002, Liu and Stiling 2006). Thus, the prevalence of *Philornis* may be higher in the Galápagos because the flies have escaped their enemies, compared to native *Philornis* in Tobago.

The number of introduced species, including parasites, throughout the world will continue to rise with increasing globalization (Hulme 2009). Once introduced parasites become problematic for hosts, the next question is how to reduce the consequences of such parasites. Control of introduced parasites can be logistically difficult and is often too little, too late (Lapointe et al. 2012). Our study suggests that understanding the ecology of native parasites may have implications for the management of their introduced relatives. For example, decreasing the prevalence of introduced parasites using native enemies could be an effective method for protecting native hosts. This study, among others (Koop et al. 2016), indicates that reducing the prevalence of *P. downsi* in the Galápagos may substantially reduce the negative effect of *P. downsi* on Darwin's finch populations.

Acknowledgments

We thank the Tobago Department of Natural Resources, Mike Rutherford, George Heimpel, Roger Moon, Ray Martinez, and Sarah Bush for various forms of assistance. We thank Marcia Couri for identification of *P. trinitensis* and John (Jack) Longino for identification of the ant species. We thank Christopher Lepczyk and two anonymous reviewers for comments that improved the manuscript. We also thank Bananaquit Apartments, Tobago Plantations, Mount Irvine Bay Golf Course, Crown Point Beach Hotel, Grafton Beach Resort, Tropikist Beach Hotel, Sunspree Resort Ltd., and Rovanel's Resort for permission to work on their properties. This work was supported by NSF Grant DEB-0816877 to D. H. Clayton. S. A. Knutie was supported by a University of Utah Graduate Research Fellowship. All applicable institutional guidelines for the care and use of animals were followed. The authors declare that there are no conflicts of interest affecting this work.

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