PHILORNIS DOWNSI INTERACTIONS WITH ITS HOST IN THE INTRODUCED RANGE AND ITS PARASITOIDS IN ITS NATIVE RANGE

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Dedication

To my mom and dad,

I was born from good parents, thank you for teaching me

that hard work requires patience and love. My work and this thesis are for you.

To my nieces, Aura and Johanna.

Abstract

The study of ecological interactions is important for the management of invasive species. In the Galapagos Islands, an invasive parasitic fly, *Philornis downsi* Dodge & Aitken (Diptera: Muscidae), is causing high rates of mortality among endemic avifauna. Long-term management of this invasive fly is not yet available, and research is needed to further understand the interactions among its host and its natural enemies to further consider management strategies. My research examines the interactions of *Philornis downsi* with one of its hosts in the Galapagos Islands and with its natural parasitoid enemies in mainland Ecuador.

I studied the interactions between the fly and one of its hosts, the Galapagos flycatcher (*Myiarchus magnirostris*) in the Galapagos Islands, as well as with the fly's natural parasitoid enemies in mainland Ecuador. My studies in the Galapagos Islands showed that *Philornis downsi* is mainly vespertine (active at dusk) and that its activity was higher when adult birds were more active around nests. And, my studies in mainland Ecuador showed that almost all parasitoids that emerged from *Philornis* pupae encountered in mainland Ecuador did not emerge from experimentally deployed pupae of non-*Philornis* fly species.

The results of my thesis will provide information on *P. downsi* interactions which could aid in its management. Additionally, understanding these interactions should simplify the efforts to mitigate the impact of the fly and avoid the possibility of future extinctions of endemic avifauna in the Galapagos Islands.

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Introduction

The invasive parasitic fly, *Philornis downsi* Dodge & Aitken (Diptera: Muscidae) arrived at the Galapagos Islands sometime before 1964 (Causton et al., 2006) from mainland Ecuador (Bulgarella et al., 2015). Its introduction has become the main threat to passerine bird populations and the target of several proposed management strategies (Causton et al., 2012; McNew & Clayton, 2018). *Philornis downsi* causes high rates of mortality among nestlings, up to 100% of the brood in some species (Heimpel et al., 2017), making extinction a real threat for some bird species including some of the iconic Darwin finches (Fessl et al., 2010). *Philornis downsi* inflicts mortality to the nestlings as a larva by feeding on the naris and ear cavities, causing scarring and beak malformations (Fessl et al 2006, Galligan et al., 2009). In later larval instars, *P. downsi* becomes hematophagous, causing blood loss, anemia, and eventually death (Fessl, 2006). *Philornis downsi* is a generalist parasite ubiquitous in the Galapagos Islands and recent models demonstrate that in 50 years, one of the most abundant species of passerine bird, the medium ground finch (*Geospiza fortis*), might go extinct due to its parasitism (Koop et al., 2016). As yet, no long-term control method has been implemented for *P. downsi*, although several management strategies are under consideration for the preservation of the Galapagos avifauna (Causton et al., 2013).

The detrimental effects of *P. downsi* on the endemic avifauna have been well documented and researched, but little is known about the biology and behavior of *P. downsi* in the Galapagos Islands and interactions with its hosts. Only three research publications on *P. downsi* behavior exist (Koop et al., 2013; Lincango et al., 2015; O'Connor et al., 2010). Expanding our knowledge of *P. downsi* biology could aid control

efforts in the Galapagos Islands. This thesis focuses on such behaviors and how understanding *Philornis*-host interactions could aid in the efforts to control *P. downsi*.

As mentioned above, different pest management strategies have been considered to control the population of *P. downsi*, including: chemical ecology using pheromonal attraction (Cha et al., 2016), sterile insect technique (Klassen & Curtis, 2005), and classical biological control (Bulgarella et al., 2017). In this thesis, I will focus on classical biological control. Biological control is defined as any reduction of plant or animal populations by natural enemies occurring in the natural systems (DeBach & Rosen, 1991). This method to control pests carries risks due to possible non-target effects, but negative effects can be ameliorated by properly researching the proposed biological control agent(s) (Heimpel & Mills, 2017).

To implement classical biological control successfully, a specialist biological control agent must be found in the native range of the pest (Heimpel & Mills, 2017). Bulgarella et al (2017) found a promising biological control agent, *Conura annulifera* Walker (Hymenoptera: Chalcididae), against *P. downsi*. *Conura annulifera* is a solitary idiobiont pupal gap-laying ectoparasitoid found in the natural range of *P. downsi* and historically emerging exclusively from *Philornis* spp. pupae (Couri et al 2006, Bulgarella et al 2017). Specificity testing was performed to ensure that *C. annulifera* was a specialist of *Philornis* species by subjecting the wasp to no-choice tests under laboratory conditions. *Conura annulifera* was paired with members of different families of insects including Muscidae, Sarcophagidae and Calliphoridae cyclorrhaphan flies, Tortricidae, Pyralidae, and Sphingidae lepidopterans, and a Braconidae wasp. Researchers found that *C. annulifera* did not emerge from any of the non-target species in no-choice tests,

concluding that *C. annulifera* was highly specific against *Philornis* species (Bulgarella et al 2017). However, standards for the introduction of a parasitoid wasp into the Galapagos Islands are more rigorous, thus it was deemed important to perform complementary specificity research in the field to corroborate laboratory specificity findings. These interactions could potentially aid in risk assessments if biological control is implemented.

Therefore, the focus of this thesis is to investigate and inform conservation scientists about interactions between *P. downsi* with its hosts and its natural enemies. The first chapter in this thesis will discuss *P. downsi* behavior outside active nests and the interactions with one of its hosts, the Galapagos flycatcher (*Myiarchus magnirostris*). Additionally, the first chapter will document the main hours during the day when *P. downsi* is the most active. Understanding these patterns could allow for in-field observation which might yield insights on its reproduction, in turn aid efforts to develop a laboratory rearing system for *P. downsi* (Lahuatte et al., 2016; Sage et al., 2018) based on observed mating or oviposition behavior. In chapter two, I studied the interactions of *Philornis* flies with *C. annulifera* and other parasitoids in the field. I paired non-*Philornis* flies with active bird nests containing *Philornis'* pupae, thus providing more information about the ecological host range and specificity of the parasitoids found in the natural range.

Chapter I: Interactions Between the Invasive Parasitic Fly *Philornis downsi* and the Galapagos Flycatcher in the Galapagos Islands

Summary

The parasitic fly *Philornis downsi* invaded the Galapagos Islands from mainland Ecuador, negatively impacting the populations of endemic avifauna, including various species of Darwin's finches. The fly targets active bird nests containing nestlings to oviposit. Once hatched, the fly larvae feed on the nestlings causing high mortality. While such negative impacts on *P. downsi* on its host populations are well documented, very little is known about the fly's diurnal behavior around active bird nests, thus complicating its management and control. To better understand *P. downsi* behavior with its host, I used video cameras to observe two Galapagos flycatcher (*Myiarchus magnirostris*) nests daily from 6:00 am to 6:00 pm for the duration of the nesting period. From these recordings, I developed an ethogram for *P. downsi* behavior and for *P. downsi*-Galapagos flycatcher interactions such as daily patterns, birds foraging time, and fly predation near the nest. My results suggest that *P. downsi* avoids adult Galapagos flycatchers by primarily landing and entering nests when adults are away. Additionally, flies visited the nest throughout the day with higher fly activity during vespertine hours. This information may be useful in the management of *P. downsi* in the Galapagos Islands.

Introduction

Philornis downsi Dodge & Aitken (Diptera: Muscidae) is an avian nest parasite that invaded the Galapagos islands from mainland Ecuador sometime before 1964 (Causton et al., 2006; Bulgarella et al. 2015; Fessl et al. 2018). This species attracted the attention of conservation scientists by causing high levels of nestling mortality of many endemic passerine bird species in the Galapagos Islands. The effects of *P. downsi* on various bird species are well understood (Dudaniec & Kleindorfer, 2006; Fessl et al., 2006) but the daily rhythms of *P. downsi* adults and their behavior around active bird nests remain relatively unstudied. Three main publications of *P. downsi* in the Galapagos Islands have provided critical information about *P. downsi* behavior. First, O'Connor (2010) showed that the larvae of *P. downsi* were active mainly at night and that there was a low level of larval removal behavior by both adult and nestling birds (the finches *Geospiza fulginosa*, *G. fortis*, and *Camarhynchus parvulus*) in infested nests. Second, Koop et al. (2013) concluded that there were no behavioral changes on the part of the medium ground finch, *G. fortis*, that resulted in a reduction of the parasite load. Lastly, Lincango et al. (2015) observed *P. downsi* adults outside a Galapagos flycatcher (*Myiarchus magnirostris)* nest and noted some behaviors and interactions of the fly entering the nest.

Studying diel patterns of nest visitation could help to answer questions about how *P. downsi* finds active bird nests as well as the time of the day when adult *P. downsi* flies are active. Such information could help to determine whether host behavior influences *P. downsi* visitation and whether host nests are used as a mating rendezvous site, as has been

found in some other fly species (e.g. Prokopy et al., 1971). Enhanced understanding of *P. downsi*-host interactions could in turn aid researchers to develop techniques to disrupt host- or mate-finding, and could also aid efforts underway to develop a laboratory rearing system for *P. downsi* (Lahuatte et al., 2016; Sage et al., 2018) based on observed mating or oviposition behavior.

This chapter provides information on *P. downsi* diurnal patterns and how adult flies avoid predation from birds and in doing so addresses four crucial questions about the interactions between *P. downsi* and one of its host birds. The questions are as follows: (1) Do *P. downsi* and adult flycatcher visits temporally overlap (i.e. are the flies and adult birds present in the nest at the same time)? (2) What is the diurnal pattern of *P. downsi* nest visitation? (3) How is *P. downsi* landing behavior around nests affected by the presence of adult birds in the nest? (4) How do the daily rhythms of birds and flies interact (i.e. do adult bird trips away from the nest predict *P. downsi* visitation)? To address these questions, I set up cameras outside of two active Galapagos flycatcher (*Myiarchus magnirostris,* Passeriformes: Tyrannidae) nests, one during the wet season and the other during the dry season. I observed *P. downsi* and bird activity in both nests.

Methods

Study Location Area and Observed Species

I characterized interactions between the Galapagos Flycatcher and *P. downsi* by observing two nests during 2015, one in March (during the wet season) and the other in June (during the dry season). I chose the Galapagos flycatcher as a model organism because it is susceptible to *P. downsi* parasitism (Fessl & Tebbich, 2011). It is also the

only land bird that nests in cavities in the Galapagos archipelago (Lanyon, 1978; Ervin, 1994) which makes it relatively easy to monitor in artificial nest boxes. Galapagos flycatchers occur in most habitats and on most of the islands and lay 3-5 eggs in a clutch (Joseph, 2004). The birds are diurnal, mainly insectivorous and relatively unafraid of humans and thus a good subject for field observation (Wiedenfeld, 2011). The Galapagos flycatcher is sexually monomorphic and its nesting behavior, courtship, and parental care are not yet known. I conducted the observations in the arid zone "El Barranco" (0^{O} 44' 14.0'' S 90^{O} 18' 4.1'' W), adjacent to the Charles Darwin Research Station, of the Santa Cruz Island in the Galapagos Islands, within artificial nesting cavities (see below).

Video Recording and Set Up

The monitored nests were built by wild Galapagos Flycatchers within pre-made bamboo poles with cavities to facilitate nesting. The bamboo poles were 3-3.5 meters high with openings of 10 cm in diameter on each separate section of the bamboo. The total number of openings in the bamboo poles was between 3 and 9 depending on the height of the bamboo pole. The Bamboo poles were secured using metal wires to *Opuntia* tree or other steady structure. Twenty-one Bamboo poles were constructed and deployed approximately 20 meters apart from each other on a trail at the El Barranco field site. I checked for Galapagos Flycatcher activity and nest status three times a week using an endoscopic fiber-optic camera with wireless monitor (shaft 17 mm diameter, fiber-optic cable length 91 cm) mounted on a pole (as in Heimpel et al, 2017). For video recording, I used GoPro 3+ cameras, which had a rigid water-proof casing. For video storage, I used SD cards with a capacity of 32 gigabytes, and an extended-life battery with eight hours of activity to power the camera. The batteries and SD cards were changed after six hours of

filming from 6:00 to 12:00 and 12:00 to 18:00. I secured the cameras to a nearby *Opuntia* tree with a flexible clamp mount with the camera aiming at the opening of the nest (Figure 1.2). The cameras were placed 30-50 cm away from the entrance of the nest, allowing free entrance and exit from the nest by the flycatcher parents. I conducted pilot studies to determine whether the camera would interfere with normal Flycatcher behavior by conducting observations before and after placement of the camera outside the nests for periods of 40 minutes. I did not observe any signs of distress during these observations and the frequency of visits and time spent inside the nest remained the same before and after the placement of the camera (I. Ramirez, unpublished).

As noted above, I observed one nest in March and one in June of 2015. I encountered the March nest while it was being constructed on 23 February. By 19 March the nest contained three nestlings and I allowed four days before setting up the camera to allow natural parental attachment to the nest. The June nest was found on 3 June at which point it already contained three nestlings. The nestlings were found with natal down and closed eyes and were thus likely less than 7 days old (Ricklefs, 1969). The true age of the nestlings was unknown however due to their premature death (see below). *P. downsi* adults were observed entering both nests.

After the chicks had all fledged or died, I collected all nesting material from both nests by detaching the bamboo pole from the *Opuntia* and retrieving the nest from the cavity by hand. Both nests were placed into plastic bags and transported to a laboratory at the Charles Darwin Research Station. I dismantled and inspected the nests within 3 hours of collection and noted any dead nestlings. Both nests contained *P. downsi* larvae and puparia, and the larval stages were categorized as first, second and third instars based

upon the size and spiracular slit morphology (Fessl et al. 2006). I categorized pupal casings (puparia) as either emerged or unemerged. The unemerged puparia were kept in groups of three at an average temperature of 26.5^{O} C with an average humidity of 66% and a photoperiod of 12:12 (L:D) inside clear plastic containers with 10 cm in diameter and 6 cm in height lined with cotton and fine mesh fabric as a lid.

Videos and Ethogram

The 12 hours of recording per day were divided into 36 videos of 20-minute length per day, which were viewed and analyzed in Windows Media Player. I watched all videos at 1x speed, slowing down and pausing the video when needed, and the observations were used to develop scenarios which included the number and timing of *Philornis downsi*-bird interactions. This process resulted in the identification of four different scenarios in which *P. downsi* and bird behavior can overlap (Table 1.1). Since flycatchers are known to be adept at catching adult flies, the overlap between adult birds and adult flies within the nest was of particular interest, thus scenario 1 denotates the event of no overlap between adult flies and birds, scenario 2 as complete overlap, scenario 3 as overlap only late during the adult bird's absence and scenario 4 as only early during the adult bird's absence.

Data Analysis

I used a log-likelihood goodness-of-fit (G) test (Sokal and Rohlf, 1981) with William's correction factor to address the question of how does *P. downsi* landing behavior differ when adult birds are present in the nest. This test compares an observed proportion to an expected or null proportion. Under the null hypothesis, I expect the

number of *P. downsi* visits to be equal when adult birds are inside or outside of the nest. I calculated the expected number of *P. downsi* visits under each condition (adult birds in or out) based on the proportion of time adult birds spent in or out of the nest.

Equation 1:

$$G = \frac{2(P \ln \frac{P}{P1N} + F \ln \frac{F}{F1N})}{1 + \frac{1}{2N}}$$

Where *P* (n=23) is the number of fly landings when an adult bird was present inside the nest, *F* (n=196) is the number of fly landings when the adult birds were absent from the nest. The values of *P1* and *F1* are the proportions of time the Galapagos flycatcher adults spent inside the nest (*P1*= 0.27) and the time they spent outside the nest (*F1*=0.73) respectively. *N* is the sum of *P* and *F,* and the denominator is William's correction factor (Sokal and Rohlf, 1981). Levels of significance were calculated using the X^2 distribution at one degree of freedom.

I used generalized linear mixed models (GLMMs) using lme4 in RStudio (R Core Team, 2013) with Poisson error structure to test whether the number of *P. downsi* nest visits was influenced by the amount of time adult birds spend outside the nest, the number of times the adult birds visited the nest and the time of day. Nest identity was coded as a random effect.

Results

Galapagos Flycatcher Fledging Success and Parasite Load

A total of 222 *P. downsi* larvae, unemerged puparia, and emerged puparia were found inside the two nests, 87 in the March nest and 135 in the June nest (Table 1.2). Bi-parental care was observed in the March nest as two adult individuals (one ringed, one un-ringed) were observed visiting the nest. Parental care was observed in the June nest as well. For both nests adult birds visited the nest 19.79 ± 0.37 (SEM; *n*= 196 observed hours) times per hour, 19.67 ± 0.4 (SEM; *n*= 130 observed hours) in March, and 20.02 ± 0.6 (SEM; *n*= 66 observed hours) in June, returning with arthropods and on a few occasions, fruits. Individual trips outside of the nest lasted 2:08 ± 0:02 (SEM; *n*=807 trips for both nests summed) minutes on average, 2:20 ± 0:02 (SEM; *n*= 486 trips) minutes in March, and 1:45 ± 0:03 (SEM; *n*= 321 trips) minutes in June. Parents spent an average of 44:00 ± 1:30 (SEM) minutes of every hour outside of the nest, equivalent to 73% of the total time of the parent's activity per hour in the March and June nests.

All nestlings fledged from the March nest on 4 April. In the June nest, a Galapagos Flycatcher parent exited the nest with a dead nestling in its beak on 10 June. While checking the nests on 11 July with the endoscopic fiber-optic camera, I found one dead nestling; no other nestlings were found in the nest. There is no video camera footage of a second nestling being carried out from the nest by a parent nor could I find a second nestling dead inside the nest or on the ground surrounding the nest cavity when I inspected it. I ensured there were no more nestlings in the nest before retrieving it on this date. I concluded that all three nestlings died on July 10-11.

Philornis downsi daily activity

I observed that *P. downsi* visited nests throughout the day, but increased visitation occurred after 15:00 hrs. peaking at 17:00 hrs. and lasting until 18:00 hrs.; 74% of all the visits occurred between 15:00 to 18:00 hrs. (Figure 1.3). *P. downsi* is the only nest parasite that enters active nests in the Galapagos Islands, thus I concluded all flies were *P. downsi*. I was unable to identify the sex of the flies due to the distance of the camera. On average, flies landed near (<10 cm) the entrance of the nest 1:37 ± 0:10 (SEM, *n*=219 landings) minutes after the adult parent left the nest and it took 00:12 ± 00:01 (SEM, *n*=151 observations) seconds on average to enter the nest after landing. Flies that landed but did not enter the nest (*n*=68 observations) flew away less than five seconds after landing. *P. downsi* adults spent an average of 1:29 ± 0:09 (SEM, *n*=151 observations) minutes inside the nest and visitation occurred 0.78 ± 0.13 (SEM, *n*=196 observed hours) times per hour. On four occasions in June, multiple flies entered and remained inside the nest for up to four minutes. In one event, two flies were inside at the same time, in two events, three flies were inside the nest at the same time, and in one event, five flies were inside at the same time. The single predation event I observed by an adult flycatcher occurred when a fly entered the nest while an adult flycatcher was present inside the nest (Scenario 4). Two other instances of flies entering while an adult bird was present inside the nest resulted in the fly not exiting the nest but I was unable to confirm the predation of the flies due to the low light intensity. *P. downsi* mating was not observed in any of the recordings.

P. downsi Overlapping Scenarios, Landing and Nest Entering Behavior

The playback of the videos showed *Philornis downsi* landing near and entering both nests. From the total of 219 *P. downsi* landings near the entrance, only 151 events resulted in *P. downsi* entering the nests, 44 of which events were observed in March and 107 in June. The 151 flies entering the nest were categorized into four overlapping scenarios (Table 1.1). The most common scenario was scenario three, in which *P. downsi* entered a nest without adult birds and exited shortly after the adult bird arrives (60% of observations; Fig. 5), and the second most common was scenario one with 33% of the observations, in which adult birds were absent during the entirety of the *P. downsi* visit. An adult bird was present when *P. downsi* entered and exited the nest (complete overlap) in 4.6% of the observations and an adult bird was present when *P. downsi* entered but was not observed to leave the nest in 1.9% of the observations (Table 1.1). *P. downsi* landed near the entrance of the nest at a significantly higher frequency when no adult bird was present inside the nest, even after accounting for the time adult birds spent foraging (g-test= 36.35, χ^2 = <0.01, df = 1).

Effect of Bird Behavior on *P. downsi* Visitation

Although *P. downsi* tend to visit nests when adult birds are absent (see above), this trend did not extend to the length of time nests were empty of adult birds, as there were fewer *P. downsi* visits to the nest when adult birds were absent for longer time periods (table 1.3). The number of adult bird visits to the nest per hour had no effect on *P. downsi* visitation and the strongest effect was the time of the day (table 1.3).

Discussion

P. downsi is mainly vespertine (active at dusk) and its activity depends on the temporal activity of adult birds inside and outside the nest. Flies were more likely to land near and enter bird nest cavities when adult birds were absent and fly visits were less frequent when adult birds spent longer periods of time away from the nest.

The vespertine activity, like crepuscular activity, could be explained by several adaptive hypotheses, including resource availability, safety from predation or abiotic conditions. *Philornis downsi* may seek hosts at dusk because those hours could be the optimal time to find resources for the fly's offspring. Thus, the fly could be displaying behaviors that evolved in the natural range to find and oviposit on hosts that could be more active during vespertine hours. Another explanation for the vespertine activity I observed in *P. downsi* could be that this fly is constrained by abiotic conditions, such as tolerance of heat. *Philornis downsi* may be active during dusk to avoid higher temperatures, as do other insects (May, 1979). *Philornis downsi* might prioritize finding food resources or a mate during morning hours rather than finding a host, thus it is only seen around nests at dusk and not at dawn around active nests. Feeding is important for fecundity and longevity in some species of dipteran insects, and nutrient deficiencies due to starvation could lead to a reduced oviposition load (Nayar & Sauermann, 1975). Another explanation could be that *P. downsi* vespertine behavior helps them to avoid predation by diurnal predators.

Despite the Galapagos flycatcher being adept at catching flies (as its name suggests), *P. downsi* predation events were rare and high levels of *P. downsi* parasitism occurred in the two nests that I investigated. The single predation event that I observed

occurred when a fly entered a nest when an adult bird was present. This rare event could imply predation avoidance by *P. downsi*. Most *P. downsi* activity occurred when no adult bird was present when the fly entered the nest. This suggests that *P. downsi* can discriminate nests with and without adult birds inside and avoids the former, avoiding predation. Therefore, I propose that *P. downsi* uses cues (noise, odor, movement or infrared radiation) associated with the presence of adult birds to find nests while avoiding predation. Additional support for this hypothesis comes from the analyses of the time adult birds spend away from their nest, where I saw that the higher frequency of such absences from nests (rate of provisioning) influenced fly visits. Shorter trips might provide more frequent cues (noise, odor or movement) that *P. downsi* could be using to find nests. When the birds are absent for longer periods of time, then these cues will be detected less frequently making them less reliable because there will be less information about the nest. Additionally, more active nests might mean young or multiple nestlings inside the nest, thus making nests with higher adult activity more productive for *P. downsi* oviposition.

It is unlikely that a single sensory cue is the sole indicator for host finding (Boulton, 2018; Brodie et al., 2014; Lehane, 2005). Several sensory cues (multi-modality) play a role in host finding and/or avoiding predation in parasitic hematophagous (blood feeding) insects. For example, *Aedes* mosquitoes respond to host silhouettes at a distance, and once approached CO_2 and infrared radiation will determine if the mosquito will feed on the target, relying on visual, chemosensory and heat cues to find and feed on its host (Sippell & Brown, 1953). A second example of multi-modality is observed in tsetse flies which rely on multiple visual cues such as color, UV light, and

movement to find their hosts in addition to odors emanating from sweat glands (Lehane, 2005). Studies on *P. downsi* chemosensory abilities could also suggest multi-modality as Cha et al. (2016) found that *P. downsi* adults were attracted to acetic acid and ethanol produced by yeast present on fermented materials which *P. downsi* feed upon. This implies that *P. downsi* uses chemo-sensorial cues to find food sources, thus potentially also using it to find a bird host. However, it seems unlikely that it uses it to discriminate the absence or presence of adult birds inside the nest. I suggest that visual and/or audio cues might play a role to discriminate adult birds inside nests and possible predation avoidance. Audio cues could inform nearby flies when an adult bird is inside the nest by nestling begging behavior, and a relative silence when an adult bird is not inside the nest. Visual cues in form of infrared (thermal) radiation, could also play a role in helping *P. downsi* to target a nest. The size of the clutch and presence of a parent inside the nest typically results in higher heat radiation suggesting an active nest (Biddle., 2018; Olson., 2006) that *P. downsi* could exploit. Nonetheless, more research is needed to fully answer this question taking in consideration that multimodality in *P. downsi* might be relevant.

In this chapter, I have presented new information about the activity and behavior of *P. downsi*. I have shown that these flies are most active at vespertine hours and that their visits to parasitize bird nests occur mostly when the parents are absent for short periods of time. This study provides valuable information that may be of use to researchers and conservation practitioners attempting to study and control *P. downsi* in the Galapagos Islands and could also help to inform management of other *Philornis* species (Bulgarella et al., 2018). The observation that *P. downsi* is a vespertine species will help guide additional field observational studies. This is relevant to studying the

mating and courtship behavior of *P. downsi*, which is critical for rearing a sustainable captive colony and developing methods such as sterile male releases (Lahuatte et al., 2016; Fessl et al. 2018). Although interactions of *P. downsi* with the Galapagos flycatcher could differ from interactions with other birds in the Galapagos Islands, and since flycatchers are avid fly predators, it could suggest a lower risk of *P. downsi* predation from other bird species. Still, the inferences in this chapter regarding cue use for host finding (multimodal cues from hosts) might aid in directing future studies into how ovipositing female *P. downsi* find their hosts while avoiding predation. This too may have implications for management, for instance providing information about auditory, visual or olfactory decoys to manipulate the fly's behavior might be useful for reducing parasitism pressure on at-risk bird populations such as the mangrove finch which is critically endangered (Sutherland, 1998).

Table 1.1. Observed overlapping scenarios and their descriptions. The scenarios of bird-fly interactions from both nests, March and June, were categorized and quantified according to overlapping time inside the nests.

Overlapping Scenarios	Scenario Description	March observations	June observations	Total observations
Scenario 1 **No Overlap**	No adult bird present when *P. downsi* enters and exits the nest	23	27	50
Scenario 2 **Complete Overlap**	Adult bird present when *P. downsi* enters and exits the nest	0	7	7
Scenario 3 **Late Overlap**	No adult bird present when *P. downsi* enters the nest, *P. downsi* exits after the adult bird arrives	20	71	91
Scenario 4 **Early Overlap**	Adult bird present when *P. downsi* enters, but the *P. downsi* never exits the nest or gets eaten	1	2	3

Table 1.2. Information on the March and June Galapagos flycatcher nests. This table indicates nestling mortality, total *P. downsi* observed and specimens found inside the nest post-collection (all stages).

	March	**June**
Nestlings Inside the Nest	**3**	**3**
Total Nestling Survival	3	0
Total Nestling Mortality	0	3
***P. downsi* found inside the nest**	**87**	**135**
Observed *P. downsi* visitations to the nest	44	107
First Instar Larvae	0	4
Second Instar Larvae	6	13
Third Instar Larvae	2	38
Unemerged puparia	77	77
Emerged Puparia	2	3

Table 1.3. Generalized linear mixed model results output of *P. downsi* visitation where the time adult birds spend outside the nest, number of bird visits to the nest, and the time of the day were the fixed effects. I used the identity of the nests themselves (March and June) as the random effect.

	Estimate	**Std. Error**	**z value**	**p value**
Intercept	-0.81022	0.66136	-1.225	0.22054
Amount of time that adult birds are away from the nest	-0.10318	0.02915	-3.539	0.00040*
Number of adult bird visits to the nest	0.02063	0.03278	0.629	0.52913
Time of Day	0.14510	0.02771	5.237	<0.0001*

Figure 1.1. An adult Galapagos flycatcher returns to its nest after a foraging trip.

Figure 1.2. Set up of the video camera with an extended-life battery (orange), protective casing and flexible clamp mount facing the entrance of an active Galapagos flycatcher nest.

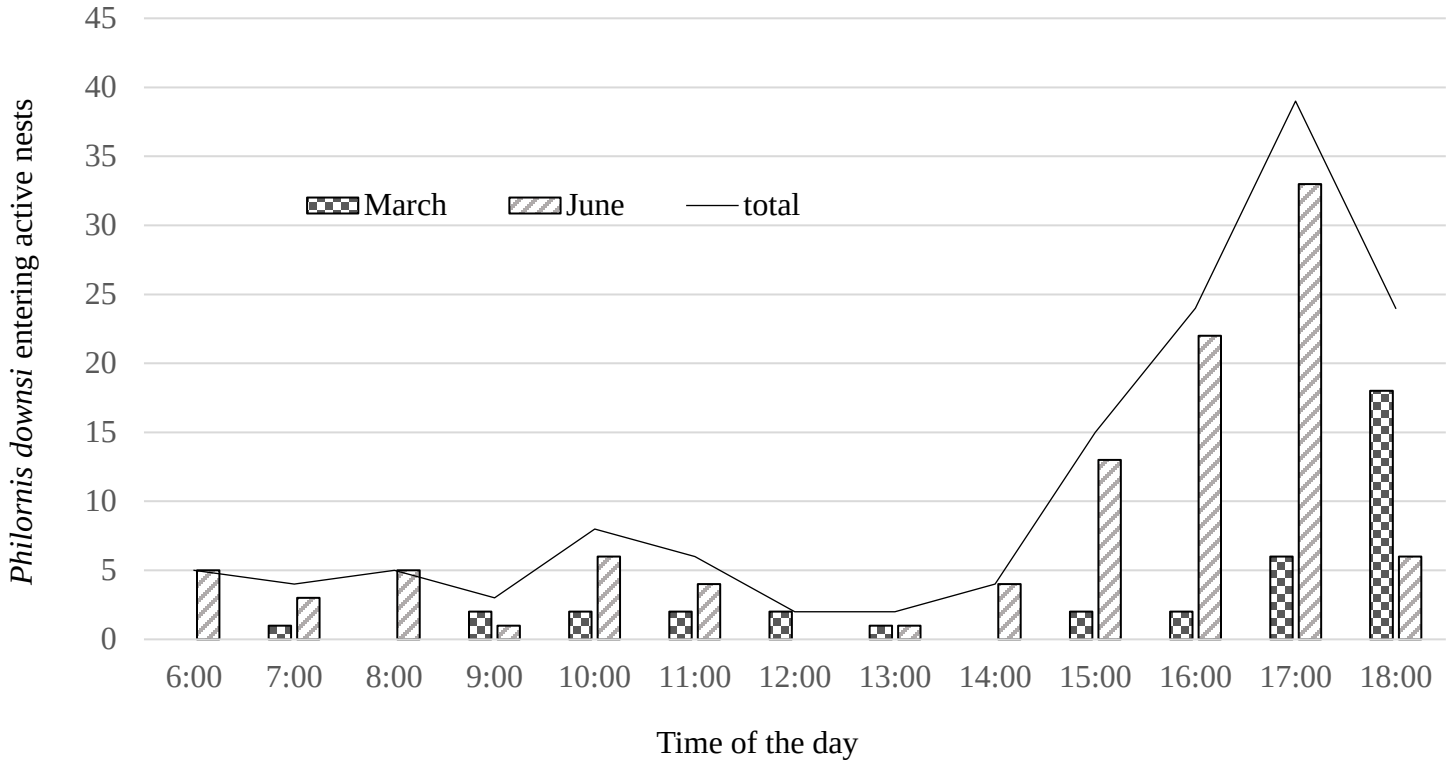

Figure 1.3. Total *P. downsi* activity of the March and June nests from 6:00 hrs. to 18:00 hours.

Figure 1.4. *Philornis downsi* fly landing adjacent to the entrance of an active Galapagos flycatcher nest that is inside an artificial cavity.

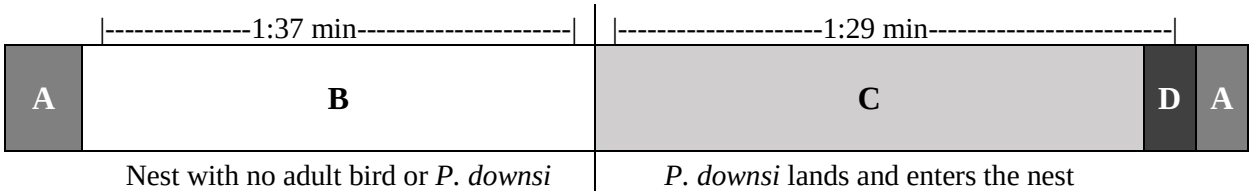

Figure 1.5. Timeline visualization of the most common scenario, scenario 3 (late overlap). A) represents the time an adult bird is inside the nest (no *P. downsi* present), B) represents the average time for a *P. downsi* fly to arrive after an adult bird leaves the nest. C) represents the time a *P. downsi* fly is inside the nest without adult birds. D) Late Overlap scenario; both fly and bird are present in the nest.

Chapter II: Experimental In-Field Assay of Host Choice: The Specificity of *Conura annulifera* And Other Parasitoids Against *Philornis downsi* In Its Native Range

Summary

Conura annulifera (Hymenoptera: Chalcididae) and other parasitoids attack neotropical *Philornis* flies (Diptera: Muscidae) in mainland Ecuador. Among the flies attacked is *Philornis downsi*, an introduced parasitic fly in the Galapagos Islands causing high mortality of endemic avifauna including Darwin's finches. The high mortality of these birds has made the control and management of *P. downsi* a high priority for conservation science. Previous laboratory studies showed high specificity of *C. annulifera* against *Philornis* flies, making *C. annulifera* a candidate biological control agent against *P. downsi* in the Galapagos Islands. I examined the specificity of *C. annulifera* and other parasitoids of *P. downsi* using an experimental in-field assay of host choice in the natural range of *P. downsi*. I reared non-*Philornis* cyclorrhaphan Diptera and placed them near and inside active bird nests where *P. downsi* pupates. I found that almost all parasitoids associated with the genus *Philornis*, including *C. annulifera*, only emerged from *Philornis* flies despite their proximity to other dipterans.

Introduction

While laboratory tests of host specificity can provide important information on the potential host range of candidate biological control agents, in-field specificity testing can provide valuable complementary information on actual host use patterns in the field. Advantages of in-field testing include the presence of natural environmental conditions, and the use of naturally foraging insects; both of these components can be difficult to achieve under laboratory conditions (Clement & Cristofaro, 1995). Ideally, some form of in-field specificity testing could complement standard laboratory specificity testing in order to provide a clearer picture of the ecological host range and specificity of the candidate agent (Heimpel & Mills, 2017). For instance, Briese et al., (2002) evaluated host specificity of candidate biological control agents against an invasive weed to Australia, *Heliotropium amplexicaule* in its natural range, Argentina. The research was divided into two phases. First, *H. amplexicaule* was paired with various genera of non-target host plants simulating a choice test, and second, researchers removed *H. amplexicaule,* simulating a no-choice test, leaving only non-target plant hosts in the plot. The most specific candidate biological control agent, the thrips *Haplothrips heliotropica*, only attacked *Heliotropium amplexicaule* during phase one, and during phase two, *Haplothrips heliotropica* was not found on non-target hosts after the removal of *Heliotropium amplexicaule* (Briese et al., 2002). This means that despite the availability of other potential hosts, *Haplothrips heliotropica* did not infest them. This research provides substantial evidence that

the thrips were specific to *Heliotropium amplexicaule* and did not infest non-target hosts even after the removal of the preferred host. Although this technique has only been used in weed biological control to date, as far as I am aware, it has the potential to be implemented in other areas of biological control, such as insect biological control.

The relatively recent introduction of *Philornis downsi* (Diptera: Muscidae) into the Galapagos Islands has provided an opportunity to test the host specificity of candidate biological control agents. *Philornis downsi* is an invasive avian parasite that feeds from the tissues and blood of altricial nestlings of land birds causing beak malformations, blood loss, and death (McNew & Clayton, 2018). This parasite invaded the Galapagos Islands sometime before 1964 (Causton et al., 2006) and initiated a conservation crisis due to the high mortality of several species of endemic and native birds including many species of Darwin's fiches (Fessl et al., 2018). The native range of *P. downsi* is mainland South America and Trinidad, and records show its presence on mainland Ecuador (Bulgarella et al., 2015). The invasion of the fly prompted the Charles Darwin Research Station and the Galapagos National Park to develop a management plan that includes importation biological control as a technique for controlling *P. downsi* (Causton et al., 2013).

In response to the management plan, Bulgarella et al (2017) found a parasitic wasp, *Conura annulifera* (Hymenoptera: Chalcididae), in western Ecuador and subjected it to specificity tests under laboratory conditions. *C. annulifera* is a solitary idiobiont gap-laying parasitoid which in previous studies of

Philornis flies suggested its specificity to this genus of flies (Bulgarella et al., 2017; Couri et al, 2006). The gap-laying habit refers to the deposition of the parasitoid egg ectoparasitically on the host pupae within the hard outer puparium, and gap-laying parasitoids are restricted to those hosts with a puparium – the cyclorrhaphan Diptera (Boulton & Heimpel, 2018). However, *C. annulifera* exhibited specificity within the Cyclorrhapha as well in laboratory studies showing that five species of cyclorrhaphan flies were rejected for oviposition and only *P. downsi* was attacked. Thus, *C. annulifera* was categorized as a candidate for introduction into the Galapagos Islands to control *P. downsi* (Bulgarella et al. 2017).

Despite the promising laboratory results, it was deemed that field-level specificity testing would aid a risk analysis of introducing *C. annulifera* to the Galapagos Islands. For this, I used a modified version of the Briese et al (2002) framework as a base model to test biological control agents against a parasitic avian fly *P. downsi* in its native range. I reared different species of cyclorrhaphan dipterans and paired them inside and outside active bird nests that had a high potential of becoming a host of *Philornis* flies (Bulgarella et al., 2017) to determine whether C. *annulifera* and other parasitoids of *Philornis* flies would incorporate them into their host range under field conditions.

Historically, importation biological control has been used once in Galapagos, to control the invasive cottony cushion scale (*Icerya purchasi*) using the vedalia beetle (*Rodolia cardinalis*) (Alvarez et al., 2012; Causton et al., 2004;

Hoddle et al., 2013), thus proving that biological control could be a feasible option to control *P. downsi*.

Methods

Location and Length of the study

The research was conducted at the Reserva Ecológica Loma Alta (1.85694^{O} S, 80.59938^{O} W) located in the Santa Elena province of western mainland Ecuador. The Ecological Reserve is composed of a dry tropical forest and a pre-mountainous cloud forest in the Chongón-Colonche mountain range (Amador O. & Martinez R, 2011; Bulgarella et al., 2015). The research was done from March to May in 2016 and February to April in 2017.

Rearing of Non-*Philornis* Flies for Parasitoid Survey and Pairing experiment

I reared cyclorrhaphan dipterans occurring naturally at the Loma Alta field site using chicken meat as a bait and rearing substrate. The meat was purchased in local shops with skin, bone, and fat. I used containers (10 cm diameter and 12 cm in height) with 0.453 kg of chicken meat to attract flies. All bait containers had clear plastic lids with ~15 holes made with nails. The diameter of the holes was ~1 cm to allow flies to enter the container to oviposit and to keep larger carrion predators from accessing the meat. I made two additional holes at the bottom of the bait containers for drainage. Fly larvae were removed from these containers prior to pupating (see below), excluding the possibility that pupal parasitoids could enter the containers during this phase. The chicken meat was placed inside the container, which then was placed outdoors under a secluded concrete building

structure, at floor level inside a small cavity in the wall, that provided protection from precipitation, predators, large scavengers, and by-passers (Figure 2.1).

I set up 2-3 bait containers every week for the length of the study; in 2016 I set up the first containers on the 16th of March and the last one on May 12; in 2017 the first containers were placed on February 17 and the last on May 12. This procedure ensured that enough non-*Philornis* pupae were available for the duration of the study. Dipteran oviposition occurred inside the containers and each container yielded approximately 50-60 larvae. Larvae traveled up towards the lid and away from the meat after the 4th day of placement, and I presumed that these larvae had completed feeding and were seeking a pupation site. I transferred these larvae to pupation containers, which were identical to the fly attraction containers but had 5 cm of a substrate composed of soil, leaves and small pebbles at the bottom and the lid was not perforated. The larvae placed into the pupation containers dug into the substrate immediately after being transferred. I noted that pupation occurred after the first day of transfer. I checked the pupation containers daily for new pupae.

Lastly, I transferred the flies that pupated the same day or the previous day to a field container. The field containers were identical to the pupation containers in dimensions except that I placed 2.5 cm of the same substrate as described above. I placed an average of 24 pupae (± 6.4) in each container (*n*= 12 containers) during 2016 and 20 (± 0 *n*= 5 containers) pupae during 2017, which were placed in the field for the pairing experiment as described below. In 2016, 219 non-*Philornis* pupae were used for the parasitoid survey and 77 non-*Philornis* pupae for the

pairing experiment and, in 2017, 100 non-*Philornis* pupae for the survey and 600 non-*Philornis* pupae for the pairing experiment.

Non-*Philornis* Paired Experiment Inside and Outside Active Bird Nests

I placed the reared pupae "inside" and "outside" the monitored active bird nests. The placement of the pupae "outside" consisted of pairing the field containers (described above) with wild nests or nests within artificial nesting cavities (see figure 2.2). The artificial nesting cavities were composed of nest boxes and bamboo poles, as described in Bulgarella et al. (2015, 2017). I tied the containers to a tree next to the entrance of the active nest (~ 50 cm) using twine (see figure 2.2) during the last week (4^{th} week) of the nesting cycle, three days before the estimated fledging date in the case of the house wren (*Troglodytes aedon)* nests.

For the "inside" pairing, I placed 30 pupae of non-*Philornis* flies inside active nests. In contrast to the pairing "outside", I only placed pupae inside nests that occurred in the pre-made artificial bamboo poles and nest boxes mentioned above. The pupae were placed using soft forceps through the lateral opening (Quiroga & Reboreda, 2012) of the wooden nest boxes with active bird nests. I placed the pupae in 3 different parts of the nesting material, 10 at the top, 10 in the middle, and 10 at the bottom of the nest (figure 2.3). I distributed the pupae in three different sections of the nest to test if parasitoids would encounter and attack exposed non-*Philornis* pupae instead of *Philornis* pupae at the top, middle and bottom. And for the bamboo poles I placed 30 non-*Philornis* pupae by tilting the pole and carefully depositing the pupae inside by using the wall of the nest inside

the pole as a slide for the pupae. Due to the nestling's size and the size of the cavity I was unable to manually distribute the pupae inside the nest. I waited until the nest was four weeks old and approximately three days from the projected fledging date to place the pupae. All pupae, inside and outside the nests were also left for an additional three days after the nestlings fledged to allow additional parasitoid visitation to the nest. Non-*Philornis* pupae were only used inside the nest in 2017, and not in 2016.

Finding and Monitoring Bird Hosts Nests

To find and monitor the progress of active bird nests, both wild and within artificial nesting cavities, I used an endoscopic fiber-optic camera with wireless monitor (shaft 17 mm diameter, fiber-optic cable length 91 cm) mounted on a pole (as in Heimpel et al, 2017). The nest boxes and bamboo poles were monitored to estimate the time nestlings would fledge the nest, and thus place the non-*Philornis* pupae inside the nests for the pairing tests. I began monitoring nests during the incubation phase and continued monitoring them until the nestlings fledge (at least twice a week). The House Wren (*Troglodytes aedon*) nest cycle took approximately 4 weeks from the incubation phase to the nestlings fledging the nest. The house wren was the most abundant bird host that I found. The nesting cycle for all other bird species varied but took no less than 4 weeks. I also monitored wild nests that occurred at the field site. I found wild nests located in trees and cavities of hollow bamboo poles used as structures for houses. Wild nests were observed, and development was recorded using the same technique and materials as the nests within artificial nesting cavities.

Mortality of two of house wren nestlings was recorded, one inside a bamboo pole and the other inside a nest box in 2016. The mortality of the nestling inside the bamboo pole was likely due to the bamboo pole falling to the ground during an earthquake measuring 7.8 on the Richter scale that occurred on April 16, 2016.

Survey of Parasitoids

I surveyed for parasitoids that attacked the reared non-*Philornis* flies during the two years of the study (2016, 2017). I used the same field containers described above attached to trees using twine that looped around the top wider part of the container, which contained an average of 17.0 (±2.3) non-*Philornis* pupae. In contrast to the pairing "outside", the field containers were placed in random places throughout the field site with no proximity to any obvious bird nests. I left these containers in the field for four days to allow parasitoid visitation.

Recovering Nests and Containers

I recovered the nests with non-*Philornis* pupae ("inside" pairing), field containers of the "outside" pairing and the survey containers. To retrieve the inside pairing samples, the bamboo poles were taken down and the nests were collected by hand and transferred to a plastic bag. The nests inside nest boxes were extracted by hand as well. All nests were taken indoors to be dismantled and all *Philornis* spp. and non-*Philornis* pupae found were counted. All pupae in the nests and the field containers were then transferred to individual emergence vials (25x95 mm, diameter and length). The emergence vials with the pupae were placed indoors in shade at room temperature, humidity, and light. After the emergence of

parasitoids and flies inside the vials, I placed them into screw-top cryovials (1.2 ml) with 75% ethanol for storage.

Identification of the Emerged Species

Non-*Philornis* flies and parasitoid species were identified after emergence, and *Philornis* species as pupae following Skidmore (1985), Couri (1999) and Bulgarella et al (2017). Non-*Philornis* fly species were identified by Dr. Bradley Sinclair from the Canadian National Collection of Insects (CNCI).

Results

Parasitism Rates in Host Bird Nest by *Philornis* Species

A total of 539 *Philornis* pupae (242 *P. downsi*, and 297 *P. niger*) were recovered from bird nests in Loma Alta during 2016 and 2017. I found a total of 29 nests during both years, five House wren (*Troglodytes aedon*) nests and a single saffron finch nest (*Sicalis flaveola*) in 2016, and in 2017 I found a total of 17 House wren nests, one Smooth-billed Ani (*Crotophaga ani*) nest, one Long-tailed Mockingbird (*Mimus longicaudatus*) nest, and one Streak-headed woodcreeper (*Lepidoclaptes souleyetii*) nest. All the bird species are known to be hosts of *Philornis* spp. (Bulgarella et al., 2015 & 2017). The average number of *P. niger* and *P. downsi* pupae found, during both years, within parasitized nests was 14.0 ± 3.4 SEM (*n*= 20 nests with *P. niger*) for *P. niger* and for *P. downsi* 11.0 ± 3.6 SEM (*n*= 20 nests with *P. downsi*). *P. niger (n*= 85 pupae*)* was more abundant than *P. downsi (n*= 33 *pupae)* in 2016, and in 2017 *P. downsi (n*= 209 pupae*)* was more abundant than *P. niger (n*= 192 pupae*)*. A total (both years) of 12 nests contained

both species of *Philornis, P. niger* with 120 pupae in total (average of 14.0 ± 4.9 SEM pupae per nest) and, *P. downsi* with 143 in total (average 17.0 ± 5.1 SEM pupae per nest) and seven nests contained a single species of *Philornis* spp, three nests with a total of 51 *P. downsi* pupae (average 17.0 ± 13.0 SEM *P. downsi* pupae per nest) and four with 51 *P. niger* pupae (average 12.7 ± 4.1 SEM *P. niger* pupae per nest) (Table 2.1). From the 29 active nests found, 17 nests were in the artificial cavities (nest boxes and bamboo poles) and used for the nest-pairing experiment. *Philornis* pupae were found in 11 out of the 17 nests that were paired with non-*Philornis* pupae, all of which had been constructed by *T. aedon* (Table 2.2).

Rates of Parasitism of non-*Philornis* Flies in Pairing Experiments and Surveys

The pairing (outside and inside) and survey resulted in non-*Philornis* flies and parasitoids emerging from the pupae in both years (n= 296 pupae in 2016, and n= 700 pupae in 2017). The non-*Philornis* flies that emerged were: *Chrysoma albiceps* (Diptera: Calliphoridae), *Hydrotaea* sp. (Diptera: Muscidae), *Lucilia eximia* (Diptera: Calliphoridae), *Peckia ingens* (Diptera: Sarcophagidae), *P. pascoensis* (Diptera: Sarcophagidae), *P. pexata* (Diptera: Sarcophagidae), *and Peckia* sp. (Diptera: Sarcophagidae), and two parasitoids: *Brachymeria podagrica* (Hymenoptera: Chalcididae) and *Exoristobia* sp. (Hymenoptera: Encyrtidae) (Table 2.3). The proportion of parasitism for *Brachymeria podagrica* was 20% being the most abundant parasitoid that emerged from the non-*Philornis* pupae. The identification of the host fly species pupae was not possible due to the

similarity of pupae among all non-*Philornis* flies. A total of 188 pupae never emerged and upon dissection, no indication of parasitoidism was found.

Rates of Parasitism of *Philornis* Flies and Parasitoid Host Specificity

The overall rate of parasitism for *Philornis* species was 3.4% (Table 2.4). Parasitoids that emerged exclusively from *Philornis* pupae during 2016 despite the presence of alternate hosts present outside the nest were: *Conura annulifera* (Hymenoptera: Chalcididae, n=7 pupae), *Spalangia* sp. (Hymenoptera: Pteromalidae, n=3 pupae) and *Trichopria* sp. (Hymenoptera: Diapriidae, n=1 pupa). During 2017, only *Trichopria* (n=6) emerged from *P. downsi* despite alternate hosts present in both inside and outside nests. Also, *Brachymeria* sp. (Hymenoptera: Chalcididae, n=2 pupae) and *Trichopria* sp. (n=12 pupae) emerged from unpaired wild nests (Table 2.1) over both years. The parasitoids *C. annulifera*, *Spalangia* sp., and *Brachymeria* sp., which are all solitary parasitoids so only a single parasitoid individual emerged from *Philornis* pupae parasitized by these species. However, *Trichopria* sp. develop gregariously, and thus multiple parasitoid individuals emerged from *Philornis* pupae (Figure 2.4).

Discussion

My results showed that almost all parasitoids that emerged from *Philornis* spp. pupae encountered at the Loma Alta Ecological Reserve did not emerge from experimentally deployed pupae of closely related dipteran species. Thus, the following parasitoid species showed patterns of specificity to *Philornis* pupae: *Conura annulifera*, *Trichopria* sp., *Brachymeria* sp., and *Spalangia* sp. These

field results mirror literature reports for *C. annulifera* (Bulgarella et al. 2017) and laboratory host-specificity studies for *C. annulifera* (Bulgarella et al. 2017) and *Trichopria* sp. (M. Bulgarella & G.E. Heimpel, unpublished). The parasitoid *Exoristobia* sp. only emerged from both *Philornis* and non-*Philornis* pupae, and laboratory studies for this species also indicated a broad host range among cyclorrhaphan Diptera (M. Bulgarella and G.E. Heimpel unpublished). Among the experimentally tested non-*Philornis* dipterans that occurred naturally in the field site were: *Hydrotaea* sp. (Muscidae), *Lucila eximia*, *Chrysoma albiceps* (both Calliphoridae), *Peckia ingens*, *Peckia pascoensis*, *Peckia pexata*, and *Peckia* sp. (all Sarcophagidae). Pupae of these species were attacked by *Exoristobia* sp. as well (at a lower rate) but primarily by *Brachymeria podagrica*, which is a well-known generalist parasitoid of cyclorrhaphan Diptera (Noyes & Sadka, 2003).

Among all the parasitoids I found, *C. annulifera* and *Trichopria* sp. appear to be the most promising biological control agents against *P. downsi* in the Galapagos Islands. *Trichopria* sp. was the most abundant parasitoid that emerged from *Philornis* pupae, however, the species identification of *Trichopria* sp. is not yet available. *C. annulifera* has shown high specificity to *Philornis* species in no-choice trials including five species of cyclorrhaphan dipterans in three families: Muscidae, Calliphoridae and Sarcophagidae (Bulgarella et al 2017). Furthermore, specificity is also achieved due to the restrictive behavior of *C. annulifera* ovipositing in the gap within the hard outer puparium of cyclorrhaphan Diptera. Cyclorrhaphan Dipterans have a unique gap between the developing fly and the hard outer puparium during pupation (Whitten, 1957). This gap allows for the

larvae of gap-laying parasitoids to avoid external environmental conditions, and, simultaneously avoid the fly's immune system (Bouletreau, 1987; Geden & Moon, 2009; Pennacchio & Strand, 2006). This gap restricts gap-laying parasitoids from expanding their host range to non-cyclorrhaphan insects making *C. annulifera* evolutionarily constrained to cyclorrhaphan Diptera (Boulton & Heimpel, 2018; Ueno, 2015).

My research supports the hypothesis that *C. annulifera* is specific against *Philornis* spp. due to its exclusive emergence from *Philornis* spp. pupae in our field assays despite the presence of a total of 919 non-*Philornis* flies inside and outside active nests and in the adjacent area. In the Galapagos Islands, 4 endemic species of the genus *Lucilia* and one from *Hydrotaea* are present (Sinclair, 2017) and our results provide an insight into the specificity of *C. annulifera* against members of these two genera. However, there are 13 other endemic species of cyclorrhaphan flies in the Galapagos archipelago (Sinclair, 2017) and additional research is needed to ensure the safety of *C. annulifera* against such species of flies. Specificity testing under quarantine conditions in the introduced range against endemic insect species in the Galapagos Islands, specially cyclorrhaphan dipterans, is a crucial step towards a safe release of *C. annulifera*.

My results provide relevant information for the management efforts to control *P. downsi* populations in the Galapagos Islands, where biological control has been considered as a strategy for its control (Causton et al., 2013). Populations of land bird species have declined in recent years, with *P. downsi* the main known cause for their decline (O'Connor et al., 2010). The mangrove finch is of special

concern due to its low numbers in the wild; 23 mating pairs are estimated to remain on Isabela Island, where *P. downsi* is found in almost every nest of this species (Wiedenfeld et al., 2007).

My experimental in-field assay of host choice allowed us to test the specificity of naturally occurring parasitoids and mirroring the findings of Bulgarella et al. (2017) on *C. annulifera*. Specificity testing for a biological control agent candidate in the pest's natural range is desirable but challenging due to limitations of standard choice testing procedures, and the possible unavailability of non-target organisms of the native range present in the pest's introduced range (Heimpel & Mills, 2017). However, despite these limitations, specificity testing in the field can be a valuable part of assessing the risk posed by the biological control agent to unintended target species. It can also allow for testing under environmental conditions very similar to the introduced range (Briese, 1999; Clement and Cristofaro, 1995; Briese et al., 2002) which are hard to replicate under laboratory conditions. Ultimately the goal of field testing is to validate the laboratory findings.

P. downsi is the only fly from the genus *Philornis* that exists in the Galapagos Islands (Sinclair, 2009), which makes introducing a highly specialized biological control agent against *P. downsi* safe if biological control were to be implemented. Additionally, the presence of such a biological control agent could serve as a line of defense against possible future *Philornis* spp. invasions (Fessl et al., 2018; Bulgarella et al., 2017). Although biological control carries intrinsic risk and specificity does not guarantee effectiveness (Brodeur, 2012), it is fundamental

to consider the successful cases where biological control proved to be safe and effective, especially for conservation. For instance, a successful implementation of classical biological control and establishment of *Rodolia cardinalis,* in the Galapagos Islands, resulted in controlling the invasive cottony cushion scale (*Icerya purchasi*) and thus preserving endemic flora (Alvarez et al., 2012; Causton et al., 2004; Hoddle et al., 2013).

Table 2.1. Total count of *Philornis* pupae collected from 24 active bird nests in the Loma Alta Ecological Reserve during 2016 and 2017, by species of bird, *Philornis* flies and parasitoids. * Denotates inside pairing experiments. $^{+}$ Denotates outside pairing experiments. (U= Unknown)

Bird Species	Nest collection Date	Nest type	*P. downsi*	*P. niger*	*C. annulifera*	*Spalangia sp*	*Brachymeria sp*	*Trichopria sp*	*Exoristobia sp*	U
2016										
Troglodytes aedon	March, 2016	Wild	0	0	0	0	0	0	0	0
Troglodytes aedon^{+}	March, 2016	Nest Box	3	40	1	0	0	1	0	0
Troglodytes aedon^{+}	March, 2016	Bamboo	4	26	0	0	0	0	0	0
Sicalis flaveola^{+}	April, 2016	Bamboo	0	0	0	0	0	0	0	0
Troglodytes aedon	April, 2016	Wild	0	14	0	0	0	0	0	0
Troglodytes aedon^{+}	April, 2016	Bamboo	6	1	6	3	0	0	0	0
Troglodytes aedon	April, 2016	Wild	2	19	0	0	0	0	0	0
Troglodytes aedon	April, 2016	Wild	0	0	0	0	0	0	0	0
Troglodytes aedon	May, 2016	Wild	31	5	0	0	0	0	0	0

Bird Species	Nest collection Date	Nest type	*P. downsi*	*P. niger*	*C. annulifera*	*Spalangia sp*	*Brachymeria sp*	*Trichopria sp*	*Exoristobia sp*	U
2017										
*Troglodytes aedon**+	March, 2017	Bamboo	0	0	0	0	0	0	0	0
*Troglodytes aedon**+	March, 2017	Bamboo	0	0	0	0	0	0	0	0
*Troglodytes aedon**+	March, 2017	Bamboo	0	0	0	0	0	0	0	0
*Troglodytes aedon**+	March, 2017	Bamboo	0	0	0	0	0	0	0	0
*Troglodytes aedon**+	April, 2017	Bamboo	0	0	0	0	0	0	0	0
*Troglodytes aedon**+	April, 2017	Bamboo	11	2	0	0	0	0	0	0
L. souleyetii	April, 2017	Nest Box	0	2	0	0	0	0	0	0
*Troglodytes aedon**+	April, 2017	Nest Box	8	14	0	0	0	2	0	0
*Troglodytes aedon**+	April, 2017	Bamboo	0	22	0	0	0	0	0	0
*Troglodytes aedon**+	April, 2017	Bamboo	2	0	0	0	0	0	0	0
*Troglodytes aedon**+	April, 2017	Bamboo	3	7	0	0	0	1	0	0
Troglodytes aedon	April, 2017	Wild	9	16	0	0	0	1	0	0

** Denotates <u>inside</u> pairing experiments. + Denotates <u>outside</u> pairing experiments. (U= Unknown)*

Bird Species	Nest collection Date	Nest type	*P. downsi*	*P. niger*	*C. annulifera*	*Spalangia sp*	*Brachymeria sp*	*Trichopria sp*	*Exoristobia sp*	U
2017										
*Troglodytes aedon**+	April, 2017	Bamboo	0	13	0	0	0	0	2	0
Troglodytes aedon	April, 2017	Wild	21	14	0	0	0	1	0	0
Mimus longicaudatus	May, 2017	Wild	43	0	0	0	2	1	0	0
*Troglodytes aedon**+	May, 2017	Bamboo	8	2	0	0	0	1	0	0
Troglodytes aedon	May, 2017	Wild	61	61	0	0	0	5	3	0
Crotophaga ani	June, 2017	Wild	6	0	0	0	0	5	0	0
Troglodytes aedon	June, 2017	Bamboo	0	0	0	0	0	0	0	3
Troglodytes aedon	June, 2017	Bamboo	0	22	0	0	0	1	0	0

** Denotates <u>inside</u> pairing experiments. + Denotates <u>outside</u> pairing experiments. (U= Unknown)*

Table 2.2. Total *Philornis* spp. pupae found inside the paired nests, and non-*Philornis* pupae used in the paired experiments

Bird Host Species	**Paired: Inside**			**Paired: Outside**		
2017	*P. downsi*	*P. niger*	Non-*Philornis*	*P. downsi*	*P. niger*	Non-*Philornis*
Troglodytes aedon	0	0	30	0	0	20
Troglodytes aedon	0	0	30	0	0	20
Troglodytes aedon	0	0	30	0	0	20
Troglodytes aedon	0	0	30	0	0	20
Troglodytes aedon	0	0	30	0	0	20
Troglodytes aedon	11	2	30	0	0	20
Troglodytes aedon	8	16	30	0	0	20
Troglodytes aedon	0	22	20	0	0	20
Troglodytes aedon	2	0	20	0	0	20
Troglodytes aedon	2	9	20	0	0	20
Troglodytes aedon	10	16	20	0	0	20
Troglodytes aedon	21	15	20	0	0	20
Troglodytes aedon	9	2	20	0	0	20
2016						
Troglodytes aedon	3	42	1	0	0	6
Troglodytes aedon	4	26	0	0	0	13
Sicalis flaveola	0	0	0	0	0	9
Troglodytes aedon	6	1	0	0	0	43

Table 2.3. Total non-*Philornis* fly species and parasitoids that emerged from paired non-*Philornis* pupae used in the experiments and the surveys in 2006 and 2017.

Emerged non-*Philornis* Fly Species	2016			2017		
	Survey	**Inside**	**Outside**	**Survey**	**Inside**	**Outside**
Chrysoma albiceps (Diptera: Calliphoridae)	0	0	0	6	23	14
Hydrotaea sp. (Diptera: Muscidae)	2	0	2	0	4	2
Lucilia eximia (Diptera: Calliphoridae)	12	0	4	5	66	51
Peckia ingens (Diptera: Sarcophagidae)	9	0	2	5	12	7
P. pascoensis (Diptera: Sarcophagidae)	14	0	4	3	16	6
P. pexata (Diptera: Sarcophagidae)	8	0	13	5	19	11
Peckia sp. (Diptera: Sarcophagidae)	83	1	31	30	63	65
Unemerged puparia	25	0	8	27	77	51
Parasitoid Species						
Brachymeria podagrica (Hymenoptera: Chalcididae)	63	0	12	19	60	53
Exoristobia sp. (Hymenoptera: Encyrtidae)	3	0	1	0	0	0

Table 2.4. Rates of parasitism of *P. downsi*, *P. niger* and Non-*Philornis* flies parasitized by seven species of parasitoids in Loma Alta in years 2016 and 2017.

	Philornis downsi		***Philornis niger***		**Non-*Philornis***	
	2016 (n = 33)	2017 (n = 209)	2016 (n = 85)	2017 (n = 192)	2016 (n = 263)	2017 (n = 545)
Conura annulifera	0.000	0.000	0.071	0.000	0.000	0.000
Trichopria sp.	0.000	0.062	0.012	0.031	0.000	0.000
***Brachymeria* sp.**	0.000	0.000	0.000	0.010	0.000	0.000
Exoristobia sp.	0.000	0.000	0.000	0.016	0.015	0.000
Brachymeria podagrica	0.000	0.000	0.000	0.000	0.285	0.220
Spalangia sp	0.000	0.000	0.035	0.000	0.000	0.000
Unknown parasitoid	0.000	0.014	0.000	0.000	0.000	0.000
Total	0.000	0.077	0.129	0.057	0.300	0.220

Figure 2.1. Bait container with chicken bait inside. The lid is perforated allowing flies to oviposit.

Figure 2.2. Placement of the field containers with non-*Philornis* pupae outside an active bird nest.

Figure 2.3. Division of the nest into 3 sections: top, middle, and bottom sections into which 10 non-*Philornis* pupae were placed. This was done to observe parasitoid location preference for host selection.

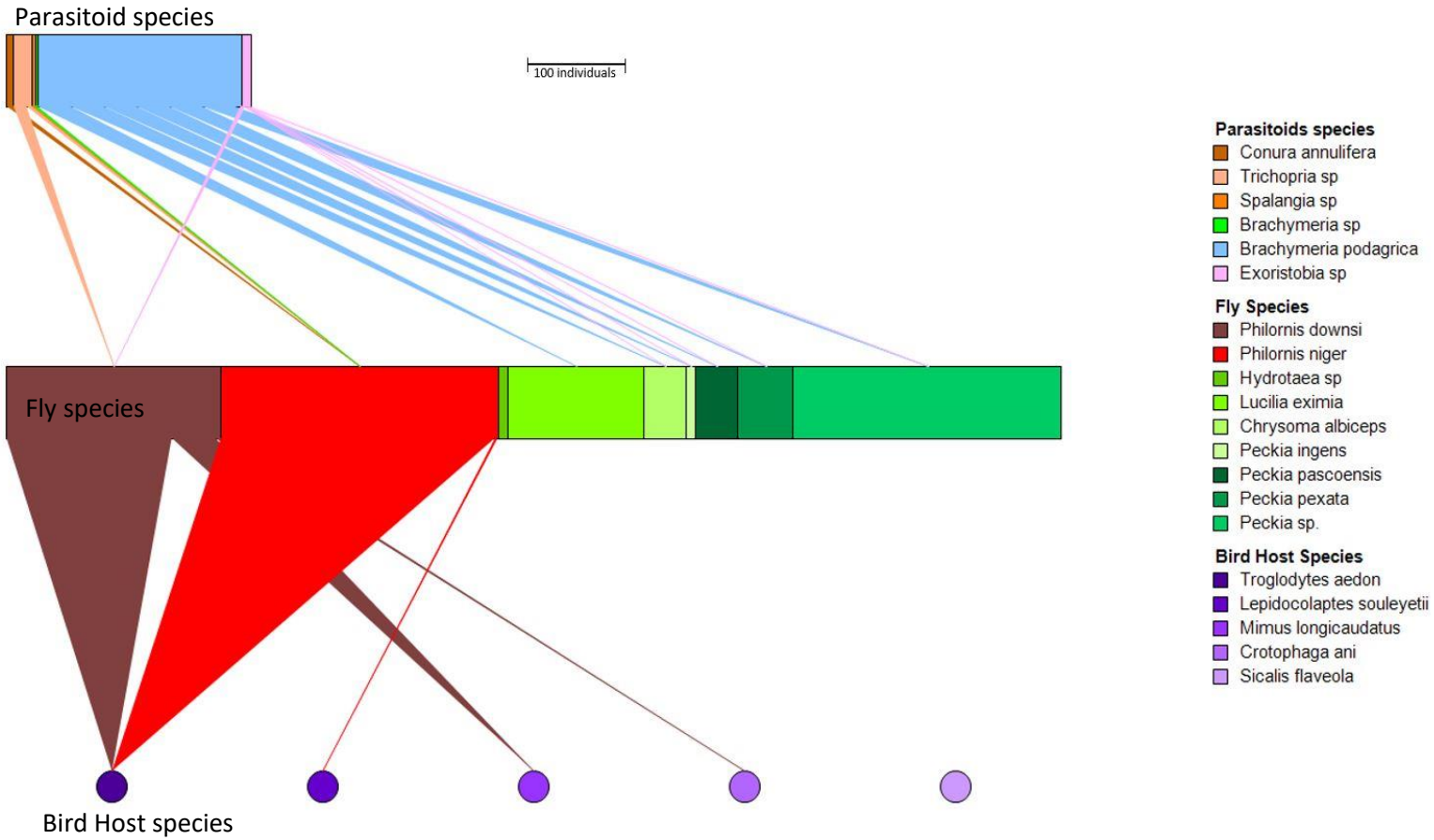

Figure 2.4: Tri-Trophic web of the observed interactions among parasitoids, flies and bird hosts in Loma Alta. Top tier: Parasitoid Species, Middle tier: Fly Species, and Bottom tier Bird Host Species.

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