

Management of an invasive avian parasitic fly in the Galapagos Islands: is biological control a viable option?

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Abstract The bird-parasitic fly, *Philornis downsi*, was first recorded in the Galápagos Islands in 1964 where it likely invaded from mainland Ecuador. This muscid fly is now the leading cause of recent declines in endemic landbird populations as its larvae feed on the nestlings of at least 19 bird species in the Galápagos, including many species of Darwin's finches. As yet, no long-term control method has been implemented for *P. downsi*, but importation (also known as classical) biological control may be a viable option. Due to historically high-profile examples of biological control agents attacking non-target species, some consider biological control to be too risky to be compatible with conservation aims. However, since biosafety practices were implemented beginning in the 1990s, these risks have been drastically reduced, and biological control is now an important tool for suppressing invasive species that are difficult to control using other means. We investigated the safety of a potential biological control agent, the parasitoid wasp, *Conura annulifera*, that attacks *P. downsi* in its native range. Here we summarise the results of a series of field, laboratory and comparative studies on *C. annulifera* (methods and results are not reported here) and outline future directions. We used a field experimental paradigm involving nest boxes baited with non-target hosts, and quarantine laboratory no-choice trials in which non-target hosts were exposed to *C. annulifera*. Our work to-date suggests that *C. annulifera* is restricted to attacking species within the genus *Philornis*. Furthermore, a phylogenetically controlled comparative study suggests that *C. annulifera* is evolutionarily constrained in its host range. These results lead us to conclude that *C. annulifera* demonstrates promise as an ecologically safe agent for the long-term biological control of *P. downsi*. Studies will now focus on an evaluation of risks to endemic and native species in the Galápagos.

Keywords: biological control, conservation, *Conura annulifera*, invasive species, *Philornis downsi*

THE HISTORY OF BIOLOGICAL CONTROL

When non-native species invade novel ecosystems, the consequences can be extreme. Anthropogenic change has increased the frequency and the success of such invasions; increased traffic to previously isolated sites increases the chance that non-native species will arrive, and the chances of colonisation are often increased in areas that are altered by human activity (Sax, et al., 2002). When colonisation occurs, non-native species are often far more successful than in their native range. Although the reasons for this are likely complex, one often-cited reason is the 'enemy release' hypothesis (ERH) (Liu & Stiling, 2006). Enemy release occurs when invaders colonise a new area, free from the natural enemies (predators, parasites and pathogens) with which they co-evolved, and are released from the effects that these enemies have on population suppression. Importation (also known as classical) biological control involves reconstructing (at least in part) the assemblage of co-evolved natural enemies present in the native range of the problematic species in order to control it (Heimpel & Mills, 2017). Although more commonly known from agricultural systems, importation biological control for conservation is a developing sub-discipline and shows promise as a long-term strategy for dealing with harmful invasive species (Van Driesche, et al., 2010; Van Driesche & Reardon, 2017). One noteworthy example is the introduction of the specialised ladybeetle, *Rodolia cardinalis*, which effectively controlled populations of the cottoncushion scale, *Icerya purchasi*, in the Galápagos archipelago. This particular introduction has more than likely been the saviour of endemic plant species that are attacked by *I. purchasi* (Hoddle, et al., 2013).

Unfortunately, historically high-profile cases of biological control failures that led to non-target effects on threatened species have received significant media attention and these examples have hampered progress in the sub-discipline of conservation-focused biological control (Van Driesche & Reardon, 2017). In order for

importation biological control to be safe and successful, it is paramount that we understand the ecology, in particular the host specificity of the putative natural enemy set for release. A majority of examples of biological control, for conservation or for agriculture, have demonstrated both its success and its safety, particularly since the 1990s when concerns over biosafety gained momentum (Barratt, et al., 2010; Van Driesche, et al., 2010; Van Driesche, 2012; Heimpel & Mills, 2017; Van Driesche & Reardon, 2017; Heimpel & Cock 2018). However, the negative reputation of biological control persists due to the memorable nature of failures that have caused detrimental effects on native fauna (see Clarke, et al., 1984; Howarth 1991). Sadly, it is these examples that are more publicly well-known due to the strong emotions that they elicit (Van Driesche, & Reardon, 2017). Yet there is still hope for the discipline, and conservation-focused biological control has initiated a paradigm shift, demonstrating that biological control can be more than just compatible with conservation aims, it can actually promote them (Van Driesche, et al., 2010; Heimpel & Cock, 2018). In order for these techniques to be incorporated into the conservation 'tool box' it is imperative that we build trust between practitioners of biological control, conservationists and the public. To do so, the biological control community must demonstrate the pragmatism and caution that go into designing safe and effective biological control programmes.

Philornis downsi

Philornis downsi (Dodge & Aitken) is a bird-parasitic muscid fly that is native to mainland South America but is invasive in the Galápagos Islands where it likely invaded from mainland Ecuador (Bulgarella, et al., 2015). It was first reported in the archipelago in 1964 and, in the last 15–20 years has become a major threat to the persistence of many passerine bird species in the Galápagos, including

the majority of species of Darwin's finches (Fessl, et al., 2018). This threat occurs because of the way the larvae of *P. downsi* feed: the adults are free-living but the larvae are obligate ectoparasitic blood-feeders on young nestlings, leading to blood loss and death (Fessl, et al., 2006; O'Connor, et al., 2010; Kleindorfer, et al., 2014; Koop, et al., 2016; Heimpel, et al., 2017). *P. downsi* is considered the greatest threat to the persistence of many land-bird species in Galápagos. The critically endangered mangrove finch (*Camarhynchus heliobates*) and medium tree finch (*C. pauper*) are particularly at risk, with any nestlings of the former now being protected by 'head-starting' (hand-rearing any eggs collected in the wild; Cunninghame, et al., 2012). The ramifications of any such extinction would be extensive. Not only would this represent a tragic loss of iconic species in a well-protected environment, it would also be a terrible loss of evolutionary history and the opportunity to study it. Rosemary and Peter Grant spent the last forty years studying the evolution of Darwin's finches and have commented on the importance and uniqueness of Darwin's finches for work of this nature:

'A final reason that makes them (Darwin's finches) so suitable (for studying evolution) is that none of the species has become extinct as a result of human intervention. This cannot be said for many other radiations elsewhere in the world.' Grant & Grant (2009)

The Grants' work has demonstrated the power of evolution and speciation and the underlying mechanisms, but much more remains to be discovered (e.g. Abzhanov, 2010 and articles therein). Losing even a single species of Darwin's finch would represent a terrible loss for evolutionary biology and could have a profound impact on future phenomena that the species' radiation may reveal to us. Moreover, losing large numbers of individuals of any of these species will have considerable impacts on the functioning of Galápagos ecosystems due to the critical roles that they play in pollination and seed dispersal (Causton, et al., 2013; Traveset, et al., 2015; Nogales, et al., 2017).

Need and potential for biological control of *Philornis downsi*

Infestation by *P. downsi* results in extreme nestling mortality in Galápagos, which has not been observed in the native range of the fly (Fessl, et al., 2018). The ERH (Liu & Stiling, 2006) – a paucity of co-evolved natural enemies in the invaded compared to the native range – is one likely reason for the increased abundance of *P. downsi* in Galápagos compared to the mainland (Bulgarella, et al., 2015; 2017; Boulton & Heimpel, 2017). The ERH serves as the theoretical underpinning of modern importation biological control, whereby one or a suite of co-evolved natural enemies is liberated into the invasive range to control the target species (Heimpel & Mills, 2017). The scarcity of natural enemies of *Philornis* spp. in the Galápagos compared to the mainland suggests that importation biological control may be a valuable tool to control *P. downsi* (Bulgarella, et al., 2017).

Although several control strategies are currently being explored and considered, importation biological control may be critical in protecting Darwin's finches and other endemic bird species in Galápagos from *P. downsi*. Other possible control methods include short-term strategies, such as nest treatment with insecticide and mass trapping using lures (Fessl, et al., 2018). The short-term approaches are considered mainly as stop-gap measures, whilst long-term measures, such as biological control and sterile male release, are developed and implemented. Of the long-term measures considered so far, biological control using natural enemies from the native range is currently the most

promising solution. The release of sterile males is another potential long-term solution but this is currently hampered by difficulties in laboratory breeding of *P. downsi* (Lahuatte, et al., 2016; Fessl, et al., 2018).

In 2012, a workshop was organised by the Charles Darwin Foundation and the Galápagos National Park Directorate in order to form an action plan for conservation of Darwin's finches and other small land birds due to the ever-increasing threat from *P. downsi* (Causton, et al., 2013). One priority research goal recognised at this workshop was to identify natural enemies in the fly's native range and investigate the potential for biological control (Causton, et al., 2013). Over the last four years, we have discovered several parasitoid wasp species attacking species of *Philornis* in mainland Ecuador (Bulgarella, et al., 2015; 2017). Before any of these parasitoids can be considered as suitable biocontrol agents, in-depth studies of their host range need to be conducted. To address this question, we have been using a holistic approach consisting of a novel field experimental paradigm, comprehensive literature review, detailed study of the physiology and evolutionary ecology of the putative biological control agents, and traditional laboratory host range tests. In this manuscript we review and summarise our published work so far and outline future directions.

FIELD OBSERVATIONS AND EXPERIMENTS

Field work at two field sites in western mainland Ecuador between 2013 and 2017 has revealed a number of parasitoid species attacking *Philornis* spp. pupae collected from nest boxes (Bulgarella, et al., 2015; 2017). In addition, we have developed a novel field experimental paradigm over the last two years that can be used as a preliminary assay to test whether the parasitoid wasp species that we have recovered are specific to *Philornis* spp. in the field.

The experimental set-up was as follows. Nest boxes that we monitor throughout the bird breeding season for *P. downsi* pupae and their parasitoid wasps were paired with bait boxes. These bait boxes contained a number of non-target host species that had been reared from the local area. We also placed pupae of non-target species inside active bird nests. Any parasitoid wasp species that attacked *Philornis* spp. in the nest boxes and nests also had the opportunity to attack non-target hosts in the adjacent bait boxes and inside active nests. Using this experimental paradigm, we were able to determine which (if any) species of parasitoid wasp did not exclusively attack *Philornis* spp. We have concentrated our further efforts on *Conura annulifera* (Hymenoptera: Chalcididae), a parasitoid that has been recorded attacking only *Philornis* spp. in these field experiments. We will concentrate on this species for the remainder of the manuscript but note that we are also considering other species for biological control of *P. downsi*, such as an unidentified species of *Trichopria* (see Bulgarella, et al. (2017) and Boulton & Heimpel (2017) for details). This study is in progress at the time of writing and the results will be published elsewhere.

Life history and evolutionary ecology of *Conura annulifera*

Previous work on the natural host range of *C. annulifera* supports our assertion that it is a specialist on the genus *Philornis*. It has been recorded in previous studies throughout South and Central America where it has been reported as parasitising only *Philornis* spp. (including *P. downsi* and *P. deceptivus*; Burks, 1960; De Santis, 1979; Delvare, 1992; Couri, et al., 2006). Moreover, studies where pupae were reared from other Diptera (Muscidae, Calliphoridae and Sarcophagidae) in regions where *C. annulifera* has been reported never yielded this parasitoid

(Bulgarella, et al., 2017). However, only five studies have reported finding *C. annulifera* in the field (Burks, 1960; De Santis, 1979; Delvare, 1992; Couri, et al., 2006; Bulgarella, et al., 2017), and so more data were needed in order to determine whether this species might constitute a *Philornis*-specific biological control agent. In the sections below, we present the evidence that we have accumulated so far in support of the possibility that *C. annulifera* is a *Philornis* genus specialist.

Conura annulifera is a solitary pupal ectoparasitoid (Bulgarella, et al., 2017). It attacks pupae of *Philornis* spp., laying a single egg on the outside of the developing pupa. More specifically, *C. annulifera* is a 'gap-layer', a parasitoid that deposits its egg between the hard external puparium and the soft body of the developing pupa. We hypothesised that the specificity of this oviposition site is likely to restrict the range of suitable hosts that *C. annulifera* can parasitise to the cyclorrhaphan Diptera, an unranked taxon that contains families such as the Muscidae, Calliphoridae, Sarcophagidae and Syrphidae (Griffiths, 1972; Boulton & Heimpel, 2017). The Cyclorrhapha are the only group of holometabolous insects that exhibit this gap (Whitten, 1957), and so it is unlikely that species outside this taxon are physiologically viable hosts for *C. annulifera*. We tested this possibility using phylogenetically controlled comparative studies for all known species of gap-layers in the superfamily Chalcidoidea and the results support our hypothesis: gap-laying species exhibit narrower host ranges than 'true' ectoparasitoids (Boulton & Heimpel, 2018). Moreover, these analyses revealed that gap-laying as a strategy may constitute an evolutionary dead-end. Compared to endoparasitoids and other ectoparasitoids, evolutionary transitions towards gap-laying were more likely than transitions away from it (Boulton & Heimpel, 2018).

This comparative work has implications for biological control in general and for the specific case of control outlined here. Our findings suggest that (1) gap-layers such as *C. annulifera* are likely to be more host specific, and so safer putative biological control agents, than 'true' ectoparasitoids, and (2) gap-layers including *C. annulifera* may represent particularly useful agents for importation biological control as they are less likely to transition, or diversify, to attack novel hosts after release outside their native range. With regards to the specific case of using *C. annulifera* to control *P. downsi* in the Galápagos, this work improves our understanding of the most at-risk non-target organisms were a release to be attempted, but it does not explicitly tell us whether *C. annulifera* is likely to be a safe species for importation biological control. To test this, more traditional host range studies were conducted, the results of which we outline in the section below.

Laboratory host range studies

Bulgarella, et al. (2017) exposed a range of non-target host pupae to *C. annulifera* that were maintained in the laboratory. This included five cyclorrhaphan Diptera (*Musca domestica*, *M. autumnalis*, *Stomoxys calcitrans* (Muscidae), *Sarcophaga bullata* (Sarcophagidae), *Calliphora vicina* (Calliphoridae)), three Lepidoptera (*Epiphyas postvittana* (Tortricidae), *Manduca sexta* (Sphingidae), *Plodia interpunctella* (Pyralidae)) and a hymenopteran (*Habrobracon hebetor* (Braconidae)). These species were chosen due to their likely physiological compatibility with parasitism by *C. annulifera* (Diptera) and because other species in the genus *Conura* have been shown to attack various Lepidoptera and Hymenoptera (see Bulgarella, et al., 2017).

In no case did the wasp produce offspring on any of these non-target species: in these lab studies, *C. annulifera*

only reproduced successfully on *P. downsi*. This suggests that, of the species presented so far, only *P. downsi* represents a viable host for *C. annulifera* (Bulgarella, et al., 2017). However, this experimental design did not allow us to address the mechanism underlying this apparent specificity. It could either be that *C. annulifera* does not attempt to attack any species other than *Philornis* (i.e., behavioural specificity) or the wasp attempts to parasitise these species but their offspring fail to develop and emerge (i.e., only physiological specificity; see Desneux, et al., 2009). For an importation biological control programme with *C. annulifera* to be truly considered safe, it is important that we rule out the possibility that *C. annulifera* would attack non-target hosts, and cause their mortality by envenomation or oviposition.

To do this, we carried out additional analyses to test whether exposure to *C. annulifera* had any influence on the successful emergence of non-target pupae compared with controls. We found no evidence that exposure to *C. annulifera* resulted in elevated mortality for non-target hosts (see Bulgarella, et al., 2017). In contrast, when *P. downsi* pupae were exposed to the wasp, mortality increased independently of successful parasitism (i.e. more unparasitised fly pupae failed to emerge in the exposed treatment than in the control), perhaps as a result of host-feeding or envenomation/attempted parasitism. This finding, plus behavioural observations, suggests that *C. annulifera* does not attempt to sting or probe any potential host other than *Philornis* spp. pupae (Boulton & Heimpel, 2017; Bulgarella, et al., 2017).

FUTURE DIRECTIONS

Although all the evidence accumulated so far suggests that *C. annulifera* is a specialist parasitoid of *Philornis* spp. and should be seriously considered as a potential agent for the biological control of *P. downsi* in the Galápagos, one crucial question regarding the host range remains. It is critical to know whether *C. annulifera* is able to attack and develop on native or endemic non-target species present in the archipelago. As is common for most oceanic islands, the Galápagos exhibits high rates of endemism in insects (Peck, 1996). Island endemics may be particularly vulnerable to the introduction of a non-native parasitoid due to their lack of shared co-evolutionary history and the necessary adaptations to evade or resist parasitism. Before we can consider biological control in the Galápagos using any natural enemy, we must evaluate the host specificity of the putative biological control agent in the context under which it is intended for use. The studies that we have conducted using *C. annulifera* thus far represent a vital first step, suggesting that importation of *C. annulifera* into a quarantine facility in the Galápagos for further host range testing is justifiable. The results of these studies also allow us to narrow down the list of most at-risk non-target organisms in the Galápagos, due to the limitations imposed by its evolutionary and behavioural ecology.

Importation biological control of *P. downsi* in the Galápagos constitutes a promising means of population suppression that may ultimately serve to protect the extremely vulnerable bird species that the fly attacks (Boulton & Heimpel, 2017). Establishment of a biological control agent such as *C. annulifera*, may, in addition to ameliorating the current situation, serve as a preventative measure from future invasions of *P. downsi* and other bird parasitic species in the genus *Philornis* that are found in Ecuador. Preventative measures such as this may be deemed particularly judicious given the probability of further invasions under the high tourism pressure that the islands currently face (Toral-Granda, et al., 2017).

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