

REVIEW

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Host-parasite ecology, behavior and genetics: a review of the introduced fly parasite *Philornis downsi* and its Darwin's finch hosts

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Abstract

Understanding host-parasite interactions requires that the multi-faceted relationships among ecological, behavioral and molecular processes be characterized and integrated. The introduced parasitic fly of birds, *Philornis downsi* (Diptera: Muscidae), was accidentally introduced to the Galápagos Islands circa 1960s and the consequences of its introduction represent one of the most challenging conservation battles faced within host-parasite ecology today. Larvae of the fly were first discovered in nests of Darwin's finches (Passeriformes: Thraupidae) in 1997. The parasitic fly larvae are killing ~55 % of Darwin's finch nestlings within nests by consuming the blood and tissue of developing birds, whereby the number of parasitic fly larvae in nests has increased by 46 % from 2000 to 2013. As nestlings increasingly die at an earlier age, which signifies earlier resource termination for the parasite, ~17 % of fly larvae also die. Several local finch populations have become extinct but some host species have increased in abundance, and as a consequence, may be sustaining *P. downsi* populations along with other non-finch hosts. The high mortality in both Darwin's finches and *P. downsi* larvae creates opportunity and imperative to understand the coevolutionary dynamics of this newly evolving host-parasite system. This review examines the rapid changes in host and parasite ecology, behavior, and genetics since *P. downsi* larvae were first discovered in Darwin's finch nests. The aim of the review is to synthesize what is known about this lethal host-parasite system and to describe rapid changes in host and parasite biology that characterize the novel association. An over-arching goal is the implementation of conservation outcomes that are informed by evolutionary biological understanding of invasive species.

Keywords: *Philornis downsi*, Darwin's finches, Galápagos Islands, Host-parasite, Coevolution, Ectoparasitism, Conservation

Background

Reflecting on his visit to the Galápagos Islands in 1835, Charles Darwin acknowledged that the islands “origin all my views” regarding evolution by means of natural selection [1]. To this day, the Galápagos Islands remain a hotspot for evolutionary biological thinking given its frequently described ‘natural laboratory’ conditions that provide exceptional opportunity to quantify ecological, genetic and behavioral determinants of evolutionary outcomes in real time [2–13]. Long-term field studies into

the evolutionary dynamics of wild populations carried out by Peter and Rosemary Grant since 1973 [14, 15], and more recently by other teams, have extended the archipelago's reputation as the generator of profound biological insights into how and why species form [3, 16]. After 40+ years of scrutiny, Darwin's finches (Passeriformes: Thraupidae) are a model field system to measure rates of evolution under different strengths of selection [17–21], quantify the evolutionary effects of introgressive hybridization [7, 22–25] and – most extraordinarily – provide the first natural case study for the formation of a new Darwin's finch ‘species’ [16].

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Perhaps ironically, despite humanity's deep appreciation of the World Heritage Galápagos Islands, the increasing levels of human visitation have put Darwin's finches on an unexpected new trajectory. A myiasis-causing fly, *Philornis downsi* (Diptera: Muscidae), was accidentally introduced to the Galápagos Islands and has been killing more than half of Darwin's finch nestlings before they can fledge [26–28]. The first adult *P. downsi* specimens from the Galápagos were identified from collections made in 1964 on Santa Cruz Island by D. Q. Cavagnaro and R. C. Schuster (California Academy of Sciences, San Francisco) [27]. B. Fessl and S. Tebbich first observed *P. downsi* larvae in Darwin's finch nests on Santa Cruz Island in 1997 [29]. There was no evidence of *P. downsi* parasitism in Darwin's finch nests on Daphne Major or Genovesa Islands during the 1970s or 1980s (personal communication PR and BR Grant). There was also no indirect evidence of *P. downsi* prior to 1962, as assessed by measurement of Darwin's finch museum specimens collected during 1899–1962 from Floreana Island [30]. We are not certain of how *P. downsi* was first introduced to the archipelago. Recent documentation of *P. downsi* parasitizing birds on the Ecuadorian mainland is generating insights into possible introduction scenarios [31]. For example, the smooth-billed ani (*Crotophaga ani*) is a known mainland host for *P. downsi* [28] that was introduced to the Galápagos during the 1960s to remove ticks from introduced cattle for agriculture [32], and perhaps inadvertently also introduced *P. downsi* to the archipelago. Since its discovery in Darwin's finch nests on Santa Cruz Island, the numbers of *P. downsi* larvae per Darwin's finch nest have been increasing and the fly's geographical distribution across the archipelago is widespread [33–40].

An international team of scientists is working together with the Galápagos National Park and Charles Darwin Foundation with the common aim to investigate the host-parasite association between *P. downsi* and Darwin's finches (Table 1). Our longitudinal study since 2000 (S. Kleindorfer's team), combined with those of others, is discovering changes in this host-parasite interaction that span ecological, genetic and behavioral aspects in both Darwin's finch hosts and *P. downsi* parasites. Collating all studies since 2000, the average number of *P. downsi* documented per finch nest has increased by 46 % from ~29 to ~51 larvae per nest (Fig. 1). On average across this period, 55 % of nestlings are documented to have experienced direct mortality from parasitism in the nest (Table 1). There was a trend for a 66 % increase in nestling mortality from 23 % dead nestlings in 2000 to 68 % dead nestlings in 2013 (Fig. 2). This longitudinal increase in the number of *P. downsi* per nest was not neatly paralleled by an increase in Darwin's finch mortality, and within studies, parasite intensity did

not always predict in-nest mortality (Table 1). These observations lead to the following questions: What ecological, behavioral and molecular processes could explain an apparent tolerance (given some host survival) to greater abundance of this novel parasite? Which finch species are most at risk of extinction, and why? What can we learn from this rapidly changing and lethal host-parasite interaction to inform conservation management?

The genus *Philornis* has a Neotropical distribution comprised of ~50 species with variable impacts on hosts [27]. On the Galápagos Islands, the introduced *P. downsi* causes mortality in its naive island hosts and has been given the highest risk ranking for invasive species [26]. Table 1 provides a summary overview of the 31 peer-reviewed publications detailing the impacts of *P. downsi* for Darwin's finch survival and fitness. While *P. downsi* affects all Darwin's finch species on the Galápagos Islands, its impacts have been studied in eight out of the 14 recognized species: two ground finch species (*Geospiza fuliginosa*, *G. fortis*), one warbler finch (*Certhidea olivacea*), and all five tree finch species (*Camarhynchus parvulus*, *C. pauper*, *C. psittacula*, *C. pallidus*, *C. heliobates*). Most published research has been undertaken on Santa Cruz Island (1998–2012), Floreana Island (2004–2014), and Isabela Island (2004–2009). The first Galápagos-wide survey of *P. downsi* prevalence across 13 islands was done by D. Wiedenfeld and colleagues spanning the years 1998 to 2005 [39]. C. Causton and colleagues developed the first framework for systematic assessment of the probability for a species to become invasive to the Galápagos Islands [26]. Table 1 divides these published studies into three main research areas for the *P. downsi* and Darwin's finch association: 'ecology', 'behavior' and 'genetics', and describes the main results of each. In this review, the above three areas of enquiry are examined in relation to: (1) direct effects on (a) host survival and (b) populations; (2) ecological effects and processes, (3) behavioral changes in (a) hosts and (b) parasites, (4) genetic insights into (a) host and parasite ecology and (b) evolutionary patterns and finally (5) broader evolutionary implications, where the repercussions of these findings for altering the evolutionary trajectories of both host and parasite, and thus the outlook for the future of Darwin's finch populations are discussed. In doing so, this review highlights the system's contribution towards the field of invasion biology and our understanding of rapid host-parasite co-evolution.

Direct effects of *Philornis downsi* parasitism on Darwin's finches

Darwin's finch survival

Philornis downsi parasitism negatively influences nestling growth and survival in Darwin's finches. Published values of in-nest mortality caused by *P. downsi* ranged

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1) Host species	2) Island	3) Year of study	4) Sample size	5) <i>P. downsi</i> prevalence	6) Mean number of <i>P. downsi</i> per nest (range)	7) In-nest mortality (%)	8) Effects on host	9) Comments	10) Type of Study	11) Exp. (E) versus Obs. (O) study	12) Reference
9 species	Santa Cruz	1998	63	97 %			N/A	First study to describe <i>P. downsi</i> in bird nests on Santa Cruz Island	Ecology	O	Fessl, Couri, Tebbich 2001 [29]
12 species	Santa Cruz	1998–2000	177	97 %	15.7 ± 7.4–49.8 ± 31.2 (0–182)	27 %	Number of larvae increased with nestling age; <i>P. downsi</i> not found in nests during incubation	Intensity compared for nestling age <8 days vs. >8d; species differed in intensity	Ecology	O	Fessl, Tebbich 2002 [28]
3 Darwin's finch species	Santa Cruz	2000, 2004, 2005	63	100 %		–32.4–95.2 %	Damage to nestling nasal cavities; calculated 18–55 % overnight blood loss in nestlings due to parasitism	Identify two stage life cycle of <i>P. downsi</i> with 1st instar within nestling nares and 2nd and 3rd instar feeding externally	Ecology	O	Fessl, Sinclair, Kleindorfer 2006 [42]
2 Darwin's finch species	Santa Cruz	2000, 2004	27	100 %	18.4 ± 2.4–23.2 ± 4.2	66 %	Parasitized nestlings had 61 % lower survival, 56 % lower body mass, 28 % lower hemoglobin concentration	11 nests pruned with 1 % permethrin (~1 <i>P. downsi</i> left per nest; range 0–5 larvae)	Ecology	E	Fessl, Kleindorfer, Tebbich 2006 [41]
Darwin's finches	All islands						<i>P. downsi</i> given highest risk ranking for invasive species that negatively impact endemic fauna	23 % (around 463 species) of Galapagos invertebrate species are introduced of which 6 are confirmed to be highly invasive and harmful to endemic species	Ecology		Causton, Peck, Sinclair, Roque-Albelo, Hodgson, Landry 2006 [26]
Small ground finch	Santa Cruz, Floreana, Isabela	2004	24	92 %	23 ± 6 (0–90)		2 % lower hemoglobin concentration per additional larva	Effect of clutch size on <i>P. downsi</i> number per nestling	Ecology	O	Dudaniec, Kleindorfer, Fessl 2006 [44]
6 Darwin's finch species	Santa Cruz	1998–2005	131	100 %	30.2 ± 4–66.8 ± 7.6	55 %	Positive association between <i>P. downsi</i> intensity and rainfall (eight year study); same intensity across habitats on Santa Cruz	Species differences in intensity	Ecology	O	Dudaniec, Fessl, Kleindorfer 2007 [33]

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Darwin's finches	13 islands	1998–2005	515		27.0 (1–182)		<i>P. downsi</i> was found on 11 of 13 islands sampled (absent from flat arid islands); more parasites in highland nests on elevated islands	Lowland nests on elevated islands had more <i>P. downsi</i> than lowland nests on flat islands	Ecology	O	Wiedenfeld, Jiménez, Fessl, Kleindorfer, Valarezo 2007 [39]
5 Darwin's finch species	Santa Cruz, Floreana, Isabela	2004–2006	64	100 %			High gene flow between islands; some genetic differentiation of <i>P. downsi</i> on Floreana; evidence for genetic bottleneck	Sequenced CO1 mitochondrial gene fragments	Genetics, Ecology	O	Dudaniec, Gardner, Donnellan, Kleindorfer 2008 [40]
Medium ground finch	Santa Cruz	2004–2006	63	64–98 %	(0–200)	16–37 %	Number of <i>P. downsi</i> per nestling (but not per nest) predicted survival; no effect of <i>P. downsi</i> on nestling size	Nests earlier in the breeding season had more <i>P. downsi</i> per nest	Ecology	O	Huber 2008 [34]
Small ground finch	Santa Cruz	2008	623				36.3 % of adult birds had malformed beaks with enlarged naris (likely caused by <i>P. downsi</i> during the nestling phase)		Ecology	O	Galligan, Kleindorfer 2009 [48]
5 Darwin's finch species	Santa Cruz	2000–2004	43	100 %	23.0 ± 3–57.0 ± 4		Larger finches built larger nests; within species, larger nests had more <i>P. downsi</i>		Behavior	O	Kleindorfer, Dudaniec 2009 [90]
Small ground finch	Floreana	2004–2006	39	94 %	8.0 ± 1.6–39.3 ± 4.6 (0–78)	3–33.4 %	Arid lowland nests had 80 % fewer <i>P. downsi</i> and 91 % lower in-nest mortality than highland nests		Ecology	O	O'Connor, Dudaniec, Kleindorfer 2010 [89]
Medium ground finch	Santa Cruz, Daphne Major	2008	67				More <i>Philornis</i> -specific antibodies in females than males, and more during the nesting period	No <i>P. downsi</i> found on Daphne Major	Ecology, Behavior	O	Huber, Owen, Koop, King, Grant, Grant, Clayton 2010 [46]
3 Darwin's finch species	Santa Cruz, Floreana	2008	11	89 %	27.1 ± 8.5 (0–74)	79.2 %	Multiple <i>P. downsi</i> entries into the nest during incubation and feeding; nestlings stood on top of each other presumably to avoid larvae; adults preened nestling nares and nest base; nestling consumed larva	In-nest video; altered time budgets and behavior in heavily parasitized nests; adults removed dead nestling from nest	Behavior, Ecology	O	O'Connor, Robertson, Kleindorfer 2010 [49]

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5 Darwin's finch species	Santa Cruz, Floreana	2004–2006	57	100 %	30.8 ± 16.5 (5–65)		Genetic relatedness among <i>P. downsi</i> within nests was 57 % lower on Santa Cruz than on Floreana Island; between 1–6 different females laid their eggs per finch nest; each female mated with ~2 different males; each female laid ~5 eggs per finch nest with a maximum of 24 from one female		Genetics, Ecology, Behavior	O	Dudaniec, Gardner, Kleindorfer 2010 [117]
Medium tree finch	Floreana	2006, 2008	30	100 %	43.1 ± 10.7 –65.6 ± 8.5 (8–96)	20–38 % total brood loss; 53 % of nests with partial or total brood loss	Parasite intensity did not predict fledging success; parasitized nestlings had grossly enlarged nares and tissue loss	Highest <i>P. downsi</i> intensity in critically endangered medium tree finch	Ecology	O	O'Connor, Sulloway, Robertson, Kleindorfer 2010 [38]
Mangrove finch	Isabela	2006–2009	15	100	40.8 ± 15.3 sd	14 %	Less rat predation after rat control (30 % predation after control versus 54 % predation before); <i>P. downsi</i> explained 14 % nestling mortality	Before and after rat control	Ecology	O + E	Fessl, Young, Young, Rodríguez-Matamoros, Dvorak, Tebbich 2010 [63]
Medium ground finch	Santa Cruz	2008	48	90 %	37.5 ± 4.9	96 %	Parasitized nestlings had ~30 % shorter primary feather length and 88 % lower fledging success	Nylon stocking placed over wire hoop as barrier to <i>P. downsi</i> ; reduced parasite intensity to ~21 larvae per nest	Ecology, Behavior	E	Koop, Huber, Laverty, Clayton 2011 [63]
Darwin's finches	All islands	2012-present					Summary of known <i>P. downsi</i> biology and impacts on endemic land birds	Launch of the <i>P. downsi</i> Action Plan	Ecology, Behavior, Genetics		Causton, Cunninghame, Tapia 2013 [72]
Medium ground finch	Santa Cruz	2010	30 (15 control, 15 exp.)		44.7 ± 6 (5–79)		No effect of <i>P. downsi</i> on attending female corticosterone level, body condition or hematocrit level	15 nests sprayed with 1 % permethrin (~0 per nest)		E	Knutie, Koop, French, Clayton 2013 [102]
Medium ground finch	Santa Cruz	2010	43 (22 control, 21 exp.)	100 %	38.5 ± 5.1	100 %	Compared with fumigated nests, females at parasitized nests had more <i>P. downsi</i> -specific antibodies, spent 42 % less time brooding and 74 % more time standing erect in the nest	14 females and 10 males from fumigated nests, 15 females and 10 males from parasitized nests	Behavior	E	Koop, Owen, Knutie, Aguilar, Clayton 2013 [47]

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Medium ground finch	Santa Cruz	2009	13 (7 control, 6 exp.)	83 %	30.5 ± 7.5	86 %	No effect of rainfall on <i>P. downsi</i> intensity (two year study)	6 nests sprayed with 1 % permethrin (zero <i>P. downsi</i>)	Ecology	E	Koop, Le Bohec, Clayton 2013 [88]
Small ground finch	Floreana	2010	14 (7 control, 7 exp.)	100 %	22.7 ± 3.9 (12–60)	100 %	In-nest video; heavily parasitized nestlings had weak begging; parents only fed strongly begging nestlings	7 nests sprayed with 1 % permethrin (zero <i>P. downsi</i>)	Behavior	E	O'Connor, Robertson, Kleindorfer 2014 [100]
4 Darwin's finch species	Floreana	2004–2013	238	100 %	27.5 ± 4.6 (2004) to 48.4 ± 6.5 (2013)	50–90 %	Across the decade, <i>P. downsi</i> intensity increased (~28 to ~48), in-nest mortality increased (~50 to ~90 %), nestlings died younger (~11 to ~5 days after hatching); pupa size got 10 % smaller (~10 mm to ~9 mm)	Compared with other finches, small ground finch nests had more <i>P. downsi</i> pupae, which indicates higher fly reproductive success	Ecology	O	Kleindorfer, Peters, Custance, Dudaniec, O'Connor 2014 [37]
3 Darwin's tree finch species	Floreana	2005, 2010	201	100 %	25 ± 3–65 ± 18		Hybrid finch nests had 50–79 % fewer <i>P. downsi</i> than medium tree finch (2005, 2010)	Contemporary hybridization in Darwin's tree Finches; evidence for hybrid fitness	Behavior, Genetics, Ecology	O	Kleindorfer, O'Connor, Dudaniec, Myers, Robertson, Sulloway 2014 [7]
2 Darwin's finch species	Santa Cruz	2010, 2012	82	100 %	25, 38	37–56 %	Extreme weather events and number of <i>P. downsi</i> influenced nesting success	Species differences in <i>P. downsi</i> intensity	Ecology	O	Cimadom, Ulloa, Meidl, Zöttl, Zöttl, Fessler, Nemeth, Dvorak, Cunninghame, Tebbich 2014 [36]
4 Darwin's finch species	Santa Cruz	2013	37 (17 control, 20 exp.) plus 26 nests checked for treated cotton		17 ± 3.9	46 %	Parasitized nests had 35 % lower fledging success	Permethrin-treated cotton from dispensers in 22 nests; 20 nests sprayed with 1 % permethrin (zero <i>P. downsi</i>)	Ecology	E	Knutie, McNew, Bartlow, Vargas, Clayton 2014 [70]
Small ground finch	Floreana	2010	14 (8 control, 6 exp.)	100 %	24.2 ± 2.7		Parasitized nestlings had larger relative (39 %) and absolute (3.3 mm) naris size compared with parasite-free nestlings (20.2 %, 1.6 mm)	6 nests sprayed with 1 % permethrin (zero <i>P. downsi</i>); museum specimens had normal naris size suggesting post 1960s arrival of <i>P. downsi</i>	Ecology, Morphology	E	Kleindorfer, Sulloway 2016 [30]

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3 Darwin's finch species	Floreana	2004–2014	582	100 %	17.7 ± 3.3–87.8 ± 19.5	Higher fly traps caught more female <i>P. downsi</i> ; higher finch nests had more <i>P. downsi</i>	28 McPhail traps placed at 1 m–7 m to test for fly sex ratio at different trapping heights	Behavior, Ecology	E	Kleindorfer, Peters, Hohl, Sulloway 2016 [71]
Medium ground finch, mockingbird	Santa Cruz	2012, 2013	127			<i>P. downsi</i> density (grams per nestling g) predicted finch but not mockingbird mortality; no <i>P. downsi</i> antibodies in nestlings; parasitized mockingbird but not finch nestlings begged more	32 finch nests and 34 mockingbird nests sprayed with 1 % permethrin	Behavior, Ecology	E	Knutie, Owen, McNew, Bartlow, Arriero, Herman, DiBlasi, Thompson, Koop, Clayton 2016 [105]

from 3 and 100 % with 55 ± 6 % mean mortality across years (Table 1, Fig. 2). Given that *P. downsi* larvae feed on the blood and tissue of the developing birds, it is common to observe enlarged nares, open-lesions, swelling and internal bleeding under the skin caused by larvae burrowing into the integument of Darwin's finch nestlings [35, 41, 42], with wounds and lesions appearing within the ears, the belly, wings and nares [30]. Experimental studies that created parasite-reduced nests (using 1 % permethrin, pyrethrum or physical barriers) found consistent evidence that *P. downsi* larvae increase in- nest mortality, with 88 % [35], 61 % [43], and 35 % [41] lower survival in parasitized versus treated nests. This corresponded with a 2 % reduction in nestling survival per additional larva [41]. Other effects of *P. downsi* on Darwin's finch development include 56 % reduction in nestling body mass [41], 30 % shorter feather length [35], and 28 % lower hemoglobin concentration [41, 44]. Low hemoglobin concentration indicates reduced oxygen carrying capacity in hosts, which may compromise the nutritional and energetic state of parasitized birds and lead to survival and reproductive trade-offs [43, 45]. One experiment explicitly quantified the blood loss inflicted by *P. downsi* by weighing nestlings at dusk and dawn and calculating blood volume consumed from the nestling [41]. This blood loss was reflected by reduced hemoglobin concentration that equated to a 0.80 decrease in nestling hemoglobin concentration with the addition of each larva [44]. Most nestlings that experienced a 25 % blood loss died within hours [42]. Two studies on host immune response have found more *P. downsi* antibodies in adult females during nesting than pre-nesting [46] and experimentally in parasitized compared with unparasitized nests [47]. These findings highlight the strong selection pressure that *P. downsi* inflicts on Darwin's finches via rapid and widespread nestling mortality as well as the significant costs for nestling growth and changes in host physiology.

In addition to direct effects on survival, *P. downsi* causes change in beak structure, thus altering host phenotype [30, 48] (Fig. 3). The 1st and some 2nd instar *P. downsi* larvae feed inside the nestlings' nares [42]. Most parasitized nestlings die in the nest but the few that fledge can have enlarged nares that persist into adulthood. Occasionally, the naris deformation was so severe that the entire upper beak was essentially absent [30, 35, 42, 49]. Relative naris size was 39 % larger among parasitized than parasite-free nestlings [30]. Because beak shape is associated with song characteristics and assortative pairing in Darwin's finches [50], changes to either trait because of changes in naris size are likely to have carry-over effects for individual and population-level patterns of mate choice [51]. Given the additional important role of beak size and shape for foraging and

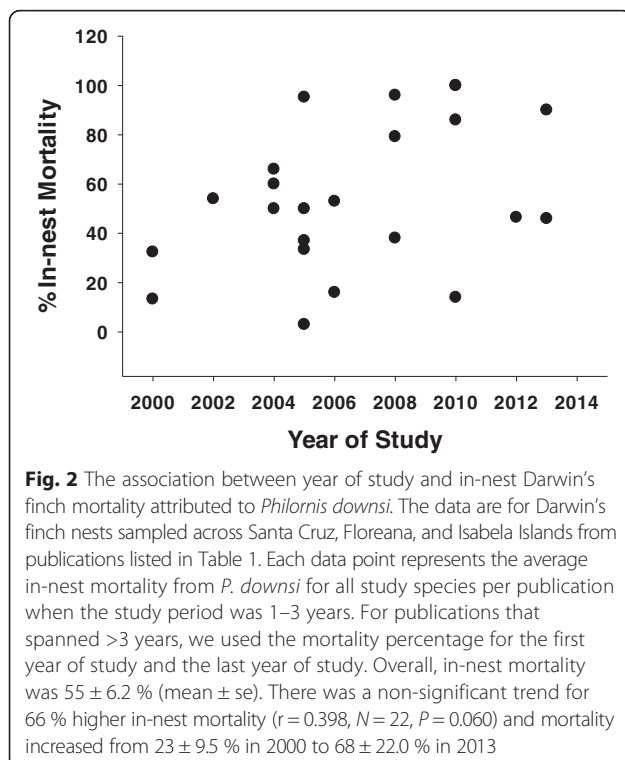
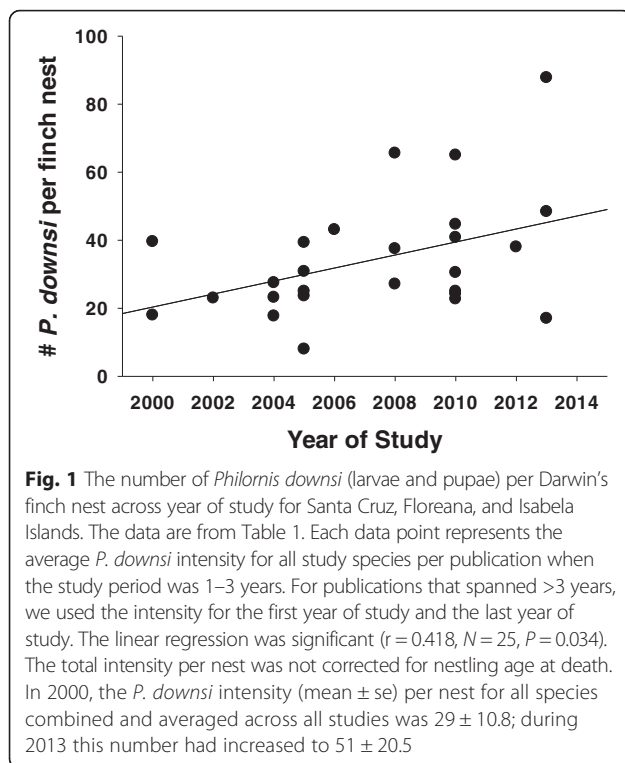
preening, altered beak phenotype as the result of previous parasite infestation is also likely to affect the efficiency of these behaviors [6, 52], which remains to be tested.

Despite the significant impacts of *P. downsi* on nestling naris size, body mass, feather length, and hemoglobin concentration, mortality patterns in relation to *P. downsi* intensity (number per nest, or number per nestling) have been surprisingly inconsistent intra-annually, both within species and across habitats, which we discuss below.

Population-level changes in Darwin's finches

No Darwin's finch species has become extinct on the Galápagos Islands since people first began observations of this system. However, there have been several local population extinctions. Floreana Island has the longest history of human settlement and three of eight Darwin's finch species have become extinct since the 1900s (large ground finch, large tree finch, warbler finch), while one species is exceedingly rare (vegetarian finch) [6, 7, 53]. Most (97 %) of the Galápagos landmass is protected in that human development is prohibited and human access is restricted. The local population extinctions and/or changes in finch abundance are happening concurrently with increasing numbers of introduced predators and pathogens [26, 54], habitat alteration due to human activity [36, 55–59], chemical weed management [36], frequency of extreme climatic events (e.g. El Nino) and climate change impacts [36, 60]. Therefore, multiple threatening processes concurrently threaten Darwin's finch populations and the high mortality caused by *P. downsi* greatly exacerbates the rate of population declines, particularly in combination with predation from introduced rats and habitat degradation [61].

Two critically endangered Darwin's finch species, the mangrove finch (*C. heliobates*) on Isabela Island and the medium tree finch (*C. pauper*) on Floreana Island, are at elevated risk from the impacts of in-nest mortality from *P. downsi*. There are ~100 mangrove finch birds left in the wild [62], making them the rarest of Darwin's finches [63]. The main threats to the persistence of the mangrove finch have been identified as 54 % nest loss from rat predation and 14 % in-nest mortality from *P. downsi* [63]. There are ~2500 male medium tree finches [64] and it has been found that since 2004: (a) the population declined by 52 % [64, 65], (b) the population age structure in 2010 was 62 % old males (5+ years) and 0 % yearling males (as assessed by plumage coloration) [7, 66], and (c) recruitment has been exceedingly low with zero fledging success since 2010 [64]. The effect of *P. downsi* on the decline of the medium tree finch is evidently severe, given the finding that nests of this species consistently had the highest number of *P. downsi* larvae (1.73 times more than small tree finch) compared with all



other sympatric Darwin's finch species [7, 38]. On Santa Cruz Island, seven of nine extant Darwin's finch species are classified as 'common' and 'of least concern' (IUCN, 2010) while two Darwin's finch species declined precipitously since 1997 [67]; the status of large tree finch and woodpecker finch was therefore changed from 'least concern' to 'vulnerable' (IUCN, 2015). The warbler finch (*C. olivacea*) declined by 45–85 % across habitat zones, and the woodpecker finch (*C. pallidus*) declined by 20–65 % across habitat zones [67]. Notably, both warbler finch and woodpecker finch had the highest *P. downsi* intensity on Santa Cruz [33].

Recent studies have modeled the impact of *P. downsi* to predict future Darwin's finch population dynamics. In the mangrove finch, Fessl et al. [63] estimated species extinction within 100 years in the absence of rat control and *P. downsi* control. In the medium ground finch, Koop et al. [68] estimated population extinction within 100 years under current *P. downsi* conditions for years with high to moderate frequency of 'poor' resource abundance. Under both high and low resource abundance, the probability of local extinction was reduced when *P. downsi* prevalence was reduced, which has significant implications for management actions that remove *P. downsi* from target nests and thereby reduce *P. downsi* prevalence (a key variable in the model). Current tools available to reduce *P. downsi* prevalence include manual application of 1 % permethrin into nests via spray bottles or injection [69] and offering permethrin-treated cotton balls that ground finches have been shown to accept for nest building [70]. Other options include strategic placement of fly traps to target egg-laying *P. downsi* females according to sex-specific flying heights [71], and investment into ongoing research to develop efficient attractants that lure *P. downsi* into traps [72, 73].

Ecological effects and processes associated with *P. downsi* parasitism

Understanding how invasive parasites, particularly those that are highly mobile insects, are influenced by and affect broader ecological processes is a complex task. In a global review of the ecological effects of invasive alien insects, only 5/403 (6 %) publications addressed the impacts of introduced insects on ecological processes, whereas 224 (56 %) examined effects of alien insects on local population dynamics [74]. Causton et al. [26] developed a novel ranking system to evaluate the invasive potential of insect species on the Galápagos Islands. With about 463 alien insect species and a marked increase in rate of introduction since the 1960s [75], alien insects constitute around 23 % of the total insect fauna on the Galápagos Islands [26]. Along with five other invertebrates, *P. downsi* was ranked in the highest category for its threat to the Galápagos biota [26]. The

(a) Nestling small ground finch (*Geospiza fuliginosa*) (left) with larger hollow naris and (b) small tree finch (*Camarhynchus parvulus*) (right) with smaller encrusted naris.



(c) Fledgling small ground finch (l.) and (d) small tree finch (r.) with enlarged naris.



(e) Adult small ground finch (l.) and (f) small tree finch (r.) with enlarged naris.

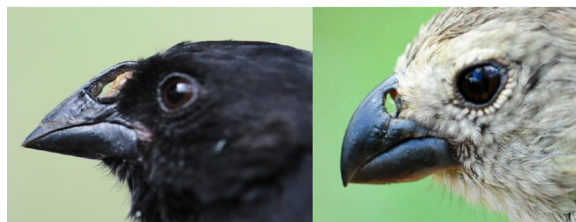


Fig. 3 a-c. Naris damage caused by *Philornis downsi* in Darwin's finch beaks is shown for host **a** nestlings, **b** fledglings, and **c** adults. The 1st instar *P. downsi* larvae feed inside the host's beak resulting in naris damage. Usually *P. downsi* in the naris creates enlarged naris size, but in some cases the naris can become smaller if *P. downsi* larvae become encrusted inside the naris. If nestlings survive, they can have enlarged naris size as fledglings and adults. Photos by Jody O'Connor (a) and Katharina J. Peters (b, c, d, e, f)

risk assessment included an evaluation of potential ecological impacts, including the trophic functional role of each invasive insect species. In this seminal evaluation, insects at higher trophic levels that cause direct impacts on flora or fauna (e.g. predators, herbivores or parasites) were given higher scores than species at lower trophic levels (e.g. decomposers and scavengers) [26].

We still lack experimental tests for how *P. downsi* is changing ecological processes on the Galápagos Islands, and lack insights into its role in shaping ecosystem processes on mainland South America [31, 76, 77]. Despite tentative evidence at present, *P. downsi* may represent a competitor or a novel food source for some invertebrates either in its larval or adult life stage, which could have downstream trophic effects on the invertebrate and wider species community [49]. Possible ecological processes affected by introduced *P. downsi* that may be considered for future study include: competitive exclusion or displacement of native insects [26, 78, 79], the potential for being a vector of insect or animal disease [26, 54,

80], host to bacterial and/or viral symbionts [81–83], and/or host to parasitoid wasps [84–87]. Regarding the latter, two species of parasitoid wasp have been observed in *P. downsi* pupae on Santa Cruz Island [72]. The possibility to develop parasitoid wasps for biocontrol is a focus of inquiry for G. Heimpel, C. Causton and colleagues [31, 72]. Given the stronghold that *P. downsi* has on the Galápagos avifauna, in addition to the host assemblages' isolation and propensity for rapid evolutionary response, the effects of the fly on ecological processes are likely to be significant.

To date, the ecological context of *P. downsi* impacts have been studied in relation to rainfall [33, 36, 88], habitat type [33, 89], and host nesting attributes [71, 90, 91]. Four studies have examined effects of rainfall on *P. downsi* intensity, and results have shown variable relationships. One study found an increase in *P. downsi* intensity in years with higher rainfall on Santa Cruz Island (in 1998–2005) [33], and one study found no difference in *P. downsi* intensity in years with different rainfall

(2008–2009) [88]. When comparing *P. downsi* intensity in the drier, arid lowlands to the wetter highlands, studies also varied, with one finding lower intensity in the lowlands compared to the highlands on Floreana Island (2004–2006) [89], and two other studies finding no difference in intensity between the two habitats on Santa Cruz Island [28, 33]. In a survey by Wiedenfeld et al. [39], the number of parasites was compared between lowland nests on low-lying and elevated islands. There were more *P. downsi* per lowland nest on elevated islands than on flat islands, suggesting that elevated islands have habitat refuges for the persistence of the fly across the year [39]. These studies imply that multiple interacting factors determine how rainfall affects *P. downsi* intensity, while the corresponding host mortality impacts are also inconclusive, given that finch populations may increase under high rainfall due to greater nesting frequency and density [14].

The way habitat structure determines host susceptibility to parasitism has been examined in the *P. downsi*-Darwin finch system, with promising implications for conservation management. The vertical structure of the *Scaevola* forest appears to harbor several ecological niches for Darwin's tree finches, as demonstrated by differences in the average heights at which species forage [66, 92] and nest [71]. To determine if the stratification of nesting heights among species could influence how many *P. downsi* infest a nest, one study placed McPhail fly traps at heights between 2 m and 7 m and found that the lowest and highest traps caught more female flies, and intermediate traps (4–5 m) caught more male flies [71]. Notably, nesting height of the critically endangered medium tree finch was ~7 m, and this species had the highest *P. downsi* intensity. The finding of more parasites in higher finch nests [71] suggests that parasite intensity could be partly explained by the encounter probability between female *P. downsi* and a host nest, rather than some aspect of the host that actively attracts the parasite. This finding may help to guide a more effective fly trapping program based on sex-specific spatial densities of adult flies.

Parasite intensity shows a positive relationship with host body mass [33] and larger host nest size [33, 90]. However, as for rainfall and habitat correlations, the association between *P. downsi* intensity and host mass was not consistent. In a study of six finch host species, comparatively high parasite intensity was documented in the smallest-bodied finch, the warbler finch (~11 g) [33]. Further, on Floreana Island, where small (13 g) and medium tree finches (17 g) are hybridizing [7], the intermediate-sized hybrid tree finch (~15 g) had the lowest parasite intensity – a pattern that also deviates from a simple association between host body size and parasite intensity [7, 64]. Within species, larger finches built

larger nests, and larger nests harbored more parasites [90]. This pattern suggests there should be selection for smaller nest size among the Darwin's finch assemblage, which remains to be tested.

The avian nest is a microhabitat harboring a set of temperature and humidity conditions that can support a diversity of invertebrates [93–96]. For insect parasites, this could result in competitive ecological interactions within the host nest. Although evidence for this is scarce, in-nest video recordings from a small ground finch nest in 2008 found black ants entering the nest and removing *P. downsi* larvae from the nest base and from nestling nares (ant species unknown; observed in 1 out of 11 recorded nests) [49]. If this phenomenon is or becomes more frequent than hitherto observed, *P. downsi* removal by ants could lead to underestimations of total *P. downsi* intensity within nests.

Behavioral changes in host and parasite

Behavior in Darwin's finches

The novel challenges brought by an alien parasite in an isolated host assemblage are likely to encourage novel behaviors as the parasite strives to adapt to the biology of its new host, which may also be dynamic [97–99]. In the Darwin's finch - *P. downsi* system, the behavior of host and parasite have been observed directly using nest inspection and in-nest video recordings, and have also been inferred based on patterns of capture and nesting patterns (Table 1).

The parasite avoidance behavior of Darwin's finch nestlings has shown changes since the parasites' discovery in 1997, when the implicit assumption was that nest mates were more or less equally affected by parasitic larvae during the nestling period [28, 44]. More recent evidence from both nest monitoring and video recordings show competitive parasite avoidance behavior among nest mates, whereby nestlings compete to stand on top of each other to avoid the emerging larvae in the nest base. Indeed, this was observed directly at 1/11 nests on video in 2008, which showed that the lower nestling was consumed by *P. downsi* while the nestlings standing on their sibling could lower their vulnerability to parasitism and survive. In 2010, this behavior was found in all nests via video observation (6/6) [49, 100]. This evidence for parasite avoidance within nests is supported by observations of nestlings dying from parasitism sequentially over multiple days, and of striking daily variation between nest mates in body mass and physical signs of parasitism [30]. Indeed, in-nest video recordings of small ground finch nestlings showed that *P. downsi* larvae focused their feeding effort on a single nestling per night [100]. Two studies have shown the impacts of *P. downsi* on the time engaged in nest brooding and positioning behavior in adults and nestlings [49, 101] (Table 1). Females at

parasitized nests spent less time brooding and more time standing erect inside the nest [47], and furthermore, females at parasitized nests inserted their beaks into nesting material, probed the nest base and preened nestlings, sometimes consuming *P. downsi* larvae [49]. Despite evidence for differences in female time budgets and behavior in relation to *P. downsi* parasitism, researchers have not found evidence for a physiological stress response in females [102]. Nestling finches have also been observed to preen and consume *P. downsi* larvae from inside the nest, as confirmed via in-nest video monitoring [49, 100], despite earlier failed attempts in 2001 to feed hosts with *P. downsi* (S. Kleindorfer personal observation). These observations suggest that host behavioral adaptations are developing in response to this novel parasite [97, 103, 104].

Parental care is indirectly affected by *P. downsi* because parasite intensity predicts nestling begging intensity, which in turn predicts whether a nestling is fed or not [100, 105]. In Darwin's finches, parasitized nestlings showed weaker begging intensity (hunched body and gape open for shorter duration) than non-parasitized nestlings both within and across nests, and parents fed the strongly begging nestlings more often [100]. The opposite was found in the Galápagos mockingbird, with parasitized nestlings having stronger begging [105]. However, as in Darwin's finches, mockingbird parents also fed the strongly begging nestlings more often than weakly begging nestlings [105]. The species difference in begging intensity among parasitized nestlings suggests that the energetic cost of *P. downsi* parasitism in Darwin's finches exceeds that for mockingbirds. Nestling begging intensity was also directly related to the selective nocturnal feeding behavior of *P. downsi* among nestlings, whereby the nestling with the highest nocturnal *P. downsi* intensity showed the weakest diurnal begging intensity [100]. Parental birds consistently cared for offspring with the highest begging vigor (an indicator of quality offspring), rather than offspring whose begging signal was significantly weakened from parasitism [100, 106, 107].

Spatial nesting behavior of Darwin's finches varies and can be divided into mixed species aggregations and solitary nesting [90, 91]. *Philornis downsi* parasitism was associated with spatial nesting behavior in a study conducted between 2000–2006 on Santa Cruz Island [90, 91]. About half of all small tree finch nests had several close neighbors from different finch species including large tree finch, woodpecker finch, small ground finch and warbler finch; the other half were solitary nests with no close heterospecific neighbors. Nests with many heterospecific neighbors had lower predation but suffered from higher *P. downsi* intensities, suggesting an increased cost of host group living due to a higher probability of repeat nest infestations by adult *P. downsi* flies

[40]. The greater number of *P. downsi* at these heterospecific nesting sites has been interpreted as an ecological trap due to the behaviors' conflicting benefit of reduced predation combined with the cost of higher parasite susceptibility [91]. The mechanism for how birds select solitary or mixed-species nesting sites is not known. Several behavioral mechanisms could be at play in nest choice, including decisions based on prior experience and/or sampling of 'public information' about clutch size or predation risk, which has been shown in other systems [108, 109].

Behavioral observations in *Philornis downsi*

Detailed observations of the *P. downsi* life cycle and reproductive behavior have been undertaken since the fly was first documented in finch nests [29, 42] (Fig. 4), which answer many questions but also raise many more. This work continues in earnest as scientists seek to fully characterize the fly's reproductive biology while searching for weaknesses in its life-cycle that may be temporal windows for targeted control measures [72]. The fly's life-cycle through to the adult fly stage has been monitored in the wild [49] and laboratory [110]. The larval stage is reported to last ~10 days, the pupa stage ~10 days, and adult flies emerge after 15 to 24 days [42, 49, 100, 110]. In-nest video recordings confirm that adult *P. downsi* flies repeatedly enter host nests during incubation and nestling feeding when the attending parent is absent from the nest [49], presumably laying eggs or possibly finding mates, but copulation has never been observed inside finch nests (or anywhere else). Additionally, video recordings show that adult *P. downsi* will stay at the nest entrance until the attending avian parent leaves, and then enter [111]. This behavior in the adult fly may interact with parental nest-attendance (e.g. female brooding), such that the number of nest infestations by female flies is affected, but this has not been examined.

What happens inside the nest after fly egg laying still remains somewhat of a mystery. The initial first instar larvae have never been observed emerging or moving from the nesting material to the nestling hosts, but they are frequently found inside the nares of two-day old nestlings, but rarely in one-day old nestlings [42]. In-nest videos and daily nest inspections have also confirmed the presence of first instar larvae inside nestling nares aged 2–5 days [49, 100]. Following this first instar stage that appears to occur predominantly inside of the hosts' beak [49, 100], second and third instar larvae generally complete their life cycle external to the host, and mainly reside in the nest base, emerging to feed on the nestlings' abdomen, wings, ears, and other exposed body parts [42]. Evidence for feeding by larvae has been provided by infrared in-nest video recordings that confirmed

twilight, nocturnal and diurnal larval feeding when the brooding female was absent from the nest [49, 100]. Often, larvae have not been visible inside the nest during the day, presumably withdrawing to the lower layers of the nest [49, 100]. Many questions remain regarding the infestation behavior, reproductive biology and development of *P. downsi*. These questions are necessary to answer given that a comprehensive understanding of the fly's reproductive biology is needed to enable laboratory breeding trials and the development of sophisticated control measures [110].

Since its discovery in Darwin's finch nests in 1997, evidence for the following behavioral changes have been observed in *P. downsi* from 1997 to 2014: (1) earlier egg-laying in finch nests (adult *P. downsi* entered nests during incubation), which was also inferred from a shift towards a higher proportion of late instar larvae at younger nestling ages across the decade, and (2) increasingly synchronous egg-laying by *P. downsi* females per finch nest, which was inferred because there was a higher proportion of instars of the same size and age-class per nestling age across all host species on Floreana Island [37]. Both of these behavioral observations suggest a temporal increase in intraspecific competition among *P. downsi* for host resources during development, but also higher virulence for nestlings at an earlier age due to the presence of more and larger larvae. Concurrent to the above observations, we have documented a 10 % decrease in *P. downsi* pupa size (from 10 mm to 9 mm) as a result of larvae pupating sooner because of earlier age of nestling death [37]. Given that, (1) smaller pupae emerge as smaller adult flies [112], and (2) fly fecundity is strongly correlated with adult fly body size [113], this finding may have profound implications for a changing life-history strategy in *P. downsi* as it responds to the ever-increasing mortality rates among its hosts. Notably, predation in Darwin's finches at all nesting stages remains high across the archipelago (though variable across locations, species, studies, and years) due to introduced rats and other predators. Predation pressure is another possible selection pressure for an earlier age of nestling parasitism as a strategy to better guarantee access to hosts. Overall, these observations signify a move towards increased virulence of *P. downsi*, and a tighter coupling of the fly's life cycle with that of its finch hosts in order to secure host resources.

The flight behavior of *P. downsi*, as revealed by differential capture of males and females across tree canopy heights may be indicative of male–female avoidance behavior, as female *P. downsi* were more common in 2 m and 7 m traps and males were more common in 4–5 m traps [71]. The possible fitness benefits of such a distribution are speculative but are supported by several lines of enquiry. For example, female reproductive success may be lower when gravid females copulate with

additional males [114, 115], and therefore gravid females could be selected to avoid males. Understanding how and why male and female *P. downsi* occur in particular places at particular times will have bearing on predictions of how *P. downsi* impacts will shape finch avoidance response, such as nesting height (discussed above).

Genetic insights into host and parasite ecology and evolution

Ecological genetic insights into P. downsi

The development of microsatellite markers for *P. downsi* by Dudaniec et al. [40, 116] enabled inter- and intra-island gene flow and genetic structure to be examined across three islands of the Galapagos: Santa Cruz, Floreana and Isabela. The genetic diversity of *P. downsi* was similar across islands and genetic differentiation was low ($F_{st} = 0.01–0.04$) between them, indicating that dispersal was largely unrestricted across these islands [40, 116]. However, a spatially-informed genetic

(a) *Philornis downsi* adult male



(b) *Philornis downsi* 2nd instar larva (left) and (c) 3rd instar larva (right)



(d) *Philornis downsi* consuming Darwin's finch nestling



Fig. 4 Images of *Philornis downsi*: **a** adult male (with yellow legs), **b** second instar larva (with smaller brownish posterior spiracles), **c** third instar larva (with larger black posterior spiracles), and **d** third instar larva consuming the blood and tissue of a Darwin's finch nestling. Photos by Bradley Sinclair (**a**), Katharina Peters (**b, c**), and Jody O'Connor (**d**)

clustering method showed there was higher gene flow between Santa Cruz and Isabela Islands than to Floreana Island [40]. This study also included both highland and lowland samples and found no genetic structure across habitats, thus confirming that *P. downsi* dispersal was also largely unrestricted within islands [40].

In addition to genetic structure across habitats and islands, microsatellite markers were used to examine relatedness patterns among fly larvae within nests (the parasite 'infra-population') to determine two reproductive behaviors, a) female re-mating frequency and b) the number of fly cohorts within a nest (i.e. number of females and males contributing to the offspring of each nest). Using a sib-ship reconstruction analysis of 1020 larvae from 57 host nests from Santa Cruz and Floreana, 1–6 females were estimated to contribute to the infra-population of a given nest and *P. downsi* females were found to mate multiple times, with an average of 1.97 males (± 0.08) siring each females' offspring [117]. Therefore, most finch nests have fly clutches that have multiple paternities and consist of multiple infestations by different female flies at a single nest. Understanding these reproductive behaviors is useful for current fly breeding trials and for informing possible control measures such as the sterile insect technique, which relies on a thorough understanding of the target insects' reproductive behavior.

Relatedness among *P. downsi* larvae within lowland nests was also much higher than in highland nests on Floreana Island (2005–2006). Greater relatedness among lowland *P. downsi* cohorts may be due to lower fly densities and thus availability of mates, or it may be due to the lower host nesting density in the lowlands compared with the highlands. Lower host nesting density might reduce the host searching efficiency of *P. downsi* flies and select for greater investment in fly egg laying per nest [117]. Notably, no other genetic dispersal or relatedness studies have been conducted on *P. downsi* since [40], however a comparative phylogenetic analysis using rDNA sequences (ITS2) from Galapagos Island, Ecuadorian and Argentinian *P. downsi* specimens showed unanimous support that the Galapagos *P. downsi* group together with all mainland specimens, suggesting a colonization route from coastal mainland Ecuador [31]. Higher resolution molecular work is still needed to provide further insight into the dispersal and adaptive variation in the fly across the archipelago. In doing so we can determine if the parasite is showing signs of evolutionary divergence from its mainland ancestors, across islands, within species, or across years.

Host evolutionary insights from genetic data

Under conditions of anthropogenic impact, species are increasingly observed to hybridize in the wild, including

under conditions of novel parasitism [118, 119]. In a population genetic analysis of tree finch host species (using microsatellite data), Kleindorfer et al. [7] documented contemporary hybridization in tree finches on Floreana Island, and showed that hybridization frequency changed across the decade [7]. Females of the critically endangered medium tree finch are increasingly pairing with males of the common small tree finch, and produce hybrid offspring [7]. The proportion of hybrids increased from 19 % in 2005 to 41 % in 2010 [7]; and by 2014, 49 % of the tree finch population on Floreana Island was of hybrid origin [64]. Hybrid offspring also showed the lowest *P. downsi* intensity compared with their parental host species [7]. These findings point to a possible role of strong and varied mortality selection from *P. downsi* acting as a selection pressure for hybridization due to increased hybrid fitness. Future study of the molecular interactions between parental species, hybrids and their parasites will further uncover coevolutionary mechanisms that may underlie this interaction.

Broader evolutionary implications and future work

Parasites are a powerful evolutionary force in nature. Host-parasite interactions are ubiquitous, with hosts continually striving to negate the iterated attacks from their parasites, while parasites are constantly evolving ways to evade the hosts' defences [120, 121]. With host-parasite interactions becoming less predictable and potentially more severe under extreme climatic events and biological invasions, unveiling their co-evolutionary dynamics is key for assessing costs to biodiversity and possible economic losses. Ecological theory predicts that parasite epidemics will cease or become less virulent when resources are depleted due to high host mortality or acquired immunity [122]. However, the rate at which hosts and parasites can evolve to an 'evolutionary stable strategy' and the type of selection that governs such phenomena remain largely unknown [123]. To better understand the ecological impacts and evolutionary consequences of harmful parasites for sensitive species, we need studies that measure dynamic host-parasite interactions over time, from both field-based *and* molecular perspectives [119]. A key part of this understanding is teasing apart the mechanisms of coevolutionary strategies at the molecular level [124, 125].

By altering species' evolutionary trajectories, parasitic insects exert a tremendous selection pressure on the structure and function of biodiversity [74, 98]. However, the mechanisms that drive and maintain parasite-induced evolutionary processes remain undescribed across genetic, functional and phylogenetic dimensions. The most common approach to study the genetic effects of alien insects has been to analyze for hybridization

between invasive and native species [126]. The other main genetic approach has been to identify the genetic structure of invasive species, mostly with the aim to trace their origin and colonization pathways [127, 128]. Clearly, there is much scope to test for a range of genetic processes within the Darwin's finch-*P. downsi* system, for example, by examining gene expression, functional genetic traits, and coevolutionary host-parasite genetic adaptations. Future molecular insights will help to gain a fuller picture of how hosts may be shifting evolutionary trajectories to combat the strong mortality selection exerted by *P. downsi*. In addition, archipelago-wide studies are needed in combination with data from mainland *P. downsi* populations to more fully understand the biological mechanisms that govern the fly's invasion, colonization, and persistence.

The first avian surveys on the Galápagos Islands were done on Isabela Island [62], Santa Cruz Island [67], and Floreana Island [53, 64, 65]. The results of these surveys show that some Darwin's finch species are locally extinct (warbler finch, large tree finch), some populations had significant decline across the past decade (e.g. mangrove finch, medium tree finch, woodpecker finch, warbler finch), and other Darwin's finch species are increasing (e.g. small ground finch, small tree finch, hybrid tree finch). In all cases of population decline, *P. downsi* has been implicated as a major causative factor. However, parasites should be selected for low virulence so that the parasite's resource (i.e. the host) does not die too soon. In other native host-parasite assemblages, parasite prevalence may be ubiquitous, but parasite virulence tends to be lower than the 55 % reported here for the *P. downsi* and Darwin's finch system [129]. There are several pathways to reduce parasite virulence, including the evolution of host tolerance and/or selection for changes in parasite characteristics [98]. But there is a temporal dimension to host-parasite coevolution. Despite the above-mentioned significant changes to both host and parasite, time may be running out for Darwin's finches.

From the parasite's perspective, a key question is how *P. downsi* can sustain its population when it causes such high mortality in its finch hosts. There is growing evidence that some land bird species on the Galápagos could act as reservoir species for the persistence of *P. downsi*: (1) *P. downsi* pupation success was highest in small ground finch nests [37]; (2) hybrid tree finch abundance increased (and hence surviving nestlings sustained parasites for longer), perhaps because nests of hybrid finches had the fewest *P. downsi* larvae across Darwin's finch species [7]; and (3) Galápagos mockingbird had low mortality costs of *P. downsi* parasitism in comparison with medium ground finch, and hence mockingbirds sustained *P. downsi* larvae for longer [105]. The average *P. downsi* pupation success across Darwin's finch nests

on Floreana Island was 83 % [37], which was measured as the percentage of third instars and pupae at the time of in-nest host death (= the termination of host resources). Second instar larvae that do not receive a food source do not pupate [105], therefore any fly larvae in nests with dead hosts that were first or second instar larvae were scored as likely to die before pupation. Controlling for host age and only comparing the number of in-nest pupae (conservative estimate of parasite success) when nestling survival was >8 days post-hatch, there were differences across host species. Across the decade (2004 vs 2013), the mean percentage of *P. downsi* pupae in host nests increased in small ground finch (58 % vs 73 %), but decreased in small tree finch (49 % vs 30 %) and medium tree finch (53 % vs 38 %) [37]. Therefore, the estimated survival of *P. downsi* differed across species, which is predicted to impact fly population biology and remains to be tested.

Conclusion

The myriad peer-reviewed studies synthesized in this review present an overall story of extreme, multi-level effects of a recent invasive parasite on a naïve island community that span insights gained from ecological, behavioral, genetic and modeling approaches. Stemming from these findings is rich evidence for a dynamic system that is being shaped by strong, parasite-induced mortality selection on hosts. In a location that is famous for being a natural laboratory for studying rapid evolution, this devastating invasive pest can also be viewed as an opportunity for understanding how host-parasite interactions are maintained and co-evolve in natural ecosystems through time. The current point in human history calls for an expansion of knowledge within this area, given the accelerating impacts of climate change on the shifting distributions of host species and pathogenic organisms [130]. It is important to emphasize that the disastrous conservation and welfare impacts caused by *P. downsi* for Galápagos birds are caused by human activity making this an anthropogenic problem that therefore justifies energetic and immediate intervention.

The answers to some fundamental questions posed by this review – why has Darwin's finch mortality not consistently increased with the rise in *P. downsi* intensity per finch nest, and which Darwin's finch species are most at risk from *P. downsi* – are multi-faceted. We have reviewed evidence that there have been changes in behavior, ecological and evolutionary genetic processes in both *P. downsi* and Darwin's finches that have contributed in different ways to changing the dynamics of the interaction. The high initial virulence of *P. downsi* has resulted in early host death, which in turn is imposing selective pressure on early *P. downsi* pupation, with

possible indirect effects on fly fecundity and size. Perhaps *P. downsi* larvae are getting smaller and consuming less blood per finch nestling than a decade ago. The timing of *P. downsi* nest infestation is also changing, and perhaps therewith also reproductive investment behavior by female flies, which is an area for further investigation. Some Darwin's finches are hybridizing, others may be reproducing at different temporal or spatial scales, which could reduce the nest encounter probability by *P. downsi*. Darwin's finch adult females have *P. downsi* antibodies that may confer some resistance, and certain host species have higher tolerance (survival) than others, for reasons not yet fully understood. There is some evidence that begging behavior by parasitized nestlings increases parental provisioning, perhaps to compensate for the physiological costs of parasitism. But not all species are equal. The critically endangered mangrove finch and medium tree finch require vigilant monitoring and likely human intervention to mitigate the impacts of *P. downsi* as their ecological opportunity is limited due to geographical restriction. Medium tree finch alleles appear to persist, for now, in the new hybrid tree finch group. But it is unclear what behavioral or genetic strategies mangrove finches could use to preserve their unique genetic legacy. To ensure the efficiency and compatibility of future research on the Darwin finch-*P. downsi* system, it is clear an increasingly collaborative effort is required that preferably integrates standardized protocols regarding field methodology, reporting and terminology. In doing so, this important research will remain comparable and informative for planned conservation actions that ultimately aim to mitigate this tremendous extinction threat to Darwin's finches.

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