Current Zoology 60 (4): 542–550, 2014

Changes in *Philornis* infestation behavior threaten Darwin's finch survival

Sonia KLEINDORFER[1*], Katharina J. PETERS[1], Georgina CUSTANCE[1], Rachael Y. DUDANIEC[2], Jody A. O'CONNOR[1]

[1] School of Biological Sciences, Flinders University, GPO Box 2100, Adelaide 5001, Australia

[2] Department of Biology, Lund University, Sölvegatan 37, Lund, 22362, Sweden

Abstract The conservation behavior framework is useful to identify key linkages between behavior and conservation practice. We apply this framework to a novel host-parasite system on the Galapagos Islands and ask if there have been changes in parasite oviposition behavior and host mortality patterns across the first decade (2004-2013) of its known association. The Dipteran parasite *Philornis downsi* was first discovered in Darwin's finch nests in 1997 and is the biggest threat to the survival of Galapagos land birds. Host mortality has increased over the past decade. In Dipterans, pupation and pupae size are determined by access to host resources. Here, we test the hypothesis that *P. downsi* flies are laying eggs in finch nests earlier in the nestling phase to maximize larval feeding time and therefore chance of pupation success before host death. The results show fewer 1st instar larvae later in the host nesting cycle in support of earlier egg laying behavior by female flies. Between 2004 and 2013, parasite intensity increased from ~28 to ~48 parasites per nest, host mortality increased from ~50% to ~90%, and host age at death decreased from ~11 to ~5 days. The earlier age at host death was correlated with fewer pupae (from ~50% to ~20%) and smaller pupae size (~10% decrease). Changes in parasite behavior reveal new fitness costs to both the parasite and Darwin's finches. These findings underscore the need for urgent conservation action to save Darwin's finches from extinction due to a novel, lethal and introduced parasite [*Current Zoology* 60 (4): 542–550, 2014].

Keywords Host mortality, Parasite size, Darwin's finches, Ectoparasitism, Camarhynchus, Geospiza

The conservation behavior framework (Berger-Tal et al., 2011; Caro and Sherman, 2011) is useful to identify key linkages between animal behavior and conservation practice; it offers a hierarchical framework to bridge the gap between the two disciplines of ethology and conservation biology. Three basic themes that link the two disciplines are identified: (1) anthropogenic impacts on behavior that impact biodiversity, (2) behavior-based species management, and (3) behavioral indicators of other processes of conservation concern (Berger-Tal et al., 2011; Brearley et al., 2013; Civitello et al., 2013; Daly and Johnson, 2011). A range of questions follow on from this context. What anthropogenic changes have changed species behavior and fitness? What species-specific behavior needs to be managed to enhance fitness? What behaviors signal that a species is experiencing a threat to its persistence? Because parasites can affect host phenotype, foraging behavior, and ecological interactions (Britton, 2013; Crane et al., 2011; Goodman and Johnson, 2011; Houte et al., 2013), understanding the role of parasite behavior for host survival is key to managing biodiversity (Edeline et al., 2008; McCallum, 2008; Murray et al., 2013; Thompson et al., 2010).

We use the conservation behaviour framework to ask if there have been changes in parasite behaviour that impact host mortality in a novel host-parasite system on Floreana Island, Galapagos Archipelago. Specifically, we seek to identify temporal changes in reproductive behaviour that could threaten host species persistence or indicate altered parasite fitness since the onset of this parasite invasion. Larvae of *P. downsi* were first discovered in Darwin's finch nests on Santa Cruz Island in 1997 (Fessl and Tebbich, 2002) though the fly was present by 1964 (Causton et al., 2006). *P. downsi* is considered the biggest threat to the survival of all Galapagos land birds (Causton et al., 2011; Dvorak et al., 2011). The non-parasitic adult *P. downsi* fly lays its eggs in nests of land birds (O'Connor et al., 2014; O'Connor et al., 2010b). These eggs hatch into larvae that consume the blood and tissue of developing chicks (Fessl et al., 2006b; Fessl et al., 2006c; Koop et al., 2011; O'Connor et al., 2010a; O'Connor et al., 2010b), and in some instances the incubating female (Huber et al., 2010). In Darwin's finch chicks, the larvae cause up to 50% blood

Received April 15, 2014; accepted July 14, 2014.

∗ Corresponding author. E-mail: sonia.kleindorfer@flinders.edu.au

loss (Fessl et al., 2006a), naris deformation (Galligan and Kleindorfer, 2009), and between 17 and 100% mean annual mortality in chicks (Dudaniec et al., 2007; O'Connor et al., 2010b). From laboratory trials conducted on the Galapagos Islands, the minimum time for *P. downsi* to complete larval development is 4 days until pupation (Causton et al., 2011; O'Connor pers. observation). There has been a trend for increased host mortality across the decade (Dudaniec et al., 2007; Huber, 2008; Koop et al., 2011; O'Connor et al., 2010b; O'Connor et al., 2010c; O'Connor et al., 2010d).

Understanding parasite behavior is key to understanding sources of selection for host and parasite (Poulin, 2011; Schmid-Hempel, 2011). In Dipterans, the duration of larval development is dependent on resource availability provided by the host. If the larvae have not consumed sufficient resources to complete development and the host dies, the larvae will either perish or be forced to pupate sooner (Poulin, 2011; Schmid-Hempel, 2011). In addition to the time window for resource acquisition, host resource availability determines pupae size: pupae are generally smaller under conditions of low resource availability (Quiroga and Reboreda, 2013; Spalding et al., 2002; Teixeira, 1999). To increase resource availability, parasites may infest a host earlier in the nesting cycle. Recently, Quiroga and Reboreda (2013) showed that the size of larvae and pupae in *Philornis seguyi* was correlated with adult fly size. In insects, there is a wealth of data for the correlation between small adult size and lower fecundity. In a comparative study of 57 oviparous insects, the common slope of the fecundity/size relationship was close to one (Honěk, 1993). Therefore, *P. downsi* pupae size is a reliable proxy for adult size and fecundity.

This study examines possible changes in the timing of parasite infestation by *P. downsi* in Darwin's finch hosts on the Galapagos Islands between 2004 and 2013. If the parasite is infesting host nests earlier during the host nesting cycle, we predict fewer 1st instar larvae later in the nesting cycle, more pupae, and larger pupae size. Here we ask, is there evidence for (1) earlier *P. downsi* infestation during the nesting cycle of host nests, (2) increased pupae size as the result of longer larval development, and lastly (3) a correlation between earlier host mortality (i.e. nestling age), pupation success and pupae size.

1 Materials and Methods

1.1 Study site and host species

This study was conducted during the months February to April during the years 2004, 2006, 2008, 2010, 2012, 2013 on Floreana Island, Galapagos Archipelago (described in Kleindorfer et al., 2014). The avian focal species are the common small tree finch *Camarhynchus parvulus*, the critically endangered medium tree finch *C. pauper*, and the common small ground finch *Geospiza fuliginosa* (Grant, 1986; O'Connor et al., 2010b; O'Connor et al., 2010c; Sulloway and Kleindorfer, 2013). The highland study sites on Floreana Island were in *Scalesia* forest at the base of Cerro Pajas volcano, which is the stronghold of the tree finch population on Floreana Island (1°17′46″ S, 90°27′06″ W) (O'Connor et al., 2010a; O'Connor et al., 2010b; O'Connor et al., 2010c). Darwin's finches begin breeding with the onset of the rains that usually occur around January or February. Males build a display nest and sing to attract a female (Kleindorfer, 2007b). Females visit the singing male and inspect the nest. If accepted, a female will subsequently lay a clutch size of 2–5 eggs per nest (Kleindorfer, 2007a); some nests had 6 eggs in 2008 and 2010. In all three focal species, the female is the sole incubator and both parents provide food to chicks. The incubation and feeding phase are ~14 days each.

The three focal species build domed shaped nests with a thick nest base that provides the nesting substrate for larvae of the introduced parasite *P. downsi* (Kleindorfer and Dudaniec, 2009). On Floreana Island, the study sites were characterized by low annual rainfall (~500 mm) in 2004 and 2006, high annual rainfall (~1,500 mm) in 2008 and 2010, and moderate annual rainfall (~800 mm) in 2012 and 2013 (Kleindorfer et al., 2014; Charles Darwin Foundation Meteorological Database: http://datazone.darwinfoundation.org/climate/).

1.2 Sample size

We monitored nesting outcome at 561 active Darwin's finch nests between 2004 and 2013 on Floreana Island. The sample size per focal species was 139 small tree finch, 196 medium tree finch, and 226 small ground finch nests. We analyzed the following subsets of data for this study: nests with *P. downsi* (238 nests), nests with data on percentage of 1st, 2nd, 3rd instar (88 nests), the percentage of *P. downsi* pupae in relation to larvae (191 nests), in-nest chick mortality (222 nests), chick age at death (150 nests), pupae size (66 nests), pupae size and chick age at death (39 nests).

1.3 Parasite species: Background information

Philornis downsi is a Dipteran parasitic fly that has a two-stage life-cycle, which is unusual for the genus: first instar larvae feed internally on the nasal and body cavities of its avian nestling hosts, and 2nd and 3rd instar

larvae feed externally on the chicks (Fessl et al., 2006c). The genus *Philornis* has a Neotropical distribution comprised of ~50 species (reviewed in Dudaniec and Kleindorfer, 2006; Quiroga et al., 2012). Possible sources of introduction of *P. downsi* to the Galapagos Islands are via known mainland hosts, such as smooth-billed ani *Crotophaga ani* that was first recorded in the Galapagos in 1962, as well as the rock pigeon *Columbia livia* that was introduced to the Galapagos in 1972. Because adult *P. downsi* feed on fruit, the fly could have been introduced to the Galapagos Islands via cargo boats laden with fruits and vegetables.

Philornis downsi is the only ectoparasite that causes measurable fitness costs in Darwin's finches; blood parasites have not been detected and intestinal protozoan parasites are rare (reviewed in Dudaniec et al., 2005; Dudaniec et al., 2006). From our previous genetic analysis of maternity, paternity, and offspring genetic structure of *P. downsi* within Darwin's finch nests, we found that each female fly mates with an average of ~2 males (range 1–5 males per female) and 1 to 6 females each contribute an average of five larvae per Darwin's finch nest (range = 1–24 eggs; Dudaniec et al., 2010). Female *P. downsi* flies generally carry ~60 eggs, therefore the female appears to only oviposit a portion of the available clutch per host nest (Causton et al., 2011; Dudaniec et al., 2010).

From in-nest video recordings, there is evidence that *P. downsi* flies enter active finch nests that contain eggs or chicks and lay eggs on the inner nest surface when parents are absent (O'Connor et al., 2010a; O'Connor et al., 2014). After host eggs hatch, the fly eggs hatch within ~6 hours (P. Lincango and C. Causton, unpublished data) and the *P. downsi* larvae crawl into the naris of the chick. The 1st and early 2nd instar larvae feed within the nares and body cavities of chicks (Fessl et al., 2006c). Late instar larvae (2nd and 3rd instar) move from inside the chick's naris and body to feed externally on the chick; the late instar larvae reside in the nest base during the day and emerge at night to feed on the chicks (O'Connor et al., 2014). Larvae pupate in the nest base after feeding on chicks for 4–7 days (O'Connor and Kleindorfer, unpublished data) and emerge as flies after 7–18 days (P. Lincango and C. Causton, unpublished data).

1.4 Host mortality

Host nesting status was determined from repeated 20-minute observations (every two days) of parental activity at each nest, as well as by nest inspection using a ladder (2004–2006) or mirror/camera on an extendable 6m pole (2008–2013). Chick age was determined by the date of hatching, and chick age at death in the nest was determined from nest inspections every two days per active nest. Darwin's finches fledge at ~14 days (O'Connor et al., 2010d) and in-nest mortality was calculated from the percentage of chicks per clutch size that died in the nest. For this study, we analyze chick age at death in relation to parasite intensity, percentage of larvae and pupae, and pupae size.

1.5 *P. downsi* instar distribution and pupae size

All Darwin's finch nests with chicks in this study had *P. downsi* parasites (100% prevalence). We collected all parasite samples per nest 1–2 days after the death or fledging of the last chick. The nesting material was dismantled and all *P. downsi* larvae, pupae and pupae cases were counted to calculate the total number of parasites (parasite intensity) per nest. The larvae were assigned to instar using a microscope in a laboratory. Chicks that had recently died were immersed in alcohol so that larvae within the body would float out and could be counted. We stored the pupae and larvae in ethanol within 24 hours of collection from the host nest.

To estimate the timing of *P. downsi* laying behavior in the host nest, we compare the percentage of larval instar phases in relation to host age at death. If *P. downsi* is laying earlier in host nests across the decade, over time we predict (1) a higher proportion of 1st instar larvae for younger hosts, (2) fewer 1st instar larvae in older hosts, and (3) more pupae in younger hosts. Because we were unable to measure 1st instar larvae during the first days of nesting when the host was still alive, we test for fewer 1st instar larvae and more pupae when hosts died by 4–6 days after hatching. We compare the proportion of larvae and pupae for different chick ages at death across the decade, which we use as a measure of pupation success. We assume higher pupation success when there are more pupae than larvae.

To test for changes in pupae size across the decade, we measured *P. downsi* pupae size from 66 Darwin's finch nests. In a controlled laboratory environment, we measured the pupae per nest to the nearest 0.1 mm using callipers. The nest sample size to calculate *P. downsi* pupae size is as follows: small tree finch (n = 22), medium tree finch (n = 18), and small ground finch (n = 26). For 40/66 nests we had information on chick age at death, which we analyzed in relation to pupae size.

1.6 Statistical analysis

Data were analyzed with SPSS 20 for Windows (SPSS Inc., Chicago, USA). We used linear regression analyses to test for changes across years in parasite in-

tensity, in-nest mortality, chick age at death (days post-hatching), proportion of pupae and larvae, and mean pupae size (mm) per nest; we also compared pupae size and chick age at death per species. We used ANOVA to test for an effect of Year and Species on parasite intensity, and MANOVA to test for an effect of Year and Species on the proportion of 1st, 2nd, and 3rd instar flies.

2 Results

2.1 Ectoparasite intensity

Total *P. downsi* intensity in Darwin's finch nests on Floreana Island increased significantly across years, from 27.5 ± 4.6 in 2004 to 48.4 ± 6.5 in 2013 (r = 0.261, n = 238, P < 0.001). To test for effects of Year and Species on parasite intensity in a single model, we used ANOVA. Parasite intensity differed significantly between years and focal species (ANOVA: Year: $F_{6,237}$ = 6.01, P < 0.001, partial eta^2 = 0.141; Species: $F_{2,237}$ = 22.87, P < 0.001 partial eta^2 = 0.173). The interaction term was significant (Species × Year: $F_{11,237}$ = 2.96, P = 0.001, partial eta^2 = 0.129). The critically endangered medium tree finch had significantly higher parasite intensity (54.7 ± 5.4) compared with the common small tree finch (28.7 ± 2.4) and common small ground finch (31.0 ± 2.1) (Tukey's post-hoc: P < 0.001), but there was no significant difference in parasite intensity between small tree finch and small ground finch (P = 0.819). Parasite intensity increased significantly across the decade in small tree finch (r = 0.252, n = 60, P = 0.050), medium tree finch (r = 0.478, n = 54, P < 0.001), and small ground finch (r = 0.178, n = 122, P = 0.049).

2.2 Host age at death

Nests with many *P. downsi* had earlier chick death (r = -0.370, n = 105, P < 0.001). Across the decade, Darwin's finch chicks died at a younger age in nests infested with *P. downsi* parasites (r = -0.65, P < 0.001, n = 150). We found the same pattern in small tree finch (r = -0.69, P < 0.001, n = 44), medium tree finch (r = -0.74, P < 0.001, n = 40), and small ground finch (r = -0.58, P < 0.001, n = 66). For all species combined, chick age at death was 10.6 ± 0.5 days in 2004 and 5.4 ± 0.3 days in 2013.

2.3 Ectoparasite oviposition behavior and pupation

To indirectly test if *P. downsi* oviposition behavior changed across the decade and occurred earlier during the host nesting cycle, we compared the percentage of *P. downsi* instar stages for the minimum period of rapid pupation (4 days) between 2004–2008 (n = 8 nests at which chicks died 4–6 days after hatching) and 2010–2013 (n = 26 nests at which chicks died 4–6 days after hatching). If the parasite is laying eggs earlier in the host nesting cycle, we should find fewer 1st instar larvae later during the nesting cycle. We found that *P. downsi* infested host nests significantly earlier in the nestling period in recent years (Table 1, Table 2). There were 6%–16% fewer 1st instar larvae in chicks that died 4–6 days after hatching comparing 2010–2013 versus 2004–2008 (Table 1, Fig. 1). There were 1%–20% fewer 1st instar larvae in chicks that died 8–10 days after hatching comparing 2010–2013 versus 2004–2008 (Table 1), but this change was not significantly different, which is likely due to small sample size as a result of high early mortality (all P>0.2) (Table 1).

Table 1 The percentage (mean ± *SE*) of *P. downsi* instar and pupae in Darwin's finch nests

(A) Early resource termination (host chicks die 4-6 days after hatching)

	Small tree finch *C. parvulus*		Medium tree finch *C. pauper*		Small ground finch *G. fuliginosa*	
P. downsi (%)	2004–2008 (n=2)	2010–2013 (n=10)	2004–2008 (n=3)	2010–2013 (n=10)	2004–2008 (n=3)	2010–2013 (n=6)
1st instar	18.8±7.5	2.6±2.0	13.7±2.0	1.3±1.3	16.1±3.1	10.1±9.1
2nd instar	16.2±4.8	37.7±9.9	14.1±3.4	22.3±8.9	12.7±1.5	37.7±5.4
3rd instar	40.0±12.7	56.7±10.6	38.5±6.6	65.8±9.5	51.0±4.5	46.1±8.4
Pupae	25.0±25.0	19.8±7.3	45.1±13.1	8.2±4.0	39.4±20.6	34.3±15.0

(B) Late resource termination (host chicks die 8-10 days after hatching)

	Small tree finch *C. parvulus*		Medium tree finch *C. pauper*		Small ground finch *G. fuliginosa*	
P. downsi (%)	2004–2008 (n=4)	2010–2013 (n=4)	2004–2008 (n=3)	2010–2013 (n=2)	2004–2008(n=10)	2010–2013 (n=3)
1st instar	3.8±3.8	2.6±2.6	21.4±6.7	0±0	11.9±19.9	0±0
2nd instar	10.5±4.3	27.0±13.1	15.2±2.4	3.5±3.5	10.9±5.8	1.0±1.0
3rd instar	41.8±6.9	45.4±11.9	43.7±3.6	67.6±22.0	36.2±11.5	44.8±29.3
Pupae	49.4±8.7	30.4±10.1	53.1±12.3	38.2±14.1	58.4±8.4	72.5±19.3

Table 2 MANOVA results for effects of Year and Species on the percentage of *P. downsi* instar in Darwin's finch nests across sampling periods (2004–2008, 2010–2013) on Floreana Island

		F value	*P* value	Partial Eta^2
Year	1st instar (%)	6.910	0.014	0.198
	2nd instar (%)	3.218	0.084	0.103
	3rd instar (%)	1.328	0.259	0.045
Species	1st instar (%)	0.606	0.553	0.041
	2nd instar (%)	0.293	0.748	0.021
	3rd instar (%)	0.052	0.949	0.004
Year × Species	1st instar (%)	0.442	0.647	0.031
	2nd instar (%)	0.276	0.761	0.019
	3rd instar (%)	0.760	0.477	0.051

Data are shown for early resource termination (host chicks die 4-6 days after hatching) in three Darwin's finch host species: small tree finch *Camarhynchus parvulus* (*n*=12), medium tree finch *C. pauper* (*n*=13), and small ground finch *Geospiza fuliginosa* (*n*=9). Only the percentage 1st instar changed significantly across the decade.

Pupation success was higher when hosts survived for longer. Using multiple regression analysis, there were more pupae when chicks survived for longer (r_{part} = 0.516, P = 0.003) and more larvae when chicks died younger (r_{part} = -0.540, P = 0.001). The percentage of pupae decreased significantly across the decade in small tree finch (r = -0.470, P < 0.001, n = 57) and medium tree finch (r = -0.423, P = 0.002, n = 49), but not small ground finch (r = 0.135, P = 0.217, n = 85). Because we stored the pupae and larvae in ethanol within 24 hours of collection from the host nest, we cannot assess rates of larval pupation after nest collection. Here we report on the finding of significantly fewer 1st instar larvae, fewer pupae, and smaller pupae size when hosts died younger – which became increasingly evident as the decade progressed.

2.4 Ectoparasite pupae size

Mean *P. downsi* pupae size decreased significantly across the decade (r=-0.54, P<0.001, n=66). Pupae size was 9.8±0.8 mm in 2006 compared to 8.6±0.2 mm in 2013. The pattern was comparable in small tree finch (r=-0.86, P<0.001, n=22), medium tree finch (r=-0.57, P=0.011, n=18), and small ground finch (r=-0.33, P=0.090, n=26) (Fig. 2). Larvae pupated at larger size when chicks survived for longer (r=0.34, P=0.031, n=39). Thus, *P. downsi* showed a change in behavior to earlier cessation of parasitism (i.e., via pupation) during the host life cycle that was associated with earlier host mortality.

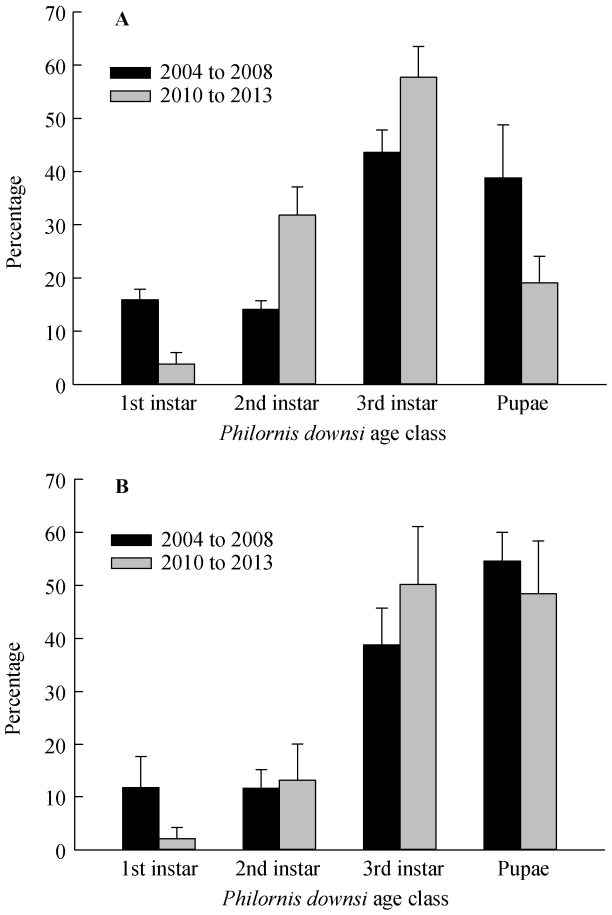

Fig. 1 The percentage (mean ± *SE*) of *P. downsi* instar and pupae in Darwin's finch nests across two sampling periods (2004–2008, 2010–2013) on Floreana Island

A. Early resource termination (host chicks die 4–6 days after hatching). **B.** Late resource termination (host chicks die 8–10 days after hatching). The minimum feeding time for *P. downsi* larvae to pupate is four days. From 2010 onwards, there were fewer 1st instar larvae as the nesting cycle progressed.

3 Discussion

The introduced fly *P. downsi* is considered the biggest threat to the survival of Galapagos land birds. The results of this study on Floreana Island support this view. Across the decade from 2004 to 2013, *P. downsi* parasite intensity nearly doubled (~28 to ~48 parasites per nest), in-nest mortality nearly doubled (~50% to ~90% in-nest mortality), and chicks died in half the time (~11 to ~5 days after hatching). The earlier age at host death predicted smaller pupae size. Also, across the decade, pupae size got 10% smaller (~10 mm to ~9 mm). In Dipterans, pupa size and adult fecundity co-vary nearly 1:1; therefore a 10% reduction in pupae size equates to a 10% decrease in parasite fecundity. The earlier death of the chicks and the smaller pupae size could be the result of a change in parasite behavior to infest the nest

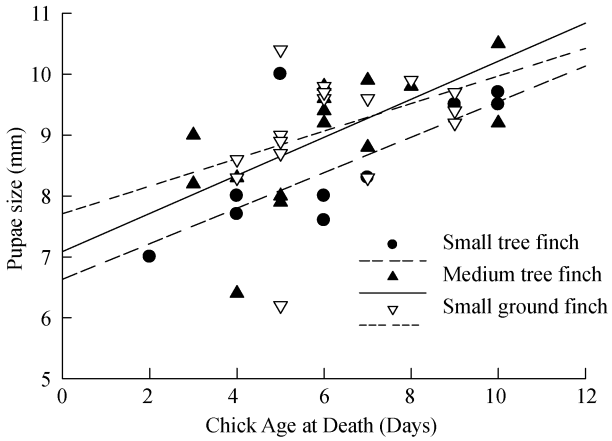

Fig. 2 The positive relationship between chick age at death and *P. downsi* pupae size for common small tree finch *Camarhynchus parvulus* (*n*=22 nests), critically endangered medium tree finch *C. pauper* (*n*=18 nests), and common small ground finch *Geospiza fuliginosa* (*n*=26 nests) on Floreana Island. Later host death predicted larger *P. downsi* pupae size (*r*=0.34, *P*=0.031, *n*=39)

earlier during the nestling phase. If *P. downsi* were infesting Darwin's finch nests earlier, one would predict fewer 1st instar larvae as the nesting cycle progressed, which was supported by our data. The combination of higher *P. downsi* intensity and a more synchronous age class of parasites means that Darwin's finch hosts were exposed to older (and hence larger) parasites consuming their blood and tissue from an earlier age, when nestlings are more vulnerable. This could explain why we found increased mortality across the decade in Darwin's finch hosts, as well as earlier age of death.

Parasites are predicted to exploit their hosts prudently to ensure maintenance of their resource base – without killing them too soon (Frank, 1996; Hanken and Wake, 1993). But this is clearly not the case with *P. downsi*. Since it was first discovered in Darwin's finch nests in 1997, the introduced parasite has been killing Darwin's finches at an ever-earlier age. Generalist parasites like *P. downsi* infest a range of host species, and have been found in Darwin's finch nests across habitats on Santa Cruz and Floreana Island (Dudaniec et al., 2007; Fessl et al., 2010; O'Connor et al., 2010a; O'Connor et al., 2014). Maladaptive virulence (whereby parasite behavior results in reduced parasite fitness and ability to reinfect) is predicted to be more common in generalist parasites (Leggett et al., 2013) because the generalist parasite is more likely to infest a novel host without shared co-evolutionary history. This study finds preliminary evidence for variable maladaptive virulence in *P. downsi*. Parasite pupation success (percentage of pupae) decreased across the decade in the two tree finch species but not in the small ground finch – despite the fact that parasite intensity across the decade increased significantly in all three focal species, as did host mortality. To summarize: host mortality patterns showed a similar pattern but parasite pupation showed a different pattern between host species. What attributes in the small ground finch compared with tree finches lead to higher pupation success in the novel parasite? This question should be the subject of future enquiry.

The Galapagos Islands are a natural laboratory to study host-parasite impacts and offer a unique window to study the importance of pathogens as selective agents in a relatively simple and pristine ecological context. Previous work on Santa Cruz Island strongly suggests that *P. downsi* intensity increases with high rainfall (Dudaniec et al., 2007), but this finding is not consistent (Koop et al., 2013). Rainfall on the Galapagos Islands is unpredictable within and across years (Grant and Grant, 2014). Future study should examine the role of rainfall and other ecological predictor variables for *P. downsi* intensity on Floreana Island. The aim of this study was to identify changes in parasite behavior and host mortality. Clearly much work remains to be done to more fully understand the ecological context of host-parasite associations (Auld et al., 2013; Duffy et al., 2012), including the data presented here.

In the current study, we document a potential trade-off between the parasite life-cycle (i.e. size at pupation) and host mortality, such that adult parasite fecundity (inferred from smaller pupae size) becomes reduced with earlier chick death. This observation suggests that a co-evolutionary arms race between maximizing parasite fecundity and keeping host resources available is occurring in this system. This brings about further consequences for reproductive investment by *P. downsi* female flies, which may co-infest nests with up to six additional females with each depositing up to approximately 24 eggs per nest (Dudaniec et al., 2010). With earlier parasite oviposition in host nests, earlier host death, and increased fecundity costs for parasites, it is feasible that *P. downsi* may be under selection to oviposit fewer eggs per nest, perhaps with fewer co-infesting females, in order to maximize fitness through reduced larval competition under a narrowing, temporary resource. In turn, this could have consequences for Darwin's finch hosts that must balance the benefits of the parasite-dilution effect observed for larger clutch size (Dudaniec et al., 2006) with a more synchronous parasite life-cycle that is

evolving under increasing levels of virulence. Rapid evolution of parasite life history traits has been observed in other systems (Duffy and Sivars-Becker, 2007; Jones et al. 2008; Kelehear et al., 2012) and requires further study in this system.

Theory predicts that parasites should become locally adapted – that is, have a fitness advantage in sympatric hosts over allopatric hosts that cannot be invaded by other non-adapted parasites (Kaltz and Shykoff, 1998). There is growing experimental evidence for local parasite maladaptation, indicating specificity for parasite attack and host defense in sympatric versus allopatric populations (Adiba et al., 2010; Lemoine et al., 2012). In the novel *P. downsi* and Darwin's finch association on Floreana Island, we found evidence for local parasite maladaptation across the decade given fewer pupae in tree finch nests, and evidence for local adaptation given more pupae in small ground finch nests. These findings would be complemented by further sampling, as well as replicated allopatric associations (Blanquart et al., 2013), which is possible within this naturally replicated island system.

The critically endangered medium tree finch, a species endemic to Floreana Island, warrants special concern as it had the highest mean *P. downsi* intensity of any Darwin's finch species studied to date (O'Connor et al., 2010d). Alarmingly, this species had 100% in-nest mortality (no fledging success) since 2012 (Kleindorfer, unpublished data). Even on the same island and in the same forest, the medium tree finch had more *P. downsi* compared with two sympatric Darwin's finch species (small tree finch, small ground finch), which raises further questions to be answered regarding host-specific virulence.

The central question posed at the beginning of this study was about changes in parasite behavior that may signal elevated extinction risk in naïve Darwin's finch hosts. The timing of *P. downsi* infestation behavior became earlier in the nesting cycle over the first decade of this host-parasite association, as inferred from the percentage of 1st instar larvae. The number of *P. downsi* per host nest also increased in all three host species across the decade. We suspect that these two factors explain why we found elevated host mortality and earlier age at death as the decade progressed. Our study therefore reveals changes in parasite behavior that pose additional challenges for Darwin's finch survival. These challenges should be considered as we develop conservation management strategies for this invasive parasite.

Acknowledgements We thank the following organizations for funding: Australian Research Council, Rufford Small Grant Foundation, Mohamed bin Zayed Species Conservation Fund, Max Planck Institute for Ornithology, Australian Research Council, Royal Society for the Protection of Birds/ Birdfair, Earthwatch Institute and Australian Federation of University Women (SA). For logistical and research support, we thank the Charles Darwin Research Station, Galápagos National Park Service, TAME airlines, and the community on Floreana Island. We thank Jeremy Robertson, David Shimoda Arango Roldan, Rebekah Christensen, James Forwood, Claire Charlton, Svenja Gantefoer, Robin Schubert, Timothy Clark, Matthias Schmidt and Christine Evans for field assistance.

References

Adiba S, Huet M, Kaltz O, 2010. Experimental evolution of local parasite maladaptation. Journal of Evolutionary Biology 23: 1195–1205.

Auld S, Penczykowski RM, Ochs JH, Grippi DC, Hall SR et al., 2013. Variation in costs of parasite resistance among natural host populations. Journal of Evolutionary Biology 26: 2479–2486.

Berger-Tal O, Polak T, Oron A, Lubin Y, Kotler BP et al., 2011. Integrating animal behavior and conservation biology: A conceptual framework. Behavioral Ecology 22: 236-239.

Blanquart F, Kaltz O, Nuismer SL, Gandon S, 2013. A practical guide to measuring local adaptation. Ecology Letters 16: 1195–1205.

Brearley G, Rhodes J, Bradley A, Baxter G, Seabrook L et al., 2013. Wildlife disease prevalence in human-modified landscapes. Biological Reviews 88: 427–442.

Britton JR, 2013. Introduced parasites in food webs: New species, shifting structures? Trends in Ecology & Evolution 28: 93–99.

Caro T, Sherman PW, 2011. Endangered species and a threatened discipline: Behavioural ecology. Trends in Ecology & Evolution 26: 111–118.

Causton C, Cunninghame F, Tapia W, 2011. Management of the avian parasite *Philornis downsi* in the Galapagos Islands: A collaborative and strategic action plan. In: GNPS, GCREG, CDF, GC ed. Galapagos Report 2011–2012. Galapagos, Ecuador: Puerto Ayora, 167–173.

Causton CE, Peck SB, Sinclair BJ, Roque-Albelo L, Hodgson CJ et al., 2006. Alien insects: Threats and implications for conservation of Galapagos Islands. Annals of the Entomological Society of America 99: 121–143.

Civitello DJ, Pearsall S, Duffy MA, Hall SR, 2013. Parasite consumption and host interference can inhibit disease spread in dense populations. Ecology Letters 16: 626–634.

Crane AL, Fritts AK, Mathis A, Lisek JC, Barnhart MC, 2011. Do gill parasites influence the foraging and antipredator behaviour of rainbow darters *Etheostoma caeruleum*? Animal Behaviour 82: 817–823.

Daly EW, Johnson PT, 2011. Beyond immunity: Quantifying the effects of host anti-parasite behavior on parasite transmission. Oecologia 165: 1043–1050.

Dudaniec R, Hallas G, Kleindorfer S, 2005. Blood and intestinal parasitism in Darwin's finches: Negative and positive findings. Acta Zool. Sinica 51: 507–512.

Dudaniec RY, Fessl B, Kleindorfer S, 2007. Interannual and interspecific variation in intensity of the parasitic fly *Philornis downsi* in Darwin's finches. Biological Conservation 139: 325–332.

Dudaniec RY, Gardner MG, Kleindorfer S, 2010. Offspring genetic structure reveals mating and nest infestation behaviour of an invasive parasitic fly *Philornis downsi* of Galapagos birds. Biological Invasions. 12: 581–592.

Dudaniec RY, Kleindorfer S, 2006. Effects of the parasitic flies of the genus *Philornis* (Diptera : Muscidae) on birds. Emu 106: 13–20.

Dudaniec RY, Kleindorfer S, Fessl B, 2006. Effects of the introduced ectoparasite *Philornis downsi* on haemoglobin level and nestling survival in Darwin's small ground finch *Geospiza fuliginosa*. Austral Ecology 31: 88–94.

Duffy MA, Ochs JH, Penczykowski RM, Civitello DJ, Klausmeier CA et al., 2012. Ecological context influences epidemic size and parasite-driven evolution. Science 335: 1636– 1638.

Duffy MA, Sivars-Becker L, 2007. Rapid evolution and ecological host-parasite dynamics. Ecology Letters 10: 44–53.

Dvorak M, Fessl B, Nemeth E, Kleindorfer S, Tebbich S, 2011. Distribution and abundance of Darwin's finches and other land birds on Santa Cruz Island, Galapagos: Evidence for declining populations. Oryx 46: 78–86.

Edeline E, Ari TB, Vøllestad LA, Winfield IJ, Fletcher JM et al., 2008. Antagonistic selection from predators and pathogens alters food-web structure. Proceedings of the National Academy of Sciences 105: 19792–19796.

Fessl B, Couri MS, Tebbich S, 2001. *Philornis downsi* Dodge & Aitken, new to the Galapagos Islands (Diptera, Muscidae). Studia Dipterologica 8: 317–322.

Fessl B, Kleindorfer S, Tebbich S, 2006a. An experimental study on the effects of an introduced parasite in Darwin's finches. Biological Conservation 127: 55–61.

Fessl B, Kleindorfer S, Tebbich S, 2006b. An experimental study on the effects of an introduced parasite in Darwin's finches. Biological Conservation 127: 55–61.

Fessl B, Sinclair BJ, Kleindorfer S, 2006c. The life-cycle of *Philornis downsi* (Diptera : Muscidae) parasitizing Darwin's finches and its impacts on nestling survival. Parasitology 133: 739–747.

Fessl B, Tebbich S, 2002. *Philornis downsi*- a recently discovered parasite on the Galápagos archipelago- a threat for Darwin's finches? Ibis 144: 445–451.

Fessl B, Young GH, Young RP, Rodriguez-Matamoros J, Dvorak M et al., 2010. How to save the rarest Darwin's finch from extinction: The mangrove finch on Isabela Island. Philosophical Transactions of the Royal Society B. 365: 1019–1030.

Frank SA, 1996. Models of parasite virulence. Quarterly Review of Biology: 37–78.

Galligan TH, Kleindorfer S, 2009. Naris and beak malformation caused by the parasitic fly *Philornis downsi* (Diptera: Muscidae) in Darwin's small ground finch *Geospiza fuliginosa* (Passeriformes: Emberizidae). Biological Journal of the Linnean Society 98: 577–585.

Goodman BA, Johnson PT, 2011. Disease and the extended phenotype: Parasites control host performance and survival through induced changes in body plan. PLoS ONE. 6: e20193.

Grant PR, 1986. Ecology and Evolution of Darwin's Finches. Princeton: Princeton University Press.

Grant PR, Grant BR, 2014. 40 Years of Evolution: Darwin's Finches on Daphne Major Island. Princeton University Press.

Hanken J, Wake DB, 1993. Miniaturization of body size: Organismal consequences and evolutionary significance. Annual Review of Ecology and Systematics: 501–519.

Honěk A, 1993. Intraspecific variation in body size and fecundity in insects: A general relationship. Oikos 66: 483–492.

Houte S, Ros VI, Oers MM, 2013. Walking with insects: Molecular mechanisms behind parasitic manipulation of host behaviour. Molecular Ecology 22: 3458–3475.

Huber SK, 2008. Effects of the introduced parasite *Philornis downsi* on nestling growth and mortality in the medium ground finch *Geospiza fortis*. Biological Conservation 141: 601–609.

Huber SK, Owen JP, Koop JAH, King MO, Grant PR et al., 2010. Ecoimmunity in Darwin's finches: Invasive parasites trigger acquired immunity in the medium ground finch *Geospiza fortis*. PLoS ONE 5.

Jones ME, Cockburn A, Hamede R, Hawkins C, Hesterman H et al., 2008. Life-history change in disease-ravaged Tasmanian devil populations. Proceedings of the National Academy of Sciences 105: 10023–10027.

Kaltz O, Shykoff JA, 1998. Local adaptation in host-parasite systems. Heredity 81: 361–370.

Kelehear C, Brown GP, Shine R, 2012. Rapid evolution of parasite life history traits on an expanding range-edge. Ecology Letters 15: 329–337.

Kleindorfer S, 2007a. The ecology of clutch size variation in Darwin's small ground finch *Geospiza fuliginosa*: Comparison between lowland and highland habitats. Ibis 149: 730–741.

Kleindorfer S, 2007b. Nesting success in Darwin's small tree finch *Camarhynchus parvulus*: Evidence of female preference for older males and more concealed nests. Animal Behaviour 74: 795–804.

Kleindorfer S, Dudaniec RY, 2009. Love thy neighbour? Social nesting pattern, host mass and nest size affect ectoparasite intensity in Darwin's tree finches. Behavioral Ecology and Sociobiology 63: 731–739.

Kleindorfer S, O'Connor JA, Dudaniec RY, Myers SA, Robertson J et al., 2014. Species collapse via hybridization in Darwin's tree finches. American Naturalist 183: 325–341.

Koop JA, Huber SK, Laverty SM, Clayton DH, 2011. Experimental demonstration of the fitness consequences of an introduced parasite of Darwin's finches. PLoS ONE 6: e19706.

Koop JA, Le Bohec C, Clayton DH, 2013. Dry year does not reduce invasive parasitic fly prevalence or abundance in Darwin's finch nests. Reports in Parasitology 3: 11–17.

Lemoine M, Doligez B, Richner H, 2012. On the equivalence of host local adaptation and parasite maladaptation: An experimental test. The American Naturalist 179: 270–281.

Lincango P, Causton C, 2008. Crianza en cautiverio de *Philornis downsi* en las Islas Galapagos. Informe interno. Charles Darwin Fourndation.

McCallum H, 2008. Tasmanian devil facial tumour disease: Lessons for conservation biology. Trends in Ecology & Evolution 23: 631–637.

Murray KA, Retallick RW, Puschendorf R, Skerratt LF, Rosauer D et al., 2011. Assessing spatial patterns of disease risk to biodiversity: Implications for the management of the amphi-

bian pathogen *Batrachochytrium dendrobatidis*. Journal of Applied Ecology 48: 163–173.

O'Connor JA, Dudaniec RY, Kleindorfer S, 2010a. Parasite infestation and predation in Darwin's small ground finch: Contrasting two elevational habitats between islands. Journal of Tropical Ecology 26: 285–292.

O'Connor JA, Robertson J, Kleindorfer S, 2010b. Video analysis of host-parasite interactions in nests of Darwin's finches. Oryx 44: 588–594.

O'Connor JA, Sulloway FJ, Kleindorfer S, 2010c. Avian population survey in the Floreana highlands: Is Darwin's medium tree finch declining in remnant patches of *Scalesia* forest? Bird Conservation International. 20: 343–353.

O'Connor JA, Sulloway FJ, Robertson J, Kleindorfer S, 2010d. *Philornis downsi* parasitism is the primary cause of nestling mortality in the critically endangered Darwin's medium tree finch *Camarhynchus pauper*. Biodiversity and Conservation 19: 853–866.

O'Connor JA, Robertson J, Kleindorfer S, 2014. Darwin's finch begging intensity does not honestly signal need in parasitised nests. Ethology 120: 228–237.

Poulin R, 2011. Evolutionary Ecology of Parasites. Princeton: Princeton University Press.

Quiroga M, Reboreda J, 2013. Sexual differences in life history traits of *Philornis seguyi* (Diptera: Muscidae) parasitizing house wrens *Troglodytes aedon*. Annals of the Entomological Society of America 106: 222–227.

Quiroga MA, Reboreda JC, Beltzer AH, 2012. Host use by *Philornis sp.* in a passerine community in central Argentina. Revista Mexicana de Biodiversidad 83: 110–116.

Schmid-Hempel P, 2011. Evolutionary parasitology: The integrated study of infections, immunology, ecology, and genetics. New York: Oxford University Press.

Spalding MG, Mertins JW, Walsh PB, Morin KC, Dunmore DE et al., 2002. Burrowing fly larvae *Philornis porteri* associated with mortality of eastern bluebirds in Florida. Journal of Wildlife Diseases 38: 776–783.

Sulloway FJ, Kleindorfer S, 2013. Adaptive divergence in Darwin's small ground finch *Geospiza fuliginosa*: Divergent selection along a cline. Biological Journal of the Linnean Society 110: 45–59.

Teixeira D, 1999. Myiasis caused by obligatory parasites. Ib. General observations on the biology of species of the genus *Philornis* Meinert, 1890 (Diptera, Muscidae). In: Guimarães JH, Papavero N ed. Myiasis in Man and Animals in the Neotropical Region: Bibliographic Database. São Paulo: Editora Plêiade/FAPESP, 71–96.

Thompson R, Lymbery A, Smith A, 2010. Parasites, emerging disease and wildlife conservation. International Journal for Parasitology 40: 1163–1170.