Blood and intestinal parasitism in Darwin’s finches: negative and positive findings

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The impact of parasites on wild vertebrate populations is receiving increased attention due to findings from numerous studies that document severe parasite-induced fitness costs in hosts (Hart, 1992; Oppliger et al., 1996; Richner, 1998; Milton et al., 2001; DasaK et al., 2003). Avian studies have greatly contributed to our knowledge of parasites in relation to mortality, reduced growth, and lowered reproductive success across taxonomic groups (Oksanen, 1994; Martin et al., 2001; Fessl and Tebbich, 2002; Sazn et al., 2002). Island host-parasite interactions are of considerable interest due to their distribution patterns, which can provide insights into the maintenance of host-parasite associations across variable island environments (van Riper et al., 1986; Apanius et al., 2000).

Introduced diseases affecting island bird populations have been identified as strong determinants of population persistence, (e.g., van Riper et al., 2002; Vander Werf, 2001), which is thought to be due to reduced immunological defense against multiple pathogens among insular species (Wikelski et al., 2004). The observation of species-specific and island-specific host-parasite interactions is important for monitoring the impact of pathogens on host communities at the local level (Apanius et al., 2000). Such specific interactions may occur due to slight differences between island habitats or species compositions (Dobson and PaCaI, 1992).

Wild birds are commonly infected with coccidia (Phylum Apicomplexa), which are protozoan endoparasites that cause blood parasitism (e.g., Plasmodium, Hemoproteus, Apicoplexa) or intestinal parasitism (e.g., Isopora, Eimeria). Fitness costs induced by avian blood parasites have been reported in relation to breeding success (Siikamaki et al., 1997; Dawson and Bortolotti, 2000), clutch size (Sazn et al., 2002), parental effort (Appleby et al., 1999) and immune function (Navarro et al., 2003). Intestinal protozoan parasites are known to significantly contribute to mortality (Oksanen, 1994; Skirmnsson, 1997), affect nesting growth (Kruszewicz, 1995; Mazgajski and Kedra, 1998), cause dehydration (Todd and Hammond, 1971), weight loss (Callow, 1984), and impair the uptake of nutrients (Augustine and Ruff, 1983). The susceptibility of birds to coccidial infections has been associated with the host’s feeding behavior, ecology, and age (Long, 1970; Skirmnsson, 1997; McQuiston, 2000). Both blood parasitism and intestinal coccidiosis are associated
with reduced reproductive fitness by interacting with the expression of sexually selected morphological traits and female mate choice (Hillgarth, 1990; Buchholz, 1995; Wiesn et al., 1997; Hill and Brawner, 1998; Freeman-Gallant et al., 2001).

Darwin’s finches on the Galapagos Islands are an iconic group of birds that have received much attention within evolutionary biology and behavioral ecology (Grant and Grant, 2003). However, the study of pathogens that may be contributing to their decline has only recently been acknowledged as a priority research area (Wikelski et al., 2004; pers. comm. D. Wiedenfeld, 2004). Introduced pathogens such as the devastating ectoparasite Philornis downsi (see Fessl and Tebbich, 2002), and avian poxvirus (Lindström et al., 2004; Kleindorfer and Dudaniec, in press) are receiving increased recognition across the islands. The mangrove finch Cactospiza heleniobates has the smallest population of all of the Darwin finches with approximately 200 pairs on Isabela island (a primary reason for this restricted population size is thought to be habitat loss) (Dvorak et al., 2002), while other endemic species including Darwin’s warbler Certhidea fruticata on Floreana Island are of particular conservation concern with population fluctuations that may be partially linked with parasitism by P. downsi (Grant et al., 2005).

Previous studies have found coccidian infections in Darwin’s finches, with the discovery of six new Isospora species distributed on the islands of Santa Cruz, Isabela, and Daphne Major (McQuiston and Wilson, 1988; McQuiston and Wilson, 1989; McQuiston, 1990). Previous attempts at finding blood parasites in Darwin’s finches have been unsuccessful (Harmon unpublished p. 171 in Grant, 1999; pers. comm. N. Gottdenker, 2004), though these studies may be considered inconclusive due to limited sampling. The following study presents the results from the examination of blood and fecal samples obtained from nestling small ground finches Geospiza fuliginosa and medium ground finches Geospiza fortis and blood samples from adult small ground finches.

The aims of the study were: i) to gather evidence for intestinal parasitism in nestling G. fuliginosa and G. fortis on Santa Cruz, and on Floreana, where the presence of coccidian parasites is hitherto unknown; and ii) to determine the presence of blood parasites in these same nestlings, as well as in adult G. fuliginosa across three islands (Santa Cruz, Floreana and Isabela).

1 Methods

Blood and fecal samples were collected during the Darwin finch breeding season between January and March 2004 on the Galapagos Islands, Ecuador. Fecal samples were collected from nestling small ground Finches (SGF) on the islands of Santa Cruz (0°37’S, 90°21’W) (n = 6) and Floreana (1°28’S, 90°48’W) (n = 9), while nestling medium ground finches (MGF) were sampled on Santa Cruz (n = 7). In total, 22 individuals were sampled from 14 nests (total of 9 SGF and 5 MGF nests). Nests were randomly sampled from the lowland habitat (3 sites Santa Cruz; 1 site Floreana), except for one sample that was opportunistically collected from the highlands of Floreana. All nestlings were aged nine days, which was determined by weight (nearest 0.1 g) using a digital scale (Tanita model 1479).

Fecal samples were immediately placed in to labeled vials containing sodium acetate acetic acid formalin (SAF), while one sample was preserved in 2.5% potassium dichromate (K2Cr2O7); both are equivalent preservation methods (Pietrzak-Johnston et al., 2000). Samples were obtained between 05:00 – 12:00 hrs. Examination of fecal samples was conducted approximately six weeks after collection, allowing sufficient time for sporulation of any protozoa that were present (Duszynski and Wilber, 1997). The samples were initially scanned using a direct wet preparation smear and concentration technique (Garcia, 2001) under a 100 × objective for evidence of parasitic worms (e.g., Helminths). Protozoan trophozoites and cysts may or may not be identified to species level and are best confirmed by examination under 400 – 1000 × magnification of the permanent stained smear using modified iron hematoxylin (Palmer, 1991). Oocysts of Cryptosporidium sp., Isospora sp., Cyclospora sp. and Eimeria sp. can be identified only to genus level using this method (Palmer, 1991). For refinement of identification to the level of genera, samples were examined using Differential Interference Contrast-Ultra Violet (DIC-UV) microscopy and stained with DAPI (4’6-Diamidino-2-phenylindole) (400 – 1000 ×) and photographed (Anguish and Ghiorse, 1997). The genera of protozoan parasites can be distinguished morphologically by the number of sporocysts and sporozoites that reside in the oocyst (Campbell et al., 1992; Vesey et al., 1993; Duszynski and Wilber, 1997).

A blood sample of 0.01 ml was collected by jugular venipuncture from birds using a 0.5 ml syringe (29G 1/2”, 0.33 mm x 12.7 mm) (Campbell, 1995). A blood smear was made for 12 SGF nestlings (the other nestlings were too anemic to collect sufficient blood volume for all procedures) from nine nests using individually marked microscope slides, air dried, and fixed in 99% ethanol either immediately, or within six hours of collection. Blood smears were also obtained from mist-netted adult SGF from three islands; Santa Cruz (n = 42), Floreana (n = 35),
and Isabela (0°58'S, 90° 58'W) (n = 50). Slides were stained with Wright-Giemsa using an automatic Hema-Tek stainer (Campbell, 1995). All slides were examined for blood parasites by observing 100 microscopic fields per slide under a 100 × oil immersion objective, which equated to approximately 10 000 erythrocytes observed per individual (~100 red blood cells per field). Due to sparse cell distribution in blood smears from two nestlings, only 50 fields could be observed.

2 Results

No evidence of blood parasitism was found in any of the G. fuliginosa smears obtained from both adults and nestlings. Oocysts of an unidentified species of Isospora were found in one G. fuliginosa nestling fecal sample from the Floreana highlands (4.5% nestlings; 95% CI = 0.01% – 0.22%) (Fig. 1). All other fecal samples from both G. fuliginosa and G. fortis were negative for intestinal parasites. Images obtained from DIC-UV light microscopy enabled the identification of Isospora, which was based on the diagnostic feature of two sporocysts containing four sporozoites within each oocyst (Baker, 1982). Both the oocyst residuum (Fig. 1: A) and polar granule (Fig. 1: B) were detected, while excysted DNA from four sporozoites was also observed. The sample contained hundreds of sporulated oocysts.

3 Discussion

This study found no evidence of blood parasites in both nestling and adult SGF, which supports the findings of previous studies that have not found blood parasites in Darwin’s finches (Harmon p. 171 in Grant, 1999). Such evidence is noteworthy considering the high impact that malarial blood parasites have had on endemic Hawaiian passerines (Jarvi et al., 2001), as well as the widespread distribution of avian blood parasites (Campbell, 1995). Secondly, this study provides evidence that intestinal parasitism appears to be rare in G. fuliginosa and G. fortis nestlings, while documenting the first record of Isospora in G. fuliginosa on Floreana Island.

The discovery of Isospora parasites on Floreana Island is of particular conservation interest as the medium tree finch Camarhynchus pauper is only found on this island. In addition, nestling parasitism by the introduced fly P. downsi was discussed in relation to the local extinction of the warbler finch Certhidea fusca on this island (Grant et al., 2005). Thus, the avifauna of Floreana appears to be under increased pressure from introduced pathogens, and the discovery of another parasite heightens the cause for concern.

Isospora species are considered to be mainly one-cycled, with life stages found only in the feces. However, the genus Atoxoplasma is two-cycled and

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**Fig. 1** *Isospora* (unidentified species) found from feces of the small ground finch *G. fuliginosa* on Floreana Island (DIC-1 000X).

Note double-layered oocyst wall, the presence of two sporocysts containing four sporozoites, sporocyst residuum (A) and the single polar body to the left of the oocyst (B).
infects blood cells, while also producing *Isospora*-type oocysts in the feces, which has led to taxonomic confusion (Rossi et al., 1996; Ball et al., 1998; Martínez and Muñoz, 1998; Upton et al., 2001). The findings of the current study suggest that the species of *Isospora* infecting *G. fuliginosa* is one-cycled and not of the genus *Atoxoplasma*, given that concurrent observations of blood and feces (as recommended by Levine 1982 and Ball et al., 1998) did not reveal blood parasites.

As coccidia are transmitted through fecal material and require moist, cool conditions to survive, it is suggested that species occupying the ground cover and lower understorey are more susceptible to infection than canopy dwellers (Mazgajski and Kędra, 1998). A survey of coccidia (*Isospora* and *Eimeria* spp.) of 190 South American avian species found that species inhabiting the forest canopy had prevalence rates significantly lower than the overall prevalence rate (McQuiston, 2000). One would therefore expect Darwin’s ground finches to show increased levels of coccidian infections compared with the tree finches, though previous studies have found a similar prevalence between the two groups (McQuiston and Wilson, 1988; McQuiston, 1989). This similarity in prevalence may arise because the tree finches reside in the moist and cool highlands, which favors the persistence of coccidia, whereas the ground finches are found mainly in the less favorable arid lowlands. The fact that the current study found an infected SRF in the highlands and no infection in the lowlands could provide evidence of this, though previous studies have found *Isospora* in both habitats (McQuiston and Wilson, 1988; McQuiston, 1989, 1990).

Interpretation of a host’s infection status (i.e., mild to severe) should be related to the time at which fecal samples were collected, because there is a diurnal cycle of oocyst shedding in birds (Kruszewicz, 1995: Brawner and Hill, 1999; Hudman et al., 2000). For example, Kruszewicz (1995) did not find any *Isospora lacaezi* oocysts in house sparrow *Passer domesticus* and tree sparrow *P. montanus* nestlings when feces were collected in the morning, but found 11% prevalence when samples were obtained during the latter part of the day. Similarly, in adult house finches *Carpodacus mexicanus*, the occurrence of *Isospora* oocysts was more frequent in the afternoon (between 15:00 – 19:00 hrs) compared to before 10:00, when no fecal samples contained oocysts (Brawner and Hill, 1999). The current study may have underestimated coccidia prevalence due to a diurnal oocyst shedding cycle, as all samples were obtained in the morning. Studies that aim to correlate parasite intensity with fitness impacts of intestinal protozoan parasites need to consider this temporal variation in the ability to accurately detect infections.

Since the documentation of intestinal parasites in Darwin’s finches by McQuiston and Wilson (1988) and McQuiston (1989, 1990), there has been no investigation into the potential fitness impacts of these often highly pathogenic protozoans. Infection with one disease can often increase an individual’s susceptibility to other diseases (Long, 1970), and consequently further research on the fitness costs of parasites is required given the current suite of infectious diseases on the Galapagos (Curry and Grant, 1989; Fessl and Tebbich, 2002; Lindström et al., 2004; Wikelski et al., 2004; Kleindorfer and Dudaniec, in press). As coccidian infections have potentially high fitness impacts, and island populations often show reduced resistance to pathogens (van Riper et al., 1986; Pickering and Norris, 1996; Crooks et al., 2001), there is a pressing need for future research on the fitness costs of *Isospora* in Darwin’s finches.

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