



Haemosporidian parasite community in migrating bobolinks on the Galapagos Islands

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ABSTRACT

Bobolinks (*Dolichonyx oryzivorus*) migrate from their breeding grounds in North America to their wintering grounds in South America during the fall each year. A small number of Bobolinks stop temporarily in Galapagos, and potentially carry parasites. On the North American breeding grounds, Bobolinks carry a least two of the four *Plasmodium* lineages recently detected in resident Galapagos birds. We hypothesized that Bobolinks carried these parasites to Galapagos, where they were bitten by mosquitoes that then transmitted the parasites to resident birds. The haemosporidian parasite community in 44% of the Bobolinks we captured was consistent with those on their breeding grounds. However, the lineages were not those found in Galapagos birds. Our results provide a parasite community key for future monitoring.

1. Introduction

Avian haemosporidian parasites are a highly diverse group of dip-teran-borne blood parasites. *Plasmodium*, *Haemoproteus* and *Leucocytozoon* being the most common genres, are widely distributed around the world (Valkiūnas, 2005). However, avian haemosporidians are limited in number and diversity in remote islands likely because the diversity of avian hosts and/or the required arthropod hosts is also limited (Clark et al., 2014). Four *Plasmodium* lineages, avian haemosporidian parasites that require both a vertebrate and mosquito host, were recently documented in diverse bird species on the Galapagos Islands (Levin et al., 2009, 2013). Colonization by these parasites is of significant conservation concern, as they can lower host fitness and survival (Atkinson and Samuel, 2010; Lachish et al., 2011).

There is no evidence, however, that the *Plasmodium* parasite(s) found in the islands complete their transmission cycle in Galapagos native (Levin et al., 2013) or introduced birds (Gottdenker et al., 2005; Deem et al., 2011; Jaramillo et al., in preparation). Therefore, we sought to determine if migratory species could be carrying parasites that may be transmitted to the Galapagos avifauna. We hypothesized that these parasites were brought to the islands by Bobolinks (*Dolichonyx oryzivorus*), the only passerine bird species that regularly migrates through Galapagos, and transmitted across the avifauna by

mosquitoes. Galapagos currently has three mosquito species—one native, brackish-water species (*Aedes taeniorhynchus*); one species (*Culex quinquefasciatus*) accidentally introduced in 1985 that is a known *Plasmodium* vector elsewhere; and one species (*Aedes aegypti*) also accidentally introduced in the 1990's, but thought to feed less on birds than the other two species (Whiteman et al., 2005; Bataille et al., 2009).

Bobolinks breed across much of the northern United States and southern Canada, and winter in eastern Bolivia, Paraguay, and north-eastern Argentina (Renfrew et al., 2013). Their main migration route in South America is inland, but a likely small, but unknown number of Bobolinks move through the Galapagos each year (Perlut and Renfrew, 2016). In an earlier study, we found *Plasmodium* parasite lineages B and C, detected in 2 Galapagos passerine species, Small ground finches (*Geospiza fuliginosa*) and Yellow warblers (*Setophaga petechia*), in the blood of Bobolinks sampled on their North American breeding grounds (Levin et al., 2013). We then characterized the geographic origins of these two lineages: *Plasmodium* lineage B was of South American origin and *Plasmodium* lineage C was of North American origin, potentially California (Levin et al., 2016). Although these results offer a compelling explanation as to how parasites may have arrived on the Galapagos Islands, we sought more evidence for the role of the Bobolink in *Plasmodium* transmission to Galapagos resident birds by sampling Bobolinks for haemosporidian parasites in the Galapagos. In October 2015, we

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traveled to San Cristobal Island to try to find, capture, and sample blood from migrating Bobolinks stopping over in Galapagos. Here we describe the infection rate and parasite community of nine Bobolinks caught on the Galapagos.

2. Material and methods

On October 12–23, 2015, we searched for Bobolinks on migration stopover in native grassland and agricultural habitat in the highlands of San Cristobal Island, Galapagos, Ecuador (see [Perlut and Renfrew, 2016](#) for details). We used mist-nets and playbacks to capture nine Bobolinks at Finca de las Gemelas (422 m elevation; 0°53'26.23"S, 89°27'15.35"W), a 2.5 ha grassland patch, and in a pasture at Santa Monica, an agricultural complex 9.15 km west of Gamelas, owned by the Ecuadorian military (435 m elevation). We banded each bird with a unique U.S. Geological Survey band, recorded morphometric measurements, and collected a blood sample from the brachial vein that was placed in lysis buffer for DNA preservation ([Longmire et al., 1988](#)).

We used a standard phenol-chloroform extraction protocol following [Sambrook et al. \(1989\)](#), with a final dialysis step in TNE₂ (1M Tris pH 8, 5M NaCl, 0.5M EDTA, dH₂O), for DNA extraction of the Bobolink samples. For PCR-based molecular screening, we amplified a region of parasite mitochondrial cytochrome *b* gene following [Waldenstrom et al. \(2004\)](#). The positive control was a consistently amplifying PCR-positive Galapagos Penguin (*Spheniscus mendiculus*) and the negative control consisted of all PCR reagents without DNA. Positive samples were sequenced to identify parasite DNA using BigDye terminator v3.1 cycle sequencing kit (Applied Biosystems) in 10 µL reactions with a final primer concentration of 1 µM following a standard cycle sequencing program. Reactions were then cleaned using ethanol precipitation before sequencing on an ABI 3130 automated sequencer at the University of Missouri – Saint Louis. Parasite cyt *b* sequences were assembled in Seqman Pro 12.2.0 and added to a data set containing previous amplified sequences from Galapagos birds and overlapping cyt *b* sequences from described morphospecies from GenBank used in [Levin et al. \(2013\)](#) to identify matches.

3. Results and discussion

Our molecular analysis identified 4 of 9 individuals (44%) that amplified *Plasmodium* sp. DNA from the Bobolink blood sampled in Galapagos. Two of the sequences matched the *Plasmodium cathemerium* lineage SEIAUR01 (GenBank accession number: AY377128, [Loiseau et al., 2013](#)), which is common in Bobolinks ([Levin et al., 2013](#)) and corresponds to lineage M found in those captured in North America ([Levin et al., 2016](#)). The other two sequences match *Plasmodium* sp. lineages WW3 and RWB01 (GenBank accession numbers: KC867662 and KC867673 respectively, [Levin et al., 2013](#)) which correspond to North American lineages K and L in [Levin et al. \(2016\)](#), respectively.

The haemosporidian parasite community found in Bobolinks captured on Galapagos during migration was consistent with the parasite community in Bobolinks sampled on their North American breeding grounds ([Levin et al., 2013](#)), but did not match the lineages found in Galapagos resident bird species. The prevalence of haemosporidian DNA was higher in our samples (44%; 4 of 9 birds) than in Bobolinks sampled on their North America breeding grounds (17.8%; 78 of 438 birds; [Levin et al., 2013](#)), although sample sizes differ dramatically. We do not yet know if and how haemosporidian parasites affect Bobolinks throughout any phase of their life-cycle. Other studies have found both weak ([Risely et al., 2018](#)) and no ([Cornelius et al., 2014](#); [Sorensen et al., 2016](#)) effect of parasites on migratory birds during migration and while wintering.

These results indicate that 1) the Bobolinks that we sampled in Galapagos have been exposed to similar parasite communities as the Bobolinks in North America (e.g. population connectivity either in the breeding or wintering grounds), and 2) the Bobolinks provide a parasite

community key to use for future monitoring, as little is known about the transmission paths of these parasites nor their impact on bird survival or reproduction. Our small sample size limited the probability of capturing a Bobolink that matched one or more of the four Galapagos lineages. Although we captured nine individuals during our 12 days of fieldwork, we observed but could not capture an additional 2–6 birds ([Perlut and Renfrew, 2016](#)). None-the-less, the plausibility of our proposed pathway for parasite introduction into Galapagos—transport by migratory Bobolinks and transmission by resident mosquitoes—is supported by recent work on the altitudinal distribution of mosquitoes in the archipelago. Both *A. taeniorhynchus* and *C. quinquefasciatus* were found in the Galapagos at the same elevation (422–435 m) as the sites that Bobolinks selected ([Asigau et al., 2017](#)). We recommend 1) sampling of additional Bobolinks and native avifauna on Galapagos to monitor changes in and sources of the haemosporidian parasite community; 2) study of the pathogenic effects of these lineages across the diverse resident species known to be infected (e.g. [Levin et al., 2013](#)); and 3) study of any costs or benefits to bobolinks in carrying these parasites.

A number of studies have found haemosporidian parasites in migratory birds throughout their breeding and wintering ranges ([Pérez-Tris and Bensch, 2005](#); [Ramey et al., 2016](#); [Ricklefs et al., 2016](#)). [Cornuault et al. \(2012\)](#) used a phylogenetic approach to understand parasite origins on remote islands of the Mascarene archipelago and found that while there is evidence for a lineage introduction through human-introduced birds, most lineages were likely brought by immigration of avian hosts or vectors and have diversified *in-situ*. To our knowledge, [Cornuault et al. \(2012\)](#) and this study are the only examples of haemosporidian parasites arriving to remote islands through migratory birds.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.ijppaw.2018.05.006>.

References

- Asigau, S., Hartman, D.A., Higaschiguchi, J.M., Parker, P.G., 2017. The distribution of mosquitoes across an altitudinal gradient in the Galapagos Island. *J. Vector Ecol.* **42**, 243–253.
- Atkinson, C.T., Samuel, M.D., 2010. Avian malaria *Plasmodium relictum* in native Hawaiian forest birds: epizootiology and demographic impacts on 'apapane *Himatione sanguinea*. *J. Avian Biol.* **41**, 357–366.
- Bataille, A., Cunningham, A.A., Cedeño, V., Patiño, L., Constantinou, A., Kramer, L.D., Goodman, S.J., 2009. Natural colonization and adaptation of a mosquito species in Galapagos: implications for disease threats to endemic wildlife. *P. Natl. A. Sci. USA* **106**, 10230–10235.
- Clark, N., Clegg, S.M., Lima, M.R., 2014. A review of global diversity in avian haemosporidians (*Plasmodium* and *Haemoproteus*: Haemosporida): new insights from molecular data. *Int. J. Parasitol.* **44**, 329–338.
- Cornelius, E.A., Davis, A.K., Altizer, S.A., 2014. How Important are Hemoparasites to migratory Songbirds? Evaluating physiological measures and infection status in three neotropical migrants during stopover. *Physiol. Biochem. Zool.* **87**, 719–728.
- Cornuault, J., Bataillard, A., Warren, B.H., Lootvoet, A., Mirleau, P., Duval, T., Milá, B., Thébaud, C., Heeb, P., 2012. The role of immigration and *in-situ* radiation in explaining blood parasite assemblages in an island bird clade. *Mol. Ecol.* **21**, 1438–1452.
- Deem, S.L., Parker, P.G., Cruz, M.B., Merkel, J., Hoeck, P.E., 2011. Comparison of blood values and health status of floreana mockingbirds (*Mimus trifasciatus*) on the islands of champion and gardner-by-floreana, galápagos islands. *J. Wildl. Dis.* **47** (1),

- 94–106.
- Gottdenker, N.L., Walsh, T., Vargas, H., Merkel, J., Jiménez, G.U., Miller, R.E., Dailey, M., Parker, P.G., 2005. Assessing the risks of introduced chickens and their pathogens to native birds in the Galápagos Archipelago. *Biol. Conserv.* 126, 429–439.
- Lachish, S., Knowles, S.C.L., Alves, R., Wood, M.J., Sheldon, B.C., 2011. Fitness effects of endemic malaria infections in a wild bird population: the importance of ecological structure. *J. Anim. Ecol.* 80, 1196–1206.
- Levin, I.I., Outlaw, D.C., Vargas, F.H., Parker, P.G., 2009. *Plasmodium* blood parasite found in endangered Galapagos penguins (*Spheniscus mendiculus*). *Biol. Conserv.* 142, 3191–3195.
- Levin, I.I., Zwiers, P., Deem, S.L., Geest, E.A., Higashiguchi, J.M., Iezhova, T.A., Jimenez-Uzcategui, G., Kim, D.H., Morton, J.P., Perlut, N.G., Renfrew, R.B., Sari, E.H.R., Valkiūnas, G., Parker, P.G., 2013. Multiple lineages of avian malaria parasites (*Plasmodium*) in the Galapagos Islands and evidence for arrival via migratory birds. *Conserv. Biol.* 27, 1366–1377.
- Levin, I.I., Baumann, R.X., Kim, D.H., Perlut, N.G., Renfrew, R.B., Parker, P.G., 2016. Local parasite lineage sharing in temperate grassland birds provides clues about potential origins of Galapagos avian *Plasmodium*. *Ecol. Evol.* 6, 716–726.
- Loiseau, C., Harrigan, R.J., Bichet, C., Julliard, R., Garnier, S., Lendvai, A.Z., Chastel, O., Sorci, G., 2013. Predictions of avian *Plasmodium* expansion under climate change. *Sci. Rep.-UK* 3.
- Longmire, J.L., Lewis, A.K., Brown, N.C., Buckingham, J.M., Clark, L.M., Jones, M.D., Wagner, R.P., 1988. Isolation and molecular characterization of a highly polymorphic centromeric tandem repeat in the family Falconidae. *Genomics* 2, 14–24.
- Pérez-Tris, J., Bensch, S., 2005. Dispersal increases local transmission of avian malarial parasites. *Ecol. Lett.* 8, 838–845.
- Perlut, N.G., Renfrew, R.B., 2016. Stopover on Galapagos during autumn migration of bobolinks (*Dolichonyx oryzivorus*). *Wilson J. Ornithol.* 128, 935–938.
- Ramey, A.M., Reed, J.A., Walther, P., Link, P., Schmutz, J.A., Douglas, D.C., Stalknecht, D.E., 2016. Evidence for the exchange of blood parasites between North America and the Neotropics in blue-winged teal (*Anas discors*). *Parasitol. Res.* 115, 3923–3939.
- Renfrew, R.B., Kim, D., Perlut, N.G., Fox, J., Marra, P.P., 2013. Phenological matching across hemispheres in a long-distance migratory bird. *Divers. Distrib.* 19, 1008–1019.
- Ricklefs, R.E., Medeiros, M., Ellis, V.A., Svensson-Coelho, M., Blake, J.G., Loiselle, B.A., Soares, L., Fecchio, A., Outlaw, D., Marra, P.P., Latta, S.C., Valkiūnas, G., Heggren, O., Bensch, S., 2016. Avian migration and the distribution of malaria parasites in New World passerine birds. *J. Biogeogr.* 44, 1113–1123.
- Risely, A., Klasseen, M., Hoyer, B.J., 2018. Migratory animals feel the cost of getting sick: a meta-analysis across species. *J. Anim. Ecol.* 87, 301–314.
- Sambrook, J., Fritsch, E.F., Maniatis, T., 1989. *Molecular Cloning: a Laboratory Manual*. Cold Spring Harbor Laboratory Press, New York.
- Sorensen, M.C., Asghar, M., Bensch, S., Fairhurst, G.D., Jenni-Eiermann, S., Spottiswoode, C.N., 2016. A rare study from the wintering grounds provides insight into the costs of malaria infection for migratory birds. *J. Avian Biol.* 47, 575–582.
- Valkiūnas, G., 2005. *Avian Malaria Parasites and Other Haemosporidia*. CRC press, Boca Raton.
- Waldenstrom, J., Bensch, S., Hasselquist, D., Ostman, O., 2004. A new nested polymerase chain reaction method very efficient in detecting Plasmodium and Haemoproteus infections from avian blood. *J. Parasitol.* 90, 191–194.
- Whiteman, N.K., Goodman, S.J., Sinclair, B.J., Walsh, T., Cunningham, A.A., Kramer, L.D., Parker, P.G., 2005. Establishment of the avian disease vector *Culex quinquefasciatus* say, 1823 (Diptera: Culicidae) on the Galapagos islands. *Ecuador. Ibis* 147, 844–847.