**Haemosporidian composition, network centrality and partner fidelity among resident and non-resident avian hosts**

Daniela de Angeli Dutra¹\*, Alan Fecchio², Érika Martins Braga³, Robert Poulin¹

danideangeli@live.com\* <https://orcid.org/0000-0003-2341-2035>

alanfecchio@gmail.com <https://orcid.org/0000-0002-7319-0234>

embraga@icb.ufmg.br <https://orcid.org/0000-0001-5550-7157>

robert.poulin@otago.ac.nz <https://orcid.org/0000-0003-1390-1206>

1.Department of Zoology, University of Otago, Dunedin, New Zealand

2.Programa de Pós-graduação em Ecologia e Conservação da Biodiversidade, Universidade Federal de Mato Grosso, Cuiabá, MT 78060-900, Brazil

3.Departamento de Parasitologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Brazil

**\*Correspondence:**

Daniela de Angeli Dutra

[danideangeli@live.com](mailto:danideangeli@live.com)

**Abstract:** Migration can modify interaction dynamics among parasites and their hosts with migrant hosts able to disperse parasites and impact local community transmission. Thus, studying the relationship among migratory hosts and their parasite is fundamental to elucidate how migration shapes interaction among hosts and their pathogens. Avian haemosporidian parasites are one of the the most prevalent, diverse, and important wildlife parasites being widely used as models in ecological and evolutionary research. Here, we evaluate parasite composition, network centrality and partner fidelity among resident and non-resident hosts using avian haemosporidians as study model. Aiming to evaluate parasite composition, we performed permutational multivariate analysis of variance to compare dissimilarity in parasite composition among hosts migratory categories. Additionally, we ran multilevel Bayesian models to access the role of migration determining centrality and partner fidelity in host-parasite networks of avian hosts and their respective haemosporidian parasites. We observed similar composition and partner fidelity among resident and non-resident hosts. Conversely, we demonstrate non-resident hosts possess a more central role in host-parasite networks than residents. However, when evaluating partial and full migratory hosts separately, we observed that only partial migratory species presented higher network centrality when compared to resident birds. Therefore, migration do not lead to differences for both parasite composition and partner fidelity. However, migratory behavior is positively associated to network centrality, indicating those individuals can possess more important roles shaping host-parasites interactions.

**Key words:** Haemoesporidians, Migratory Behavior, Parasite Composition, Partner Fidelity, Avian Malaria

**Introduction**

Migration, i.e. long distant and periodical roundtrip movement of animals between distinct habitats, can alter interaction dynamics among parasites and their hosts by serving as an escape mechanism for some pathogens but also increasing parasite prevalence and richness of certain other pathogens within migrant host species (Altizer et al. 2011; Satterfield et al. 2015; de Angeli Dutra et al. 2021a; Poulin and de Angeli Dutra 2021). Migratory behavior can also modify the availability of hosts for parasites throughout seasons since migrant specimens do not inhabit one same habitat year-round. At the same time, migrants can represent an opportunity for parasites to increase their distribution worldwide, as infected migrant individuals transport their pathogens through their routes and stopovers, therefore, proving new chances for host switching into new environments and resident species (de Angeli Dutra et al. 2021b; Poulin and de Angeli Dutra 2021). Indeed, the presence of migratory individuals can also affect local parasite transmission, altering parasite prevalence and richness also within resident host community (de Angeli Dutra et al. 2021b; Fecchio et al. 2021). However, despite the fact migration can modulate parasite-host interaction, only a few studies have addressed the implications of host migration on parasite ecology and evolution (Poulin and de Angeli Dutra 2021).

Thus, innate characteristics of host-parasite interactions could be altered by host migratory behavior, such as virulence (i.e. pathogenicity level) or partner fidelity, which is the species specificity in pairwise symbioses (e.g. host-parasite systems). Previous research suggest antagonistic interactions presents lower partner fidelity than mutualistic ones, indicating host-parasite systems are not evolutionarily malleable (Fortuna et al. 2020). Additionally, infecting migratory individuals can be a challenge to parasites since they need to adapt to novel resources and conditions which could lead to looser fidelity among parasites and their migrant hosts. For example, for malaria parasites infecting migratory birds to be transmitted into their hosts’ new habitats they must to be able to infect and develop their cycle into new vector species and distinct environmental characteristics (Valkiūnas 2005). Hence, the exposure of parasites to abrupt environmental and vector changes may impact the ecological and evolutionary relationship between parasites and their migratory hosts as it host migrations represent repeated, predictable, and directional selective pressures (Poulin and de Angeli Dutra 2021). So, it is fundamental to study how host shifts between migratory and residents hosts occurring in sympatry and under different environmental conditions can alter parasite-host dynamics. This in necessary to understand how parasite life-history traits evolve under repeated and predictable changes.

Avian haemosporidian parasites, i.e. malaria and malaria-like vector borne protozoan parasites, are one of the most prevalent, diverse and studied wildlife pathogens. These parasites are an excellent ecological and evolutionary model to study host-parasite relationship due to its high prevalence, diversity, cosmopolitan distribution and variable levels of specificity to their hosts (Valkiūnas 2005). This is particularly relevant for South America, which harbors the highest diversity of birds, vectors and haemosporidian parasites worldwide (Remsen et al.; Santiago-Alarcon et al. 2012; Ellis et al. 2019). This continent also contains great vector abundance and considerable haemosporidian prevalence (Braga et al. 2011; Santiago-Alarcon et al. 2012). Furthermore, avian community composition seems to impact parasite composition as well, with avian community turnover driving haemosporidian turnover in the Amazon region (De La Torre et al. 2021). All those features together make South America an ideal region to investigate ecological and evolutionary dynamics on avian haemosporidian interaction.

Therefore, studying the role of host migratory behavior on parasite composition, network centrality and partner fidelity is fundamental to understand the role of migration on life-history traits for parasites. Here, we hypothesize resident species possess higher partner fidelity to their parasites due to their higher environmental and vector stability. Additionally, since migrant harbors higher richness of haemosporidians (de Angeli Dutra et al. 2021a) and the reduced stability may favor the infection by generalist parasites, we also expect them to present higher centrality in our networks. Moreover, since migrants are exposed to more parasite lineages as migrants are present in regions that harbor different parasite communities our second hypothesis is that parasite composition varies among resident and migratory avian hosts species. In this research, we computed and compared partner fidelity and network centrality levels between haemosporidians and their resident and partial and full migratory avian hosts using Bayesian multilevel models. Further, using permutational multivariate analyses of variance (PERMANOVA) we evaluated whether resident and migratory hosts harbors similar haemosporidian composition.

**Methods**

*Dataset*

All analyses were performed using a dataset comprising ~15200 bird blood samples obtained from 974 avian species from 85 different localities comprising seven different South American biomes - Amazonia, Atlantic Rain Forest, Cerrado, Temperate Grassland, Caatinga, Pantanal and Andean Forest. The birds were sampled from 2005 to 2018, with a subset of those samples having previously been used in published research (Lacorte et al. 2013; Ferreira et al. 2017; Fecchio et al. 2018, 2019, 2020; Anjos et al. 2021) and the rest consisting of unpublished data (Fig. 1). This large dataset was combined with data available from MalAvi (<http://130.235.244.92/Malavi/>) and represents a total of 2758 sequenced parasites representing 752 distinct lineages, all belonging to one of three genera: *Plasmodium*, *Haemoproteus* and *Leucocytozoon.* Haemosporidian infection was estimated using PCR protocols described previously (Fallon et al., 2003; Hellgren et al., 2004; Bell et al., 2015). All lineages were identified by PCR protocols described by Hellgren et al. 2004 that amplifies a cytochrome b fragment of 478 base pars. Hosts were classified into three migratory categories: (1) resident; (2) partial migrant and (3) full migrant, according to the Brazilian Committee of Ornithology Records - CRBO 2014, Somenzari et al., 2018 and BirdLife International (<https://www.birdlife.org/>).

*Haemosporidian-Host Partner Fidelity and Network Centrality Analyses*

All analyses were conducted in R version 4.0 (R Core Team, 2019). For haemosporidian-bird partner fidelity analyses and network centrality, we considered only biomes with at least 10 distinct parasite lineages, which represents 249 distinct avian host species and 40 parasite lineages from five biomes – Amazonia, Andean Forest, Cerrado, Caatinga and Atlantic Rain Forest (Supplementary Table S2). We created incidence matrixes between avian host species and parasite lineages for each biome. Using “specieslevel” function from “bipartite” package (Dormann et al. 2008) in R, we computed normalized degree and closeness and weighted centrality values for parasites infecting birds in each biome. The first value represents the number of distinct realized interaction between hosts and parasites in each biome divided by the total number of distinct potential partners (i.e. parasites) in that same region. Normalized degree values can be employed as a measure of partner fidelity, with hosts presenting higher values less specific to their partners than hosts with lower values (Fortuna et al. 2020). On the other hand, weighted centrality is a measure of centrality in a network, which is calculated as the minimum sum of the paths between a species (i.e. hosts) and all their partners (i.e. parasites) in the network, with hosts presenting higher closeness values more central. We combined all biome values into one single dataset and ran Bayesian models to compare partner fidelity and network centrality among migratory categories.

In order to run our Bayesian analyses, we employed the function “brm” from “brms” package (Bürkner 2017). In the first model, we considered normalized degree as our depend variable and avian host migratory category (resident; partial migrant and full migrant, reference level = resident) as our independent variable and used biome as random effect. Likewise, for our second model we employed weighted closeness as our depend variable, avian host migratory category (resident; partial migrant and full migrant, reference level = resident) as our independent variable and again biome as random effect. We downloaded the full avian phylogeny file from the AllBirdsHackett1.tre website (<https://birdtree.org/>), selected only the species used for our analyses and created a matrix with phylogenetic distances between bird species. This matrix was also added to all our model as random effect to account for host phylogenetic influence on partner fidelity and network centrality. Priors were determined using “get\_prior” function. We ran the Bayesian models using 4 chains with 4000 total iterations per chain (2000 for warmup, 2000 for sampling) and employed zero-one inflated beta distribution, since normalized degree and weighted closeness represents a rate data. Further, we combined partial and full migrant into one single category and repeated our Bayesian analyses. Later, we applied “bip\_ggnet” function from “ggnet” package ([briatte.github.io/ggnet/](https://briatte.github.io/ggnet/)) to plot a bipartite net representing the relationship among haemosporidian lineages and avian hosts from different migratory categories.

*Haemosporidian Composition Analyses*

For haemosporidian composition analyses, we considered only localities with 10 or more individuals sampled and at least three distinct parasite lineages per biome and at least two distinct host migratory categories, which represents 2465 haemosporidian lineages from 485 avian species (Supplementary Table S1). We created an incidence matrix between biome and host migratory category and parasite lineages. Later, applying the function “vegdist” (method Bray) from “vegan” package in R (Dixon 2003), we calculated dissimilarity indices among migratory host categories. We, then, compared dissimilarity between parasite composition among migratory categories employing performs an Analyses of Variance like permutation test (PERMANOVA) for homogeneity of multivariate dispersions. For this, we employed “permutest” function also from “vegan” package with 999 permutations. Again, we combined partial and full migrant into one migratory category and repeated the analyses above. A non-metric multidimensional scaling analyses was plotted to visualize the dissimilarity on parasite composition among avian host migratory categories.

**Results**

Among the 249 avian species evaluated in the Bayesian analyses, 227 birds species were classified as resident whereas 16 and six were considered to be partial and full migratory species, respectively. In these analyses, we assessed 81 species from Amazonia, 89 from Andean Forest, 73 from Cerrado, 68 from Atlantic Rain forest and 34 from Caatinga. Our first Bayesian model revealed avian hosts present resembling normalized degree (i.e. partner fidelity) among host migratory categories (Table 1) with normalized degree values around 0.10 (Fig. 2). Likewise, no difference was observed for partner fidelity when evaluating resident versus non-resident (i.e. partial and full migratory hosts combined, Table 2).

For our next Bayesian models evaluating network centrality, we observed that non-resident avian hosts present higher weighted closeness (i.e. network centrality) than resident individuals (Fig. 3, Table 3). On the other hand, when evaluating full and partial migratory hosts separately, only partial migratory hosts present higher values of network centrality compared to residents (Table 4). In all our Bayesian models phylogeny and biome had significant effects on partner fidelity (Table 1-4). Similarly, our network plot demonstrate most hosts and parasites are found within one main component and that non-resident hosts are more centrally distributed in our parasite-host network system (Fig. 4). Moreover, we can also observe most parasites can infect multiple hosts while avian hosts seems mainly infected by one or a few distinct haemosporidian lineages.

Out of the 2465 haemosporidian lineages evaluated in our composition analyses, most lineages (N = 1544) represent *Plasmodium* parasites, followed by *Haemoproteus* parasites with 909, being 590 classified in the subgenera *Parahaemoproteus* and 319 in the subgenera *Haemoproteus*. Only 12 lineages of *Leucocytozoon* were assessed in these analyses. Additionally, most lineages were recovered from Amazonia (N = 638), Cerrado (N = 613) and Atlantic Rain Forest (N = 482). Similarly, we observed no difference on parasite composition among distinct migratory avian host categories when considering both resident versus partial and full migratory hosts separately (Fig. 5, Table 5) or combined (Table 6).

**Discussion**

**Acknowledgments**

We thank the MalAvi curators for maintaining the database and for making all data available, as well as all researchers who shared their data. We are also grateful to all funding agencies that made this research possible.

**Funding**

Daniela Dutra was supported by a doctoral scholarship from the University of Otago. During the project, Alan Fecchio was supported by a postdoctoral fellowship (PNPD scholarship) from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). Érika Braga was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

**Availability of data and material**

A part of the data that support the findings of this study is openly available at https://onlinelibrary.wiley.com/doi/10.1111/mec.15094 and http://130.235.244.92/Malavi/ (Bensch et al., 2009). The other portion of the data that support our findings can be shared by Prof. Érika Martins Braga under reasonable request.

**Authors’ contribution**

Daniela Dutra and Robert Poulin conceived the idea and designed the study. Daniela Dutra performed the data analyses. Daniela Dutra, Érika Braga and Alan Fecchio collected the data. Daniela Dutra wrote the manuscript with input from all other authors. All authors contributed critically to the drafts and gave final approval for publication.

**Reference**

Altizer S, Bartel R, Han BA (2011) Animal migration and infectious disease risk. Science (80- ) 331:296–302. https://doi.org/10.1126/science.1194694

Anjos CC, Chagas CRF, Fecchio A, et al (2021) Avian malaria and related parasites from resident and migratory birds in the brazilian atlantic forest, with description of a new Haemoproteus species. Pathogens 10:1–21. https://doi.org/10.3390/pathogens10020103

Bell JA, Weckstein JD, Fecchio A, Tkach V V. (2015) A new real-time PCR protocol for detection of avian haemosporidians. Parasites and Vectors 8:1–9. https://doi.org/10.1186/s13071-015-0993-0

Braga ÉM, Silveira P, Belo NO, Valkiunas G (2011) Recent advances in the study of avian malaria: An overview with an emphasis on the distribution of Plasmodium spp in Brazil. Mem Inst Oswaldo Cruz 106:3–11. https://doi.org/10.1590/S0074-02762011000900002

Bürkner PC (2017) brms: An R package for Bayesian multilevel models using Stan. J Stat Softw 80:27239. https://doi.org/10.18637/jss.v080.i01

de Angeli Dutra D, Fecchio A, Braga ÉM, Poulin R (2021a) Migratory birds have higher prevalence and richness of avian haemosporidian parasites than residents. Int J Parasitol 1–6. https://doi.org/10.1016/j.ijpara.2021.03.001

de Angeli Dutra D, Filion A, Fecchio A, et al (2021b) Migrant birds disperse haemosporidian parasites and affect their transmission in avian communities. Oikos. https://doi.org/10.1111/oik.08199

De La Torre GM, Campião KM, Bell JA, et al (2021) Avian community composition affects ornithophilic mosquito and avian malaria turnover across an interfluvial system in southern Amazonia. J Avian Biol 52:1–12. https://doi.org/10.1111/jav.02701

Dixon P (2003) Computer program review VEGAN , a package of R functions for community ecology. J Veg Sci 14:927–930

Dormann C, Gruber B, Fründ J (2008) Introducing the bipartite package: analysing ecological networks. Interaction 1:0.2413793

Ellis VA, Sari EHR, Rubenstein DR, et al (2019) The global biogeography of avian haemosporidian parasites is characterized by local diversification and intercontinental dispersal. Parasitology 146:213–219. https://doi.org/10.1017/S0031182018001130

Fallon SM, Ricklefs RE, Swanson BL, Bermingham E (2003) Detecting Avian Malaria: An Improved Polymerase Chain Reaction Diagnostic. J Parasitol 89:1044–1047. https://doi.org/10.1645/ge-3157

Fecchio A, Bell JA, Bosholn M, et al (2019) An inverse latitudinal gradient in infection probability and phylogenetic diversity for Leucocytozoon blood parasites in New World birds. J Anim Ecol. https://doi.org/10.1111/1365-2656.13117

Fecchio A, Bell JA, Collins MD, et al (2018) Diversification by host switching and dispersal shaped the diversity and distribution of avian malaria parasites in Amazonia. Oikos 127:1233–1242. https://doi.org/10.1111/oik.05115

Fecchio A, Chagas CRF, Bell JA, Kirchgatter K (2020) Evolutionary ecology, taxonomy, and systematics of avian malaria and related parasites. Acta Trop 204:105364. https://doi.org/10.1016/j.actatropica.2020.105364

Fecchio A, Lugarini C, Ferreira A, et al (2021) Migration and season explain tick prevalence in Brazilian birds. Med Vet Entomol. https://doi.org/10.1111/mve.12532

Ferreira FC, Rodrigues RA, Ellis VA, et al (2017) Habitat modification and seasonality influence avian haemosporidian parasite distributions in southeastern Brazil. PLoS One 12:0178791. https://doi.org/10.1371/journal.pone.0178791

Fortuna MA, Nagavci A, Barbour MA, Bascompte J (2020) Partner fidelity and asymmetric specialization in ecological networks. Am Nat 196:382–389. https://doi.org/10.1086/709961

Hellgren O, Waldenstro J, Bensch S (2004) A New Pcr Assay For Simultaneous Studies Of Leucocytozoon, Plasmodium, And Haemoproteusfrom Avian Blood. J Parasitol 90:797–802. https://doi.org/10.1645/GE-184R1

Lacorte GA, Flix GMF, Pinheiro RRB, et al (2013) Exploring the Diversity and Distribution of Neotropical Avian Malaria Parasites - A Molecular Survey from Southeast Brazil. PLoS One 8:1–9. https://doi.org/10.1371/journal.pone.0057770

Poulin R, de Angeli Dutra D (2021) Animal migrations and parasitism: reciprocal effects within a unified framework. Biol Rev 10.1111/br:

Remsen JVJ, Areta JI, Bonaccorso E, et al A classification of the bird species of South America. In: Am. Ornithol. Soc. http://www.museum.lsu.edu/~Remsen/SACCBaseline.htm

Santiago-Alarcon D, Palinauskas V, Schaefer HM (2012) Diptera vectors of avian Haemosporidian parasites: Untangling parasite life cycles and their taxonomy. Biol Rev 87:928–964. https://doi.org/10.1111/j.1469-185X.2012.00234.x

Satterfield DA, Maerz JC, Altizer S (2015) Loss of migratory behaviour increases infection risk for a butterfly host. Proc R Soc B Biol Sci 282:20141734. https://doi.org/10.1098/rspb.2014.1734

Somenzari M, Amaral PP do, Cueto VR, et al (2018) An overview of migratory birds in Brazil. Pap Avulsos Zool 58:3. https://doi.org/10.11606/1807-0205/2018.58.03

Valkiūnas G (2005) Avian Malaria Parasites and other Haemosporidia

Fig. 1: Parasite lineage localities. Collection localities comprise a total of 66 localities by combining our dataset and the MalAvi database.

Fig. 2: Mean (±credible intervals) normalized degree avian hosts possess according to the migratory category in which they are classified. R = resident, M = full migrant, PM = partial migrant.

Fig. 3: Mean (±credible intervals) weighted centrality avian hosts possess according to the migratory category in which they are classified. R = resident, M = full migrant, PM = partial migrant.

Fig. 4: Network representing avian-haemosporidian interactions. Distinct colors represent avian hosts from distinct migratory categories or parasites. Circles represent avian hosts while triangles haemosporidian parasites.

Fig. 5: Non-metric multidimensional scaling plot illustrating the dissimilarity on parasite composition among avian host migratory categories. R = resident, M = full migrant, PM = partial migrant.

Table 1: Parameter estimates, standard errors, and credible intervals for the Bayesian model testing the differences in partner fidelity of haemosporidian parasites among avian host from distinct migratory categories. (Residents only = reference category)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Estimate** | **Std. error** | **Cred. Inter (95%)** | |
| Intercept | -2.27 | 0.16 | -2.59 | -1.94 |
| Full migratory host species | 0.12 | 0.17 | -0.23 | 0.45 |
| Partial migratory host species | 0.06 | 0.11 | -0.15 | 0.27 |
| Biomes | 0.28 | 0.17 | 0.09 | 0.76 |
| Avian host phylogeny | 0.08 | 0.06 | 0.00 | 0.22 |

Table 2: Parameter estimates, standard errors, and credible intervals for the Bayesian model testing the differences in partner fidelity of haemosporidian parasites among avian host from distinct migratory categories. (Residents only = reference category)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Estimate** | **Std. error** | **Cred. Inter (95%)** | |
| Intercept | -2.28 | 0.16 | -2.60 | -1.94 |
| Non-resident host species | 0.08 | 0.09 | -0.11 | 0.26 |
| Biomes | 0.28 | 0.18 | 0.09 | 0.78 |
| Avian host phylogeny | 0.08 | 0.06 | 0.00 | 0.21 |

Table 3: Parameter estimates, standard errors, and credible intervals for the Bayesian model testing the differences in network centrality of haemosporidian parasites among avian host from distinct migratory categories. (Residents only = reference category)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Estimate** | **Std. error** | **Cred. Inter (95%)** | |
| Intercept | -4.48 | 0.31 | -5.50 | -4.23 |
| Non-resident host species | 0.30 | 0.10 | 0.10 | 0.49 |
| Biomes | 0.57 | 0.30 | 0.24 | 1.39 |
| Avian host phylogeny | 0.32 | 0.09 | 0.16 | 0.50 |

Table 4: Parameter estimates, standard errors, and credible intervals for the Bayesian model testing the differences in network centrality of haemosporidian parasites among avian host from distinct migratory categories. (Residents only = reference category)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Estimate** | **Std. error** | **Cred. Inter (95%)** | |
| Intercept | -4.87 | 0.32 | -5.45 | -4.17 |
| Full migratory host species | 0.31 | 0.18 | -0.06 | 0.65 |
| Partial migratory host species | 0.29 | 0.12 | 0.06 | 0.53 |
| Biomes | 0.56 | 0.29 | 0.23 | 1.34 |
| Avian host phylogeny | 0.32 | 0.09 | 0.16 | 0.50 |

Table 5: Degrees of freedom, sum and mean square and F and P value for permutational multivariate analysis of variance (PERMANOVA) testing the difference in parasite composition among avian host from distinct migratory categories. (Residents only = reference category)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **Degrees of Freedom** | **Sum Square** | **Mean Square** | **F value** | **P value** |
| Groups | 2 | 0.003365 | 0.0016823 | 0.783 | 0.46 |
| Residuals | 16 | 0.034376 | 0.0021485 |  |  |

Table 6: Degrees of freedom, sum and mean square and F and P value for permutational multivariate analysis of variance (PERMANOVA) testing the difference in parasite composition among resident and non-resident avian species. (Residents only = reference category)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **Degrees of Freedom** | **Sum Square** | **Mean Square** | **F value** | **P value** |
| Groups | 1 | 0.000212 | 0.000212 | 0.0745 | 0.79 |
| Residuals | 12 | 0.034095 | 0.002841 |  |  |