**Determinants of haemosporidian single- and co-infection risks in western Palearctic birds**

Romain Pigeaulta,b,1,\*, Mathieu Chevaliera,c,1, Camille-Sophie Cozzaroloa,d, Molly Baura, Mathilde Arlettaza, Alice Ciboise, André Keiserf, Antoine Guisana, Philippe Christea,2, Olivier Glaizota,f,2

a *Department of Ecology and Evolution, CH-1015 Lausanne, Switzerland*

b *Laboratoire EBI, Equipe EES, UMR CNRS 7267, 86000 Poitiers, France*

c *Ifremer, Centre de Bretagne, DYNECO-LEBCO, CS 10070, 29280 Plouzané, France*

d *Biogéosciences, UMR 6282 CNRS, université Bourgogne Franche-Comté, 6 boulevard Gabriel, 21000 Dijon, France*

e *Natural History Museum of Geneva, C.P. 6434, CH-1211 Genève 6, Switzerland*

f *Musée cantonal de zoologie, CH-1014 Lausanne, Switzerland*

1 These authors contributed equally to this work

2These authors share senior authorship

\*Corresponding author. Romain Pigeault.

Tel.: +33 (0)5 49 45 37 30; Fax: +33 (0)5 49 45 40 15.

*E-mail address*: romain.pigeault@univ-poitiers.fr

**Supplementary Data S1.**

**1. Parasite detection**

For each individual, haemosporidian parasites (i.e. *Haemoproteus, Leucocytozoon* and *Plasmodium*) were detected from tissue samples using molecular methods. Specifically, a nested PCR targeting a fragment of cytochrome b gene of the parasite mitochondrial genome (Hellgren et al., 2004) was performed in triplicate on all samples after DNA was extracted from tissues using a DNeasy Blood & Tissue Kit (Qiagen, Switzerland) following the manufacturer's instructions. Nested PCR products were visualized on agarose gels after electrophoresis to identify infected samples. This nested PCR protocol does not allow discrimination between *Plasmodium* and *Haemoproteus* infections, nor detection of co-infections with these two genera of parasites. Therefore, all positive samples were sequenced in both directions as in Rooyen et al. (2013) and parasite genera were then identified by performing a local BLAST search in the MalAvi database (http://mbio-serv2.mbioekol.lu.se/Malavi/, (Bensch et al., 2009)). Eighteen samples positive to *Plasmodium* and/or *Haemoproteus* infection showed double nucleotide peaks on the sequence chromatographs and were manually analyzed to discern mixed lineage infection (i.e. concurrent infection with parasites from more than one lineage of the same genus) or co-infection by *Haemoproteus* and *Plasmodium* (i.e. mixed-genera infection). We re-amplified and re-sequenced all samples for which the chromatograph could not reliably identify the parasite sequences. Birds not infected by any parasites were classified as “not infected”, birds infected with a single parasite genus were classified as “single infection” and those infected with at least two different genera as “mixed-genera infection” (Pigeault et al., 2018).

**2. Species attributes**

*2.1. Species position along the slow-fast continuum of life-history variation*

Species position along the slow-fast continuum of life-history variation was represented by the first axis of a principal component analysis (PCA) performed on nine variables describing bird reproductive traits (clutch size, number of broods per year, average length, width and weight of the egg, incubation period, fledging age, age at first breeding and maximum lifespan (Supplementary Table S1)). The first axis explained 62.7% of the variability and represented a gradient from fast (negative values) to slow (positive values) life-history strategies. The variables that contributed most to the construction of this axis were the average length, width and weight of the egg (contributions of the variables: 16.57%, 16.61%, 14.12%, respectively), the incubation period (14.49%), the fledging age (14.09%) and the age at first breeding (11.29%).

*2.2. Climatic niche breadth and climatic niche position*

Kernel density estimation (KDE) has proved useful to characterize complex and potentially irregular shapes (Blonder et al., 2014) and is increasingly used to characterize climatic niches (e.g. Broennimann et al., 2012). For each species, the bandwidth of the KDE was estimated from the data using a multivariate generalization of the plug-in bandwidth selector (Hpi; Wand and Jones, 1994). Envelopes were then defined as the minimum threshold of probability density that included 99% of points (to leave out environmentally atypical occurrences). From species climatic envelopes, we extracted the niche area (i.e. an estimate of niche breadth) and computed the niche centroid as the mean of point coordinates falling inside the delimited niche. We then extracted the coordinates of the centroid on each of the two PCA axes (Fig. 1 in the main text).

Subsequently, we also used two other algorithms to delineate the niches realized by the species: convex hulls (Chull) and alpha hulls (Ahull) to test if we obtained similar results (see Supplementary Figs. S2, S3). Chull are defined as the smallest convex set that contains all samples. Ahull and KDE are extensions of Chull where non-convex and irregular shapes are allowed. While Chull are sensitive to outliers, Ahull and KDE are sensitive to the density of points in the environmental space. Chull is parameter-free whereas Ahull and KDE depend on a parameter that controls the shape of the envelope (the bandwidth; see Section 2.2.2 in the main text). For the Ahull, we adopted an iterative procedure starting with an alpha value of one and then incrementing the value of the parameter by steps of 0.2 until 99% of points were included in the envelope.

**References**

Bensch, S., Hellgren, O., Pérez-Tris, J., 2009. MalAvi: A public database of malaria parasites and related haemosporidians in avian hosts based on mitochondrial cytochrome b lineages. Mol. Ecol. Res., 9(5), 1353–1358. https://doi.org/10.1111/j.1755-0998.2009.02692.x

Blonder, B., Lamanna, C., Violle, C., Enquist, B. J., 2014. The n-dimensional hypervolume. Global Ecol. Biogeograph., 23(5), 595–609. https://doi.org/10.1111/geb.12146

Broennimann, O., Fitzpatrick, M. C., Pearman, P. B., Petitpierre, B., Pellissier, L., Yoccoz, N. G., Thuiller, W., Fortin, M.-J., Randin, C., Zimmermann, N. E., Graham, C. H., Guisan, A., 2012. Measuring ecological niche overlap from occurrence and spatial environmental data. Global Ecol. Biogeograph., 21(4), 481–497. https://doi.org/10.1111/j.1466-8238.2011.00698.x

Hellgren, O., Waldenström, J., Bensch, S., 2004. A new pcr assay for simultaneous studies of *Leucocytozoon*, *Plasmodium*, and *Haemoproteus* from avian blood. J. Parasitol., 90(4), 797–802. https://doi.org/10.1645/GE-184R1

Pigeault, R., Cozzarolo, C.-S., Choquet, R., Strehler, M., Jenkins, T., Delhaye, J., Bovet, L., Wassef, J., Glaizot, O., Christe, P., 2018. Haemosporidian infection and co-infection affect host survival and reproduction in wild populations of great tits. Int. J. Parasitol., 48(14), 1079–1087. https://doi.org/10.1016/j.ijpara.2018.06.007

Rooyen, J. van, Lalubin, F., Glaizot, O., Christe, P., 2013. Avian haemosporidian persistence and co-infection in great tits at the individual level. Malaria J., 12(1), 40. https://doi.org/10.1186/1475-2875-12-40

Wand, M. P., Jones, M. C., 1994. Kernel Smoothing. CRC Press, New York.