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Does the niche breadth or trade-off hypothesis explain the abundance–occupancy relationship in avian Haemosporidia?

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Abstract

Two hypotheses have been proposed to explain the abundance–occupancy relationship (AOR) in parasites. The niche breadth hypothesis suggests that host generalists are more abundant and efficient at colonizing different host communities than specialists. The trade-off hypothesis argues that host specialists achieve high density across their hosts' ranges, whereas generalists incur the high cost of adaptation to diverse immuno-defence systems. We tested these hypotheses using 386 haemosporidian cytochrome-*b* lineages (1894 sequences) recovered from 2318 birds of 103 species sampled in NW Africa, NW Iberia, W Greater Caucasus and Transcaucasia. The number of regions occupied by lineages was associated with their frequency suggesting the presence of AOR in avian Haemosporidia. However, neither hypothesis provided a better explanation for the AOR. Although the host generalist Plasmodium SGS1 was over three times more abundant than other widespread lineages, both host specialists and generalists were successful in colonizing all study regions and achieved high overall prevalence.

Keywords: abundance–occupancy relationship, blood parasites, haemosporidia, host specialization, host–parasite interactions, niche breadth, trade-off

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Introduction

The positive abundance–occupancy relationship (AOR) is one of the most general ecological phenomena (Gaston 1996; Gaston *et al.* 2000). This relationship results from locally common species often being widespread and locally rare species usually having small ranges. It appears robust at intraspecific (temporal dynamics of population density and range size of a single species) and interspecific (comparison of population densities and range sizes across species) levels and at different geographical scales and regions, irrespective of varying methodologies used to estimate species abundance (Gaston *et al.* 2000). Positive AOR has been documented in a large variety of free-living organisms (Gaston 1996; Gaston *et al.* 2000). Although much less attention has been given to AOR in parasitic animals, most studies also support it, for example (Barger & Esch 2002; Krasnov *et al.* 2004; Jenkins & Owens 2011; Poulin *et al.* 2012; Thieltges *et al.* 2013), with some, however, showing the lack of AOR in parasites (Morand & Guégan 2000).

Two hypotheses have been proposed to explain the evolutionary mechanisms driving the AOR in parasitic

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animals. The niche breadth hypothesis (NBH) (Brown 1984), when applied to parasitic animals, suggests that parasites with the ability to infect multiple host species are more abundant and efficient at colonizing different host communities and thus acquire larger ranges and abundance than host-specific parasites (Krasnov *et al.* 2004; Hellgren *et al.* 2009). In contrast, the trade-off hypothesis (TOH) suggests that due to the high cost of adaptation to diverse defence mechanisms used by different host species, host generalists parasitize each of their individual host species much less efficiently than host-specific parasites. By extension, host-specific parasites need only adapt to a single host species' defence system and can thus achieve high prevalence across the entire range of that single host (Poulin 1998).

Both hypotheses appear to be supported by empirical data. However, the support for one hypothesis over the other across relevant studies may not be rooted in the underlying mechanisms driving AOR in parasitic animals, but rather in different analytical approaches utilized. For example, when a parasite's niche breadth is estimated on the basis of the number of host species, inclusive of the host specificity index and its variance (Poulin & Mouillot 2003), the parasite abundance is positively correlated with the niche breadth supporting NBH (Krasnov *et al.* 2004; Hellgren *et al.* 2009). In contrast, when prevalence or parasitaemia of a parasite is concerned, they appear to be positively correlated with the degree of its host specialization challenging NBH, but being consistent with TOH (Poulin 1998). None of these studies, however, address both the abundance of parasites and their prevalence in individual host species in relation to the degree of parasites' host specialization.

In this study, we adopt a novel approach that accounts for both the number of host species used by a parasite lineage and its prevalence in each host species to test competing hypotheses attempting to explain AOR in avian haemosporidian parasites. We use a data set containing 386 avian haemosporidian lineages of *Haemoproteus* (187), *Leucocytozoon* (148) and *Plasmodium* (51) identified among 1894 individual haemosporidian mtDNA cytochrome-*b* (cyt-*b*) sequences. First, we assess the AOR by testing whether the number of regions occupied by parasite lineages is related to their abundance (number of birds infected by a lineage) in our data set and identify the most abundant and geographically wide-ranging haemosporidian lineages. Then, we identify the number of host species utilized by these lineages and their prevalence in each host species to elucidate whether abundant and widespread avian haemosporidian parasites are host generalists as predicted by NBH or host specialists as predicted by TOH.

Materials and methods

We obtained blood (73%), heart or liver (27%) samples from 2318 individual birds of 103 species (Appendix S1, Supporting information) sampled in four geographically and faunistically distinct regions: northwest Africa (NWA; 318 birds of 43 species), northwestern Iberia (NWI; 708 birds of 46 species), western Greater Caucasus (WGS; 489 birds of 30 species) and Transcaucasia (TRC; 803 birds of 65 species; Fig. 1). Three-quarters of the samples (75%) in each region were collected in May in NWA, during April–September in NWI, June–July in WGC and May–July in TRC (Appendix S2, Supporting information). Although 30 additional species (99 samples) were also screened for haemosporidian parasites, we excluded them from the analyses because there were no infected individuals among them (Appendix S3, Supporting information). The Latin names of avian host species follow the Clements Checklist of Birds of the World: Version 6.8 (Clements *et al.* 2013).

There were no significant differences in the proportion of infected birds detected using blood or tissue samples in 13 of 14 species for which ≥10 samples of each kind were used in this study (Appendix S4, Supporting information). Only in a single species (*Sylvia atricapilla*), the prevalence of haemosporidia appears to be significantly higher among blood than tissue samples. This, however, could be a artefact of sampling across biogeographical regions and years.

Only local birds (resident birds or breeding adults and juveniles of migratory species) sampled over multiple years, and localities within geographical regions were used in this study. In all localities, birds were captured with 15 m × 2.5 m nylon mist nets with 16 mm mesh size (Ecotone, Gdynia, Poland). Each mist net was opened for 2–3 days in the same place from 1 h before sunrise to 1 h after sunset and then moved to a new location. Birds were sampled in the forest and brush

Fig. 1 Sampling regions and years, number of host species and individual birds sampled for this study. Region abbreviations: NWA, northwest Africa, NWI, northwest Iberia, WGA, western Greater Caucasus and TRC, Transcaucasia.

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habitats below and above timberline primarily targeting forest avian communities. To mitigate spatial, temporal and habitat variation in parasite and host communities, regions were sampled during three or more years at randomly chosen localities across each region within the target habitat. Blood was sampled by brachial venipuncture with a sterile needle and collected into a heparin-free glass capillary. Heart and liver samples were taken during specimen preparation. All samples were immediately transferred into 2 ml vials with 96% ethanol and stored until DNA extraction.

Total DNA was extracted from avian blood or tissue samples preserved in 96% ethanol using the JETQUICK Tissue DNA Spin Kit (Genomed, Löhne, Germany) or using the E.Z.N.A. Tissue DNA Kit (Omega Biotek, Norcross, Georgia, USA) according to the manufacturer's protocol. To test DNA samples for presence of haemosporidian parasites, we conducted PCRs targeting a fragment of the mtDNA cytochrome-*b* gene (cyt-*b*). Each DNA sample was PCR tested with three primer pairs. Each pair used the same forward primer (UNIVF 5′-CAYATAYTAAGAGAAYTATGGAG-3′; sites 187–209 from the 5′ end of cyt-*b*) and one of three different reverse primers: UNIVR1 (5′-GCATTATATCWGGATGWGNTAATGG-3′; sites 715–739), UNIVR2 (5′-ARAGGAGTARCATATCTATCWAC-3′; sites 745–768) or UNIVR3 (5′-ATAGAAAGMYAAGAAATACCATTC-3′, sites 781–804). S.V.D. designed these primers by incorporating 2–3 degenerative sites into standard MalAvi primers (HAEMNF, HAEMR2 without last T, HAEMNR2, and HAEMNR3, respectively) to increase the overall diversity of avian haemosporidian haplotypes among those available in GenBank while producing a PCR product completely overlapping the 479 bp (sites 235–713) fragment of avian haemosporidian cyt-*b* adopted as a lineage identification standard for the MalAvi database (Bensch *et al.* 2009). The three primer pairs were necessary because neither individual primer pair is capable of targeting of the entire known haplotype diversity.

Each sample was screened with the three primer pairs. If a DNA sample was negative in the specific primer-pair PCR screening, it was repeated once for NWA and NWI samples, and up to two times (if the first repeat was also negative) for WGC and TRC samples to ensure that negative samples were truly negative.

PCRs were conducted in a total volume of 12.5 μl and contained 1x GoTaq Flexi buffer, 2 mM $MgCl_2$, 0.2 mM of each dNTP, 0.3 mM of each primer and 0.313u of GoTaq Flexi DNA polymerase (Promega Madison, Wisconsin, USA), and 2 μl of DNA template. The PCR profile was the same for all primer pairs. It started with 3 min of denaturation at 94 °C, followed by 41 cycles of denaturation at 94 °C for 30 s, annealing at 52 °C for 30 s and extension at 72 °C for 45 s. The PCR ended with 10 min of elongation at 72 °C.

PCR products were purified using ExoSAP (United States Biochemical Corporation, Cleveland, Ohio) according to the manufacturer's protocol and sequenced directly on an ABI 3730 Genetic Analyzer (Applied Biosystems Inc., Foster City, CA, USA) at the Macrogen Europe (Netherlands) or University of Florida ICBR facilities. PCR fragments were sequenced in both directions if they were positive for UNIVF-UNIVR1 primer pair and only with UNIVR2 or UNIVR3 for their respective pairs to ensure complete coverage of the 505 bp region between primers UNIVF and UNIVR1. Sequences were aligned using Sequencher 5.0.1 (Gene Codes, Ann Arbor, Michigan) and trimmed to 505 bp length.

Multiple infections present in a single PCR were resolved by employing several approaches. If the other primer pair(s) produced an unambiguous sequence identical to the one present in the multiple-infection PCR (MIP) of the same sample, this unambiguous sequence was subtracted to reveal the remaining sequence in the MIP. In some cases, the height of the peaks in the MIP's chromatogram was consistently and significantly different along the entire sequence length, which also allowed us to resolve multiple infections. If the peaks had a similar height and there were no unambiguous sequences available for a particular sample with the MIP, we aligned the MIP with all unambiguous sequences available and eliminated, one by one, sequences that had differences with the MIP in positions that did not contain double peaks or if the state in a double peak site was different from those present in the MIP. After this consecutive elimination, we were left with fragments whose consensus produced the same pattern of double peaks as the one we observed in the MIP. Therefore, we considered the MIP as being composed of these remaining lineages. In all but two samples with MIPs, we were able to resolve all infections using a combination of these approaches, including a few cases when a primer pair amplified three different haplotypes. All the sequences were checked for indels, stop codons and nucleotide composition to make sure that the functional copy of cyt-*b* was amplified. All haplotypes found only in multiple infections and that were not present in GenBank or MalAvi were double-checked to assure that they were indeed new lineages and not misreads of the MIPs.

Unique haplotypes were identified from the individual sequences in DnaSP 5.10.00 (Librado & Rozas 2009) and compared with GenBank and MalAvi databases to identify whether they match known parasite lineages and morphospecies, and to characterize their geographical distribution.

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We employed the following terminology in this study. Abundance of the host species refers to the sample size of each host species across all four study regions in our data set. Abundance of the parasite lineage refers to the number of birds parasitized by each lineage in our data set. We assign the 13 most abundant parasite lineages in our data set (see Results) to two host specialization categories: host specialists and host generalists. Lineages that parasitized primarily a single host species, but were detected in a single or very few individuals of a few other host species, were considered host specialists. Lineages that infected several or more individuals in several host species were considered host generalists.

The similarity of regional host and parasite samples was assessed by calculation of pairwise Dice's indices (Dice 1945) modified to use abundance instead of presence/absence of species (MDI). In the Dice's index formula 2C/(A+B), C is the sum of shared individuals for each host species sampled in the two regions being compared, and A and B are the total numbers of individuals sampled across all host species in each of the two regions, respectively. The correlation between matrices of complement MDI values (1-MDI) was assessed using Mantel's test (Mantel 1967) with 100 000 permutations using the ade4 package in R.

Generalized linear models were built using CurveExpert Professional v.1.6.8 (available at http://www.curveexpert.net/) with model fit assessed by the amount of variance explained. Models were compared using Akaike information criterion – AIC (Akaike 1974). Nonparametric statistics, necessary due to discrete or nonnormally distributed data, were conducted in R v.3.0.1 (available at http://www.R-project.org). Spearman's rank correlation was used to test relationships among sets of variables. For testing AOR, the number of times a parasite lineage was observed in our data set was correlated with the number of occupied biogeographical regions. For this test, we omitted lineages observed fewer than four times because their samples size was lower than the number of regions. The relationship between lineage abundance and host specialization was assessed using the Mann–Whitney *U*-test. The number of generalists and specialists were compared with that expected at random using Fisher's exact test.

Due to the different number of individuals examined for different host species, a negative binomial regression model was employed to examine differences in lineage prevalence between host specialists vs. generalist (Agresti 2002). Model fit was assessed by testing the deviance compared with its asymptotic chi-square distribution with nonsignificant results indicating reasonable fit. The negative binomial regression was conducted by PROC GENMOD in SAS version 9.2 (SAS Institute Inc., Cary, NC). Statistical significance was defined as a test resulting in a P-value <0.05.

When our lineages completely matched the sequences available in MalAvi database (Bensch *et al.* 2009), we provided MalAvi names in parentheses behind our lineage IDs. For example, 'H077 (*Leucocytozoon* PARUS19)' indicates that our lineage identified as H077 completely matched the shorter sequence in the MalAvi database identified as PARUS19, which is a *Leucocytozoon*. We refrained from using only MalAvi names because our fragment is 26 bp longer than MalAvi sequences, and on several occasions, two of our haplotypes matched the same MalAvi sequence but were treated as different in this study.

Results

Our regional host samples (Fig. 1) differed in their composition (Appendix S1, Supporting information). The greatest difference was between NWA and TRC (MDI = 0.143), whereas WGR and TRC were the most similar (MDI = 0.421; Table 1). A similar pattern, albeit with slightly less variance, was observed for parasite MDI values, which varied between 0.203 for NWA and TRC and 0.329 for NWA and NWI (Table 1). The compliment MDI values for host and parasite comparisons were correlated ($r = 0.835$, Mantel's test $P = 0.042$). These results suggest relatively low similarity of our regional samples in both host species and haemosporidia composition and representation.

The number of individual birds parasitized by different lineages varied between 1 and 207. Most lineages (208 of 386) were detected only once, and the frequency of lineages declined proportionally to their sample size to the power −1.9 ($y = 207.134x^{-1.859}$, $R^2 = 0.998$, d.f. = 34, $P < 0.0001$; Fig. 2). The number of individuals infected by Haemosporidia per host species increased with the host species sample size ($y = -1.844 + 0.611x$, $R^2 = 0.793$, d.f. = 101, $P < 0.001$); however, a power curve ($y = 0.039x^{1.578}$, $R^2 = 0.841$, d.f. = 101, $P < 0.001$) provided a significantly better fit ($\Delta_{AICC} = 26.754$ $P < 0.001$) for this relationship than a linear regression, suggesting that haemosporidians parasitized a higher proportion of

Table 1 MDI values for regional host (above diagonal) and parasite (below diagonal) samples

Region	NWA	NWI	GWC	TRC
NWA		0.357	0.188	0.143
NWI	0.329		0.339	0.328
GWC	0.220	0.252		0.421
TRC	0.203	0.236	0.315	

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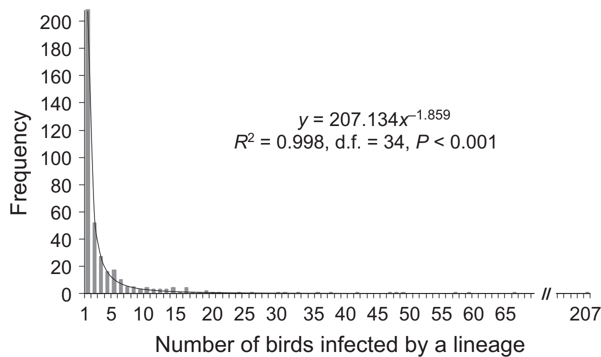

Fig. 2 Distribution of number of individual birds infected by a single haemosporidian lineage.

individuals in abundant host species than in less common ones (Fig. 3).

The number of regions occupied by parasite lineages was associated with their abundance, suggesting that differences in host composition or sampling stochasticity had little effect on AOR (Fig. 4). This association was significant for each of the three haemosporidian genera individually (*Haemoproteus*: Spearman's rho = 0.639, $n = 51$, $P < 0.0001$; *Leucocytozoon*: rho = 0.608, $n = 36$, $P = 0.0002$; *Plasmodium*: rho = 0.603, $n = 12$, $P = 0.037$) and for all three genera combined (rho = 0.622, $n = 99$, $P < 0.0001$). Only four of 360 lineages observed ≤24 times were found in all four regions, whereas 11 of 13 lineages observed ≥30 times were found in all four regions and two were found in three regions.

One of the two common and widespread lineages that were found only in three of the four regions, H077 (*Leucocytozoon* PARUS19), was not observed in our NWI samples, although it has been reported from both Portugal and Spain (Jenkins & Owens 2011). The other of these two lineages, H030 (*Haemoproteus* CCF6), was not found in WGC. Although we are not aware of other studies of avian Haemosporidia in the Greater Caucasus, CCF6 has been found to the west and east of WGC, in Bulgaria (Dimitrov *et al*. 2010) and in the Ural Mountains (Palinauskas *et al*. 2013), respectively. Therefore, the presence of PARUS19 has been confirmed in all four study regions, and the occurrence of CCF6 in all four regions is likely. Sampling stochasticity, perhaps, is responsible for our failure to discover them in all four regions.

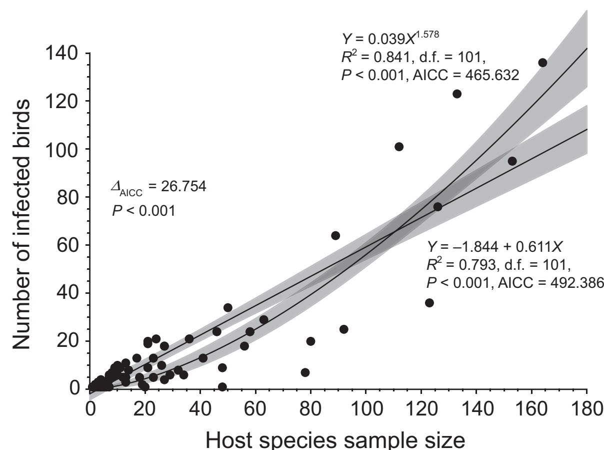

Fig. 3 The relationship between number of individual birds in the host species infected by haemosporidian parasites and the sample size of the host species.

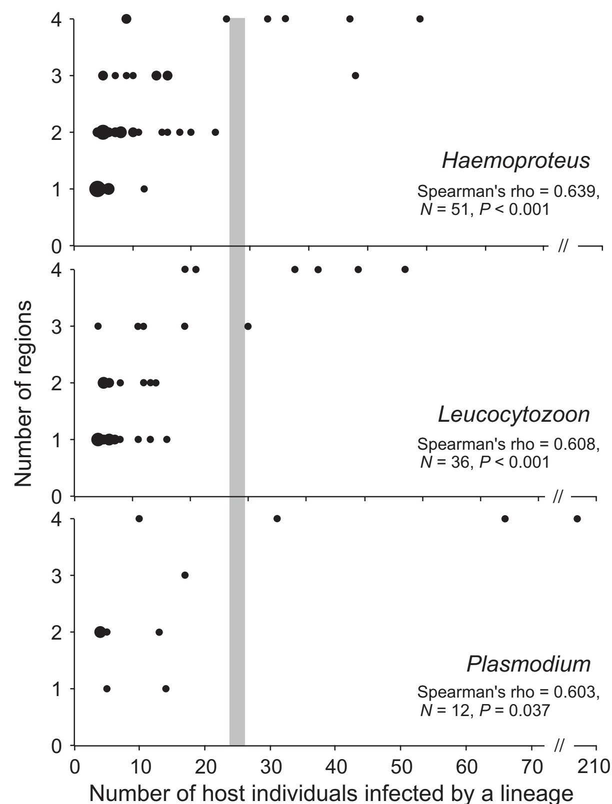

Fig. 4 Number of regions occupied by lineages of *Haemoproteus* (top), *Leucocytozoon* (middle) and *Plasmodium* (bottom) vs. the number of individual birds infected by them. Circle size is proportional to the number of overlapping data points.

There was little bias in the representation of different haemosporidian genera among the 13 most common and widespread lineages. More diverse genera *Haemoproteus* (187 lineages) and *Leycocytoozon* (148 lineages) each had five lineages in this category, and less diverse *Plasmodium* (51 lineages) had 3 (Fig. 4).

The number of host species parasitized by the 13 abundant and widespread lineages varied from two to 40, suggesting a great deal of variation in host specialization among these lineages. Although all 13 lineages were found in at least two host species, eight of them: H029 (*Haemoproteus* TURDUS2, $n = 47$), H030 (*Haemoproteus*

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CCF6, n = 48), H032 (*Haemoproteus* CCF2, n = 36), H105 (*Haemoproteus* SYAT01, n = 33), H065 (*Leucocytozoon* PARUS22, n = 57), H061 (*Leucocytozoon* PARUS4, n = 49), H073 (*Leucocytozoon* PARUS16, n = 38) and H235 (*Plasmodium* SYAT05, n = 66) parasitized primarily a single host species and were detected only in a single or very few individuals of a few other host species (Fig. 5). In contrast, five lineages: H022 (*Haemoproteus* PARUS1, n = 59), H027 (*Leucocytozoon* SFC8, n = 42), H077 (*Leucocytozoon* PARUS19, n = 30), H011 (*Plasmodium* SGS1, n = 207) and H010 (*Plasmodium* GRW11, n = 31) infected multiple individuals in multiple host species (Fig. 5). Therefore, among the 13 abundant and widespread lineages, eight were associated primarily with a single host (host specialists) and five were true host generalists. This observed ratio did not differ significantly from the expected ratio under the assumption of no relationship between host specialization and parasite's abundance and geography (observed ratio 8:5 vs. no relationship 7:7; Fisher's exact P = 0.704).

Lineage sample size (the number of times a lineage was observed in our data set) was not affected by its host

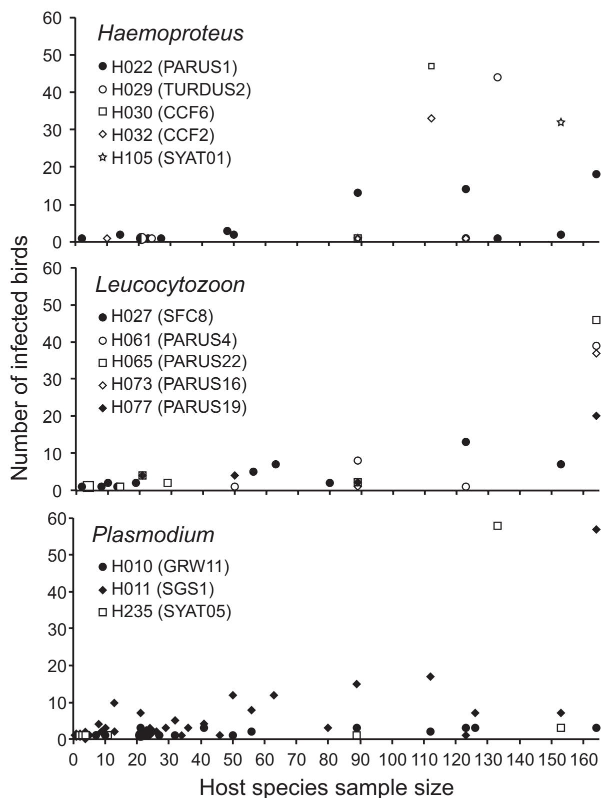

Fig. 5 Number of individual birds per host species infected by the 13 most abundant and widespread lineages of *Haemoproteus* (top), *Leucocytozoon* (middle) and *Plasmodium* (bottom) vs. host species sample size. Black filled symbols identify host generalists; open symbols identity host specialists. Individual lineages identified by a combination of symbol colour and shape.

specialization (Mann–Whitney U = 18, P = 0.833), suggesting that neither host specialization strategy allows a parasite lineage to become more abundant than lineages employing an alternative strategy.

The maximum host-specific prevalence (the prevalence in the host species with highest prevalence) between host-specialist lineages and generalists differed significantly (P = 0.018; model fit $\chi^2_{(11)}$ = 13.3, P = 0.270). Host specialists had a higher maximum host-specific prevalence than generalists. However, H011 (SGS1) was over three times more frequent (n = 207 vs. 30–66) and parasitized a 2.5 times greater number of host species (40 vs. 2–17) than any other abundant and widespread lineage (Appendix S5, Supporting information). The prevalence of this lineage was 35% in *Parus major* and varied from 14% to 24% in several distantly related host species with $n \geq 50$. Furthermore, in some host species with low sample size, the prevalence of H011 (SGS1) was very high. For example, 10 of 13 (76.9%) *Cettia cetti* and four of eight (50.0%) *Phoenicurus moussieri* were positive for H011 (SGS1). These data suggest that in contrast to other haemosporidian lineages in our data set, H011 (SGS1) is an especially successful generalist that is capable of achieving moderate to high prevalence in multiple distantly related host species.

To determine whether our data set is representative of the general patterns of host species use and prevalence of haemosporidian parasites, we compared our data with the information available for the same lineages in the MalAvi database (Bensch *et al.* 2009). Both the number of host species infected by the 13 most abundant and widespread haemosporidian lineages (Spearman's rho = 0.882, n = 13, P = 0.002) and the prevalence for 21 lineage/host pairs for which we were able to find data in MalAvi were strongly correlated (Spearman's rho = 0.668, n = 21, P = 0.003; Appendix S5, Supporting information), suggesting that our data do not appear to be biased by our sampling of host species and geographical localities.

Discussion

Although AOR is one of the most prominent ecological phenomena among free-living organisms (Gaston 1996; Gaston *et al.* 2000), its underlying causal mechanisms have not been tested thoroughly in parasites, and in particular, in avian Haemosporidia. We are aware of a single study testing the presence of AOR in a single haemosporidian genus (*Leucocytozoon*), which involved only two host species (*Cyanistes caeruleus* and *Parus major*) sampled in western Europe (Jenkins & Owens 2011). The authors found support for AOR in their study system and rejected phylogeographical structuring of *Leucocytozoon* lineages.

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Our study expanded testing of AOR in avian Haemosporidia to 386 lineages of all three genera, 103 host species and four biogeographically distinct regions of the southwestern Palearctic. Despite the presence of biogeographical barriers to host movements, relatively low similarity in composition and representation of both host species (MDI = 0.143–0.421) and haemosporidian lineages (MDI = 203–329; Table 1) among our regional samples, the AOR in all three genera of avian Haemosporidia was supported (Fig. 4). The vast majority of lineages that were observed fewer than 24 times were restricted to some of the sampled geographical regions, whereas 11 of 13 parasite lineages observed at least 30 times were found in all sampled regions. The two parasite lineages observed at least 30 times but found in only three of the four sampled regions have been reported by others from the same or surrounding regions (Dimitrov *et al.* 2010; Jenkins & Owens 2011; Palinauskas *et al*. 2013).

The two hypotheses that were proposed to explain the AOR in parasitic animals received mixed support in our study. The lineage H011 (*Plasmodium* SGS1) parasitized 2.5 times greater number of host species than any other lineage. Its overall prevalence was three times higher than that of any other lineage, and it was common in all four study areas. Furthermore, it had greater prevalence in two host species than any host-specialist lineage had in their respective host species. These findings are consistent with the NBH, which predicts that host generalist parasites should be more abundant and widespread than host-specific parasites due to a much larger number of potential host individuals than are available to a host-specialist lineage (Krasnov *et al.* 2004; Hellgren *et al.* 2009).

However, host specialists and generalists were similarly represented among the 13 most abundant and widespread lineages (8:5), and lineage abundance was independent of the host specialization. These results are inconsistent with either hypothesis.

Yet, haemosporidian lineages disproportionally parasitized the most abundant host species more frequently than host species with low sample sizes in our data set, which suggests the presence of a density-dependent host specialization. Also, when H011 (*Plasmodium* SGS1) is excluded from the analysis, the prevalence of host specialists in their respective host species was significantly higher than the prevalence of host generalists in any of their host species. These findings are consistent with the predictions of TOH that suggests the presence of a trade-off between ability to parasitize multiple host species and achieving high prevalence in them (Poulin 1998).

Thus, it appears that neither of the two hypotheses we tested provide a satisfactory explanation for AOR in avian Haemosporidia. However, incomplete sampling of abundant avian species resulting from our emphasis on sampling forest birds and restriction of sampling techniques primarily to mistnetting could have biased our conclusions against the NBH. The four apparently host generalist haemosporidian lineages (H022, H027, H077 and H010) that had significantly lower prevalence in any of their host species than the prevalence of host-specialist lineages in their hosts could have a much higher prevalence in abundant avian species we did not sample. Among such abundant potential host species that are present in all four study areas are, for example, corvids (*Pica pica*, *Corvus corone/cornix*, *C. monedula*), *Acrocephalus* warblers (*A. scirpaceus* and *A. arundinaceus*) and larks (*Alauda arvensis* and *Galerida cristata*). Besides more complete sampling of potential host species in the study areas we sampled, sampling additional biogeographical regions may also strengthen support for NBH particularly where those areas harbour different potential avian hosts.

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References

Agresti A (2002) *Categorical Data Analysis* John Wiley & Sons, New Jersey.

Akaike H (1974) A new look at the statistical model identification. *IEEE Transactions on Automatic Control*, **19**, 716–723.

Barger MA, Esch GW (2002) Host specificity and the distribution–abundance relationship in a community of parasites infecting fishes in streams of North Carolina. *Journal of Parasitology*, **88**, 446–453.

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. *Molecular Ecology Resources*, **9**, 1353–1358.

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Brown JH (1984) On the relationship between abundance and distribution of species. *American Naturalist*, **124**, 255–279.

Clements JF, Schulenberg TS, Iliff MJ *et al.* (2013) The eBird/Clements checklist of birds of the world: Version 6.8.

Dice LR (1945) Measures of the amount of ecologic association between species. *Ecology*, **26**, 297–302.

Dimitrov D, Zehtindjiev P, Bensch S (2010) Genetic diversity of avian blood parasites in SE Europe: cytochrome b lineages of the genera *Plasmodium* and *Haemoproteus* (*Haemosporida*) from Bulgaria. *Acta Parasitologica*, **55**, 201–209.

Gaston KJ (1996) The multiple forms of the interspecific abundance-distribution relationship. *Oikos*, **76**, 211–220.

Gaston KJ, Blackburn TM, Greenwood JJD *et al.* (2000) Abundance–occupancy relationships. *Journal of Applied Ecology*, **37**, 39–59.

Hellgren O, Pérez-Tris J, Bensch S (2009) A jack-of-all-trades and still a master of some: prevalence and host range in avian malaria and related blood parasites. *Ecology*, **90**, 2840–2849.

Jenkins T, Owens IPF (2011) Biogeography of avian blood parasites (*Leucocytozoon* spp.) in two resident hosts across Europe: phylogeographic structuring or the abundance–occupancy relationship? *Molecular Ecology*, **20**, 3910–3920.

Krasnov BR, Poulin R, Shenbrot Georgy I, Mouillot D, Khokhlova Irina S (2004) Ectoparasitic 'Jacks-of-All-Trades': relationship between abundance and host specificity in Fleas (*Siphonaptera*) parasitic on small mammals. *The American Naturalist*, **164**, 506–516.

Librado P, Rozas J (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, **25**, 1451–1452.

Mantel N (1967) The detection of disease clustering and a generalized regression approach. *Cancer Research*, **27**, 209–220.

Morand S, Guégan J-F (2000) Distribution and abundance of parasite nematodes: ecological specialisation, phylogenetic constraint or simply epidemiology? *Oikos*, **88**, 563–573.

Palinauskas V, Iezhova TA, Križanauskienė A *et al.* (2013) Molecular characterization and distribution of *Haemoproteus minutus* (*Haemosporida*, *Haemoproteidae*): a pathogenic avian parasite. *Parasitology International*, **62**, 358–363.

Poulin R (1998) Large-scale patterns of host use by parasites of freshwater fishes. *Ecology Letters*, **1**, 118–128.

Poulin R, Mouillot D (2003) Parasite specialization from a phylogenetic perspective: a new index of host specificity. *Parasitology*, **126**, 473–480.

Poulin R, Blanar CA, Thieltges DW, Marcogliese DJ (2012) Scaling up from epidemiology to biogeography: local infection patterns predict geographical distribution in fish parasites. *Journal of Biogeography*, **39**, 1157–1166.

Thieltges DW, Marcogliese DJ, Blanar CA, Poulin R (2013) Trematode prevalence-occupancy relationships on regional and continental spatial scales in marine gastropod hosts. *Marine Ecology Progress Series*, **490**, 147–154.

S.V.D., G.V., and R.J.L. designed the study, S.V.D., S.A.A., V.M., and R.J.L. collected samples, S.A.A., V.M., J.H. conducted lab work, S.V.D., N.M., R.J.L., and G.V. analysed data, S.V.D. designed a new protocol for Haemosporidia screening and wrote the first draft of the manuscript. All authors participated in revisions with the greatest contribution from N.M. and S.V.D.

Data accessibility

DNA sequences: GenBank. GenBank accessions KJ488555–KJ488940.

Fasta sequence alignment of unique haemosporidian haplotypes and individual level data listing bird ID, species, region, sampling date and haemosporidian lineages found: Dryad doi:10.5061/dryad.r8bj6.

Supporting information

Additional supporting information may be found in the online version of this article.

Appendix S1 Host species, their regional sample sizes, and number of individuals infected with each haemosporidian lineage.

Appendix S2 Dates of host sampling in four study regions. The regions are: NWA - northwest Africa, NWI – northwest Iberia, TRC - Transcaucasia, WGC - western Greater Caucasus.

Appendix S3 Species and their regional sample sizes that were excluded from the study due to the lack of haemosporidian infections.

Appendix S4 Proportion of individuals infected by haemosporidia detected by PCR screening of blood or other tissue types (heart, liver, pectoral muscle) in 14 species with at least 10 samples of each type.

Appendix S5 Host species and prevalence of the 13 most abundant and geographically widespread haemosporidian lineages from our data set and comparative data from MalAvi (accessed Jan. 11, 2014).

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