Host associations and evolutionary relationships of avian blood parasites from West Africa

Jon S. Beadell a,*,1, Rita Covas b,2, Christina Gebhardt a, Farah Ishtiaq a, Martim Melo b, Brian K. Schmidt c, Susan L. Perkins d, Gary R. Graves e, Robert C. Fleischer f

a Center for Conservation and Evolutionary Genetics, National Zoological Park, Smithsonian Institution, 3001 Connecticut Avenue, Washington, DC 20008, USA
b Department of Vertebrate Zoology, MRC-116, National Museum of Natural History, Smithsonian Institution, P.O. Box 37012, Washington, DC 20013-7012, USA
c Center for Conservation and Evolutionary Genetics, National Zoological Park, Smithsonian Institution, 3001 Connecticut Avenue, Washington, DC 20008, USA
d Sackler Institute for Comparative Genomics and Division of Invertebrate Zoology, American Museum of Natural History, Central Park West at 79th Street, New York, NY 10024, USA

ABSTRACT

The host specificity of blood parasites recovered from a survey of 527 birds in Cameroonian and Gabon was examined at several levels within an evolutionary framework. Unique mitochondrial lineages of Haemoproteus were recovered from an average of 1.3 host species (maximum = 3) and 1.2 host families (maximum = 3) while lineages of Plasmodium were recovered from an average of 2.5 species (maximum = 27) and 1.6 families (maximum = 9). Averaged within genera, lineages of both Plasmodium and Haemoproteus were constrained in their host distribution relative to random expectations. However, while several individual lineages within both genera exhibited significant host constraint, host breadth varied widely among related lineages, particularly within the genus Plasmodium. Several lineages of Plasmodium exhibited extreme generalist host–parasitism strategies while other lineages appeared to have been constrained to certain host families over recent evolutionary history. Sequence data from two nuclear genes recovered from a limited sample of Plasmodium parasites indicated that, at the resolution of this study, inferences regarding host breadth were unlikely to be grossly affected by the use of parasite mitochondrial lineages as a proxy for biological species. The use of divergent host–parasitism strategies among closely related parasite lineages suggests that host range is a relatively labile character. Since host specificity may also influence parasite virulence, these results argue for considering the impact of haematozoa on avian hosts on a lineage-specific basis.

1. Introduction

Host specificity is a key factor influencing the virulence of parasites (Garamszegi, 2006), their geographical ranges and the extent to which parasites may emerge into novel hosts (Taylor et al., 2001; Woolhouse and Gowtage-Sequeria, 2005; Hellgren et al., 2007a,b). Host–parasite associations presumably reflect the physiological and immunological constraints imposed by hosts, as well as the ecological factors, such as the distribution and abundance of hosts, parasites and vectors, which limit opportunities for transmission of parasites between different hosts. While host specialization may limit the availability of resources and increase the risk of extinction, it may also allow for increased contact among individuals of a parasite species restricted to a narrow host range. This, in turn, could increase opportunities for out-crossing, leading to increased genetic diversity and possibly the evolutionary flexibility required to colonize new hosts (Combes and Theron, 2000). On the other hand, parasites with broad host distributions are generally thought to have low fitness in any one host, but may achieve higher abundance and face reduced extinction risk relative to specialists. Given these trade-offs, the evolutionary path followed by a parasite species likely represents a fine balance between the selective pressures favoring either specialist or generalist strategies (Woolhouse et al., 2001). Small perturbations to these pressures over time or across space could lead to reversals in host–parasitism strategies and variability in strategies even amongst closely related parasites.

Avian haematozoa present an interesting system for studying the host–parasitism strategies (specialization or promiscuity) of closely related parasites due to their high diversity and the diverse host fauna that is potentially available to a parasite in any particular geographical location. Avian malaria (family Plasmodiidae) and related haematozoan parasites in the families Haemoproteidae and Leucocytozoidea have been detected on every continent...
2. Materials and methods

2.1. Sample collection, parasite detection and identification

Avian tissue samples were collected by B. Schmidt and C. Gebhard (National Museum of Natural History, Washington DC, USA) from 396 individuals in the Gamba Complex, Gabon during the period February 2002 to April 2003 (Angehr et al., 2006). Blood was collected from an additional 131 individuals mist-netted in Cameroon (Mann’s Spring, Buéa and Limbe) and Gabon (Cap Estiers and Kango) by R. Covas and M. Melo (University of Edinburgh, UK) at various seasons between 2002 and 2004 (Fig. 1). Sampling of birds followed the Ornithological Council’s Guidelines to the Use of Wild Birds in Research (Gaunt et al., 1997). We extracted DNA from these samples using DNeasy kits (Qiagen) following the manufacturer’s protocol. Parasite screening and identification of lineages was performed using methods described previously (Bensch et al., 2004; Beadell and Fleischer, 2005). Briefly, we screened for haematozoan parasites using primer sets F2/R2, 850F/1024R and 213F/372R in a 25 μl PCR. The latter primer set allowed for differentiation of parasites in the genera Plasmodium, Haemoproteus and Leucocytozoon via restriction fragment length polymorphism (Beadell and Fleischer, 2005). From samples in which we detected an infection, we amplified and sequenced a 533 bp fragment of the cyt b gene using primers 3760F/4292rw2. In cases where a low quantity of DNA or degraded template prevented the amplification of this fragment, we sequenced smaller fragments using primers F1/F2/R2/Rw (351 bp; Ishtiaq et al., 2006) or F2/213F/292F (256 bp). Sequences were aligned using Sequencer 4.1 and those sequences that matched identically were defined as unique lineages. For a subset of the samples for which we obtained mitochondrial sequences, we also amplified a portion of the nuclear genes dihydrofolate reductase-thymidylate synthase (DHFR-TS; 236 bp; Bensch et al., 2004) and diacylglycerol O-acyltransferase (transferase). We amplified the latter with primers trF1 (5’-GCC WAC TAT GTG TTT TCA ATT-3’) and either trR1 (5’-GCT AAA TTY CAC CAA TGA TGA-3’; 294 bp) or trR2 (5’-GCT ATT GAT CAT ATT TTT ARC ATY CCT TC-3’; 231 bp) using conditions identical to those used in amplifying mitochondrial DNA but with an annealing temperature of 50 °C.
2.2. Phylogenetics

We used a neighbor-joining tree to confirm the identification of parasite mitochondrial lineages to genus (Plasmodium, Haemoproteus and Leucocytozoon) based on their association with GenBank sequences from morphologically identified specimens. Subsequently, for lineages of Plasmodium spp. and Haemoproteus spp. for which we had recovered at least 351 bp of sequence, we used PAUP* (Swofford, D.L., 1998. PAUP*, phylogenetic analysis using parsimony (*and other methods), Version 4.0. Sinauer, Sunderland, MA) to estimate phylogenetic relationships within genera using maximum likelihood (ML) and a general time reversible (GTR) model of nucleotide substitution, which was chosen using the program MODELTEST (Posada and Crandall, 1998). Because these genera appear to be sister taxa (Perkins and Schall, 2002; Martinsen et al., 2008), we rooted the Plasmodium spp. tree with Haemoproteus spp. and vice versa. For both trees, we estimated support for branches based on 300 bootstrap replicates.

In order to explore the evolutionary relationships among certain lineages of Plasmodium more closely, we also generated phylogenetic trees based on DHFR and transferase haplotypes. As a possible consequence of lower copy number, we were not able to amplify nuclear gene fragments from many of the parasites for which we had recovered a mitochondrial signature. In addition, we excluded nuclear sequences for samples in which we detected any evidence of multiple infection (e.g., multiple peaks in the chromatograms obtained for either nuclear sequences or the mitochondrial sequence) in order to help ensure that sequences derived from the same parasite. We estimated relationships using ML and either a GTR (DHFR) or HKY (Hasegawa et al., 1984; transferase) model of nucleotide substitution. Bootstrap support was estimated based on 500 replicates.

2.3. Host specificity analysis

We evaluated the host associations of parasite lineages at multiple levels of parasite relatedness. In all analyses, we defined host specificity both with respect to host species and host family. For avian family definitions, we followed classifications outlined in the Handbook of the Birds of the World (del Hoyo et al., 2003). Initially, we asked whether the host ranges of individual lineages of either Haemoproteus or Plasmodium were consistent with the random assignment of host species or host families to parasite lineages. To determine the significance of the observed constraint for each lineage, we randomly reassigned hosts to parasite lineages 1000 times and then compared the observed number of host species or families in which a particular lineage was found with the numbers obtained from randomization. During randomization, we preserved the observed number of times each parasite lineage was detected. We tested for a difference in the number of significantly constrained lineages between the genera Haemoproteus and Plasmodium using a chi-squared test. For each parasite genus, we also compared the average observed number of host species or host families per parasite lineage with the average obtained from randomizations. We tested whether the average number of hosts per lineage of Haemoproteus and Plasmodium was different using a Wilcoxon–Mann–Whitney test.

In order to determine whether spillover of parasite lineages into atypical hosts in ecological time may be obscuring longer-term evolutionary signals of host specificity, we compared the host distribution of individual parasite lineages that were detected in two or more host individuals (branch tips) with the host distribution of pairs of sister lineages (i.e., lineages joined by first-step nodes with bootstrap support > 70%). At each level of relatedness (branch tips and first step nodes), we calculated the proportion of parasite lineages occurring in a single host species or in a single host family, and we tested for a significant difference in this metric between parasite genera using a chi-squared test or Fisher’s exact test when appropriate.

In order to probe host specificity at deeper levels within the parasite phylogenetic tree, we subsequently performed a logistic regression of host species (same or different) or host family (same or different) versus LogDet parasite distance (Ricklefs and Fallon, 2002; Beadell et al., 2004). We used only lineages with at least 351 bp of sequence data and missing data was ignored when calculating pairwise distances between sequences of different length (PAUP*). Each parasite lineage was included in the dataset once for every time it was detected; therefore the representation of a lineage in a particular host species was proportional to the frequency with which it occurred in that particular host. Thus, a lineage that occurred, for example, four times in one host species and just once in a second host species (e.g., WAH21), would generate a greater signal of host specificity than a lineage occurring just once in each host species (e.g., WAH10). Because multiple pairwise distance calculations violate assumptions of independence, we determined the significance of regression coefficients following a permutation protocol described previously (Beadell et al., 2004). We randomly reassigned host individuals to the parasite phylogeny 1000 times (again preserving the total number of individuals in which a lineage was observed and the observed number of infected individuals of each host species), performing logistic regression upon each iteration. We then tested for significance by comparing the observed regression coefficients with the distribution obtained from randomization. We tested for a significant regression at the level of host species and at the level of host family in both Haemoproteus spp. and Plasmodium spp., as well as in several smaller groups of parasite lineages within each genus.

3. Results

3.1. Prevalence

We screened a total of 527 individual hosts representing 93 species and 29 avian families. We detected Haemoproteus spp. in 119 individuals (23%), Plasmodium spp. in 238 individuals (45%) and Leucocytozoon spp. in 35 individuals (7%). In several cases, our methods (sequencing or restriction fragment analysis) failed to identify the parasite genus and these infections were classified as “genus unknown”. Prevalence of parasites in well-represented (>20 individuals) avian families is summarized in Table 1. Data for each host species examined is summarized in Supplementary Table S1. Because we recovered Leucocytozoon only infrequently, we restricted further analyses to the two most common parasite genera, Haemoproteus and Plasmodium.

3.2. Phylogenetics

We obtained sequence information from a total of 84 Haemoproteus infections (38 unique lineages) and 171 Plasmodium infections (47 unique lineages; GenBank accession numbers are listed in Supplementary Table S2). We recovered between one and eight parasite lineages from individual host species, corresponding to an average of 2.1 parasite lineages per species of infected host. For one lineage of Haemoproteus (one infection) and five lineages of Plasmodium (six total infections), we were unable to recover sequence of at least 351 bp; therefore these lineages were excluded from subsequent analyses requiring a phylogenetic framework. Phylogenetic relationships between mitochondrial lineages of Plasmodium spp. and Haemoproteus spp. are presented in Figs. 2 and 3, respectively.
3.3.1. Host specificity at tips of phytogeny

Turdidae
Alcedinidae
Table 1
lineages that were testable (i.e., recovered at least twice),
amongst these markers and provides support for defining parasite
we also examined separately from the remaining parasites. Inter-
the host species or family level are identified in Figs. 2 and 3.
Plasmodium
can be attributed to both vicariance and host-switching,
to multiple independent colonization events. If diversification
of parasites can be attributed to both vicariance and host-switching,
then signals of host specificity for clades of parasites arising from
different host-switching events are likely to coalesce to different
points in time. Therefore, when analyzing host specificity (see be-
low) we investigated signals of host specificity arising from several
partitions: the entire genus Plasmodium, Plasmodium excluding
clade PA and clade PA alone. Similarly, within Haemoproteus, we
identified one well-supported sub genus-level clade (HA) which
we also examined separately from the remaining parasites. Inter-
estingly, clade HA encompassed most of the Haemoproteus lineages
derived from non-passerines (Alcedinidae, Indicatoridae, Capiton-
dae and Strigidae) in our sample.

Evolutionary relationships of parasite lineages deduced from
two nuclear genes were similar to those estimated from mitochon-
drial sequences (data not shown). Our analyses were limited be-
cause we recovered nuclear data from only a small subset of
mitochondrial lineages and bootstrap support for phylogenetic
relationships was low due to the small size of the nuclear frag-
ments targeted. Nonetheless, a plot of linkage between mitochon-
drial and nuclear markers illustrates the strong correspondence
amongst these markers and provides support for defining parasite
taxonomic units using mitochondrial lineages alone (Fig. 4). As evi-
denced in Fig. 4, few squares are shaded outside of the boxes out-
lined along the diagonal, indicating that the mitochondrial lineages
examined are likely to represent reproductively-isolated units. The
few exceptions are largely attributable to the sharing of nuclear
haplotypes between closely related mitochondrial lineages.

3.3. Host specificity

3.3.1. Host specificity at tips of phylogeny

Assessing lineages individually, we found that of the Plasmo-
dium lineages that were testable (i.e., recovered at least twice),
three of 23 occurred in significantly fewer host species, and two
of 23 occurred in significantly fewer host families than expected
by chance (assessed at Bonferroni corrected P < 0.0022). For Haem-
proteus spp., seven of 20 lineages occurred in significantly fewer
host species, and three of 20 lineages occurred in significantly few-
er families than expected by chance (Bonferroni corrected
P < 0.0025). The number of lineages exhibiting significant
constraint at the host species level (χ2 = 2.89, df = 1, P = 0.0989) or at
the host family level (χ2 = 0.41, df = 1, P = 0.52) was not signifi-
cantly higher for Haemoproteus spp. compared with Plasmodium
spp. Individual lineages exhibiting significant constraint at either
the host species or family level are identified in Figs. 2 and 3.

Individual lineages of Haemoproteus (n = 37) were found in an
average of 1.3 ± 0.7 (SD) host species and 1.2 ± 0.6 host families
(Table 2). Both values were significantly lower (P < 0.001) than ex-
pected if hosts were randomly distributed across parasite lineages.
Similarly, on average, lineages of Plasmodium (n = 42) were found
in significantly fewer species (average = 2.5 ± 4.4, P < 0.001) and
fewer families (average = 1.6 ± 1.8, P < 0.001) than expected by
chance. When all lineages were included, Wilcoxon–Mann–Whit-
ney tests revealed no significant differences between either the
average number of host species (Z = −1.67, P = 0.096) or average
number of host families (Z = −0.66, P = 0.5083) infected by lineages of
Haemoproteus and lineages of Plasmodium. However, when con-
sidering only those lineages that were detected in two or more
individuals (i.e., lineages that were not constrained to just a single
host species or family by default), the difference between the aver-
age number of host species in which lineages of Haemoproteus
(1.6 ± 0.8, n = 20) and Plasmodium (3.7 ± 5.6, n = 23) were found
was significant (Z = −2.0, P = 0.045). Again, no significant difference
in specificity was observed between Haemoproteus and Plasmodium
at the level of host family (Z = −0.76, P = 0.45).

3.3.2. Host specificity at greater depth within phytogeny

The proportion of Haemoproteus parasite lineages that were
detected at least twice, yet were found in just a single host species
(60%, n = 20), was higher than in Plasmodium (30%, n = 23)
although the difference was only marginally significant (P = 0.052; Table 3).
No significant difference was apparent when comparing the host
species constraint of lineages joined by first-step nodes. At the
family level of host specificity, no significant differences were ob-
served between Haemoproteus spp. and Plasmodium spp., either
when comparing identical lineages at branch tips (average diver-
gence = 0%) or lineages joined by well-supported first-step nodes
(average divergence = 1.9% for Haemoproteus and 1.4% for Plasmo-
dium). In both Haemoproteus and Plasmodium spp., the proportion
of lineages constrained to a single host family tended to be higher
than those constrained to a single host species when assessed at
either branch tips or first-step nodes (Table 3).

Logistic regressions of host species or family versus parasite ge-
etic distance allowed us to extend our analysis of host specificity
to even greater depths within the parasite phylogeny. We per-
formed separate logistic regressions on Haemoproteus spp. and
Plasmodium spp. and on two subsets of lineages within each genus.
These subsets were composed of either (i) one well-supported
clad of parasites nested within each genus (Clade HA or PA) or
(ii) all of the remaining lineages in that genus (Group HB or PB).
Coefficients generated for all regressions were significant
(P < 0.01) compared with 1000 randomizations of the data and
the resulting trends are presented in Fig. 5. As an index of the phy-
logenetic depth at which a signal of host specificity was lost (i.e.,
the genetic distance at which parasites were no longer likely to
be found in the same host taxa), we solved logistic regression equa-
tions (ln[P/(1−P)] = a + b × x) obtained for each subset for the dis-
tance (x) at which there was a 0.5 probability that two parasite
lineages would be found in the same host (Table 4). Haemoproteus
spp. exhibited a signal of host specificity at the host family level when the genus was examined in its entirety ($x = 0.015$), as well as when partitioned into subsets ($x = 0.013$ for clade HA and $x = 0.017$ for the entire genus minus clade HA). The signal at the host species level was lost at smaller parasite divergences ($x = 0.001$ for the entire genus and $x = 0.005$ for the entire genus minus clade HA). In contrast, only one Plasmodium subset (clade PA) exhibited a measurable signal of host specificity ($x = 0.026$) and this was at the level of host family. In all cases, the signal of host specificity was generally stronger at the level of host family than at the level of host species.

4. Discussion

Our results demonstrate subtle differences in the host strategies of Haemoproteus spp. and Plasmodium spp. and corroborate patterns emerging from previous regional surveys that have revealed broad variability of host-parasitism strategies employed by both genera of avian haematozoa (Beadell et al., 2004; Ricklefs et al., 2004; Fallon et al., 2005; Krizanauskiene et al., 2006). Application of methods similar to those described here to a haematozoan parasite fauna from Australia and Papua New Guinea indicated that lineages of Haemoproteus generally appeared
to be more constrained at the family level than lineages of *Plasmodium* (Beadell et al., 2004). In the present study we demonstrated that, on average, lineages of *Haemoproteus* were more constrained at the level of host species than were lineages of *Plasmodium*. This difference in host specificity, however, was not evident following removal of the three lineages of *Plasmodium* with the broadest host distribution (WA20, WA9 and WA15). Furthermore, the difference in specificity did not extend to the level of host family. In fact, probing host–parasite associations across the parasite phylogeny using logistic regression suggested that at least one large collection of *Plasmodium* lineages (clade PA) exhibited a signal of host specificity at the host family level (0.026) that was equivalent to the signal obtained for a well-defined group of Australo-Papuan *Haemoproteus* lineages (0.029) and stronger than any signal recovered for groups of West African *Haemoproteus* (range = 0.013–0.017). Thus, at least some lineages of *Plasmodium* appear to be constrained to certain host groups to the same extent as lineages of *Haemoproteus*.
Fig. 4. Linkage plot. Shaded squares indicate sharing of nuclear haplotypes (dihydrofolate reductase–thymidylate synthase (DHFR-TS) below the diagonal; transferase above the diagonal) between individual parasite mitochondrial lineages (identified at left and depicted with corresponding alternate shading in the same order across top). Boxes outlined along the diagonal indicate the squares that would be shaded if each mitochondrial lineage were associated with a unique nuclear signature. Hashed squares indicate sharing of nuclear haplotypes among different mitochondrial lineages. In some cases, identification of shared nuclear haplotypes was not possible (N) due to unsuccessful amplifications of one locus or the other in certain samples.

Table 2
Average host constraint observed across lineages of avian blood parasites

<table>
<thead>
<tr>
<th></th>
<th>Haemoproteus</th>
<th>Plasmodium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Host species per lineage</td>
<td>1.3 (1.6)</td>
<td>2.5 (3.7)</td>
</tr>
<tr>
<td>Host families per lineage</td>
<td>1.2 (1.4)</td>
<td>1.6 (2.1)</td>
</tr>
</tbody>
</table>

Values in parentheses were obtained from averaging across only those lineages that were detected more than once.

Table 3
Host-specificity of haematozoan parasites indicated by the proportion of parasite lineages which derived from the same host species or the same host family at two levels of parasite relatedness (branch tips and first-step nodes)

<table>
<thead>
<tr>
<th>Parasite genus</th>
<th>Lineages at branch tips</th>
<th>Lineages joined by 1st-step nodes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Same host species (%)</td>
<td>Same host family (%)</td>
</tr>
<tr>
<td></td>
<td>Same host species (%)</td>
<td>Same host family (%)</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>n</td>
</tr>
<tr>
<td>Haemoproteus</td>
<td>60</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td>86</td>
<td>7</td>
</tr>
<tr>
<td>Plasmodium</td>
<td>30</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td>23</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>44</td>
<td>9</td>
</tr>
</tbody>
</table>

Because of the extreme variability in observed host–parasitism strategies, particularly in certain lineages of Plasmodium, comparing average strategies among haematozoan genera may not be valuable. We recovered individual lineages of Plasmodium from between one and 27 different avian host species. The extreme diversity of hosts observed for a single lineage is in keeping with the 39 species of host infected by lineage GRW4 worldwide (Beadell et al., 2006) and the 27 hosts infected by lineage PA in the Antilles (Fallon et al., 2005). We recovered lineages of Haemoproteus from a maximum of three host species (three families), but other regional surveys have detected certain lineages of Haemoproteus in up to seven (Krizanauskienė et al., 2006) and even 26 (Fallon et al., 2005) host species, suggesting that some Haemoproteus lineages may exhibit similarly broad host distributions. In contrast to these generalist lineages, we also identified at least several individual lineages that exhibited significant host constraint at both the host species and host family level. More intensive and thorough sampling of the West African avifauna will undoubtedly expand the host ranges of many of the apparent specialist haematozoan lineages, but the signals of host specificity extending deeper within the Haemoproteus phylogeny suggest that many of these lineages are likely to be true specialists. Thus, both Haemoproteus and Plasmodium appear to harbor lineages with strongly divergent host–parasitism strategies.

Why do related parasites exhibit such striking difference in host specificity? As outlined previously, specialists presumably benefit from relatively high fitness in the limited number of hosts that they utilize and may be able to evolve more quickly in response to changes in host defense or physiology. Generalists, on the other
Fig. 5. Logistic regression curves modeling the probability that two parasite lineages exhibit the same host range at different levels of genetic divergence. For both *Haemoproteus* and *Plasmodium*, separate trend lines were calculated using data from the entire genus (black line), a well-supported group of parasites within each genus (HA or PA, dotted line; see Figs. 2 and 3) and the entire genus excluding HA or PA (dashed line). Trend lines that intersect the y-axis below 0.5 indicate that, on average, parasite lineages in the group being considered exhibited less than a 50% chance of being found in just a single host species (above) or family (below).

**Table 4**

<table>
<thead>
<tr>
<th>Phylogenetic partition</th>
<th>Parasite genetic distance at which host-specificity signal lost</th>
<th>Host species level</th>
<th>Host family level</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Haemoproteus</strong></td>
<td></td>
<td>Host species level</td>
<td>Host family level</td>
</tr>
<tr>
<td>Genus</td>
<td>0.001</td>
<td>0.015</td>
<td></td>
</tr>
<tr>
<td>Clade HA</td>
<td>n/a</td>
<td>0.013</td>
<td></td>
</tr>
<tr>
<td>Genus excluding HA</td>
<td>0.005</td>
<td>0.017</td>
<td></td>
</tr>
<tr>
<td><strong>Plasmodium</strong></td>
<td></td>
<td>n/a</td>
<td></td>
</tr>
<tr>
<td>Genus</td>
<td>n/a</td>
<td>n/a</td>
<td></td>
</tr>
<tr>
<td>Clade PA</td>
<td>n/a</td>
<td>0.026</td>
<td></td>
</tr>
<tr>
<td>Genus excluding PH</td>
<td>n/a</td>
<td>n/a</td>
<td></td>
</tr>
</tbody>
</table>

Values indicate the genetic distance beyond which two sampled parasite lineages have less than a 50% chance of deriving from the same host species or same host family. Larger values are indicative of a longer history of association between hosts and parasites.

hand, may be less prone to extinction because they maintain larger populations distributed over a greater number of hosts. Thus, the strategy adopted by a parasite represents a fine balance between the selective pressures favoring either specialist or generalist strategies (Woolhouse et al., 2001). Consequently, host–parasitism strategies may shift rapidly so that even closely related parasites may exhibit very different host ranges. Alternatively, it is possible that the generalist strategy does not exist, or that the generalist strategy represents an unstable and ephemeral transition state (Stireman, 2005). Large observed haematozoan host ranges may reflect the spillover of parasites into hosts in which the full transmission cycle may never be completed. Or, they may simply reflect our inability to distinguish between cryptic parasite species. This phenomenon could explain the extreme lack of specialization observed in *Plasmodium* lineage WA9, detected in 27 different species representing nine host families, and lineage WA15 which was detected in 15 species also representing nine different host families. We cannot rule out the possibility that these lineages actually represent a group of species that are so young that mutations have not yet accumulated to allow differentiation of the numerous specialists that may compose the group.

If mitochondrial lineage diversity is generated too slowly to reflect host specialization, one might question whether mitochondrial lineages are a useful taxonomic unit for investigating host specificity. Certainly, we may be limited in our ability to distinguish recently evolved parasite species. In this case, the broad host ranges of parasite lineages such as WA9 or WA15 may place a downward bias on our estimates of host-specificity. But, at the other end of the spectrum, we may ask whether mitochondrial lineages are valid proxies for long-established reproductively-isolated parasite species. If not, how does this affect estimates of host specificity?

Evidence from two nuclear genes suggests that mitochondrial lineages do provide a reasonable taxonomic metric, at least at the level of mitochondrial differentiation examined here. Although
we cannot determine whether lineages that share a given mitochondrial haplotype are currently reproductively-isolated, sequencing of fragments of the DHFR–TS and transferase genes provided no evidence to refute the common ancestry of parasites sharing a single mitochondrial signature. In other words, the apparent generalism attributed to WA9, for example, did not appear to be the consequence of a single mitochondrial lineage having introgressed into multiple evolutionarily distinct parasites. Nuclear sequences from individuals possessing the WA9 mitochondrial signatures were either identical (transferase) or differed by a single nucleotide (DHFR), suggesting that the parasites sharing this mitochondrial lineage also share a similar nuclear genome. Evaluated more broadly across other Plasmodium lineages, the correspondence between mitochondrial haplotype and nuclear sequences provided support for the use of mitochondrial lineages as the taxonomic basis for evaluating host associations. Mitochondrial lineages tended to be associated with distinct nuclear genotypes; however, the correspondence was not perfect. In at least one case (WA20), a single mitochondrial lineage encompassed three distinct nuclear signatures, evident in both transferase and DHFR. If this mitochondrial lineage is really composed of three reproductively-isolated species, then the host range of this seemingly generalist lineage could be inflated. In several other cases, we identified distinct mitochondrial lineages that shared at least one nuclear haplotype (e.g., WA37 and WA38 (transferase), WA19 and WA20 (DHFR and transferase), WA20 and WA21 (DHFR)). This sharing may represent instances of incomplete lineage sorting in which mitochondrial lineages do actually represent reproductively-isolated species. Alternatively, this sharing of nuclear haplotypes could indicate that the associated mitochondrial lineages simply represent intraspecific diversity. In this case, artificially separating these lineages would inflate estimates of host specificity. For lineages WA37 and WA38, the distinction is irrelevant given that both lineages were found in the same host species. Similarly, changes in host distributions arising from the genetic associations of lineages WA19, WA20 and WA21 would not dramatically alter the signal of host generalism arising from these lineages.

The apparent validity of using mitochondrial lineages as a foundation for investigating host–parasite associations is due in part to the resolution provided by our opportunistic sampling. Among the lineages that we sampled, the average genetic distance between pairs of most closely-related Plasmodium lineages (first-step nodes) was about 1.4%. For Haemoproteus lineages, the average minimum divergence was about 1.9%. As points of reference, the well-defined and closely-related species Plasmodium falciiparum and Plasmodium reichenowi exhibit a divergence of about 2.3% across the mitochondrial genome (Joy et al., 2003), while morphospecies of Haemoproteus can exhibit as little as 0.7% divergence (Hellgren et al., 2007a,b). Mean intra-morphospecies divergence can be substantially higher but at least some of this divergence may represent differentiation among cryptic biological species that share a similar morphology (Beadell et al., 2006; Hellgren et al., 2007a,b). Therefore these data, combined with the strong correspondence between mitochondrial and nuclear haplotypes, suggest that the majority of the parasite lineages in our sample represent species-level taxonomic units. Further integration of genetic and morphological studies (Martinsen et al., 2007; Hellgren et al., 2007a,b), combined with experimental studies of parasite transmission (Lezhova et al., 2005), should help to resolve the species limits of avian blood parasites and lend context to the lineage-level host ranges provided by regional surveys.

Our data suggest that host–parasitism strategies within the genera Haemoproteus and Plasmodium are variable and can show extreme differences even among closely-related lineages. While we demonstrated that at least some parasites within both genera have been constrained at the level of host-family and even host-species over their evolutionary history, we found evidence of apparent broad host generalism, particularly in certain lineages of Plasmodium. Importantly, wide variability in host specificity among lineages of avian haematozoa may be linked to wide variation in virulence (Garamszegi, 2006). Although several studies have demonstrated negative consequences of haematozoan infection for survival, clutch size, incubation period, fledging success, motor activity and fat accumulation (Bennett et al., 1992; Gustafsson et al., 1994; Nordling et al., 1998; Merino et al., 2000; Valkiunas, 2005), few have accounted for possible differences in the virulence of different lineages infecting a particular host population (but see Zeh tingljev et al., 2008). Variability in host specificity, and therefore virulence, of closely related parasite lineages should be accounted for when estimating their impact on host fitness, immunity or life history. Regional surveys have uncovered numerous parasite lineages with extremely divergent host–parasitism strategies; these would now make good candidates for experimentally testing the assumed linkage between host-specificity and virulence.

Acknowledgements

We thank the governments of Cameroon and Gabon for allowing us to conduct our surveys and providing the necessary permits. Fieldwork in Cameroon and Gabon was only possible thanks to the support of Roger Fotso, Joe Mbelle, Okah Monya, Nohou Ndam, Francis Njie, Martin A.E. Omenotori, Landry Thchignonou, Patrice Christy and the staff of the Ipassa Research Station. We also thank Liliana Gil-Colos, Eben Gering and Jen Reed for help in the lab. MM was supported by a post-doctoral grant from the French Research Ministry, Fieldwork by CG and BS was supported by the Alexander Wetmore Fund of the Division of Birds, National Museum of Natural History, the Smithsonian Institution’s Monitoring and Assessment of Biodiversity (SIMAB) program, Shell Gabon, and the Shell Foundation. Laboratory work was supported by NIH grant GM063258 to RCF.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ijpara.2008.06.005.

References


