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A jack-of-all-trades and still a master of some: prevalence and host range in avian malaria and related blood parasites

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Abstract. A parasite's ability to be a specialist vs. a generalist may have consequences for its prevalence within one or more if its host species. In this study we investigated the relationship between host specialization and prevalence in the highly species diverse avian blood parasites of the genera *Plasmodium* and *Haemoproteus*. Contrary to trade-off hypotheses that may explain host specialization, within both genera the parasites with the ability to complete their life cycles and be transmitted across a wide host range (broad compatibility) were also the most common parasites within their compatible host species. These patterns remained unchanged when the host species with the highest prevalence were excluded, which reduces the possibility that the observed pattern was caused by parasites reaching high prevalence in a single main host, and being "spilled over" to other host species. We hypothesize that a positive relationship between parasite host range and prevalence might be explained by an overall higher encounter rate for the parasites with broad host range, which compensates for possibly reduced performance of parasites in each host species. Overall, these results show that parasites with the ability to successfully infect a wide variety of host species of broad ancestry also can have the ability to be the most prevalent in single host species.

Key words: avian malaria; blood parasites; encounter rate; Haemoproteus; *haemosporidians; host–parasite interaction; host range;* Plasmodium; *prevalence; specialists vs. generalists.*

Introduction

Can a parasite with the ability to infect a wide range of hosts also reach higher prevalence in a given host species, compared to a parasite specialized to that host species? Whether a parasite is restricted to a few host species, i.e., is a specialist, or can complete its life cycle in many different host species, i.e., is a generalist, may affect how virulent the parasite is to each host (Daszak et al. 2000, Pfennig 2001, Garamszegi 2006), genetic variability and the response to selection within each host (Antolin 2008), and how prevalent the parasite is in its susceptible host species (Daszak et al. 2000, Dobson 2004, Keesing et al. 2006). Much of the knowledge of how a parasite's host specificity affects its prevalence in different host species is based on theoretical work (e.g., Holt et al. 2003, Dodson 2004, Kessing et al. 2006), and is rarely tested empirically in natural systems. Here we examine empirically the relationships between parasite host range and prevalence in host species within natural communities of avian blood parasites and bird hosts.

The ability of parasites to be spread in host populations depends on various factors, including how compatible the parasite is to the hosts and how often the parasite encounters the host species (Combes 1997). A parasite's compatibility to a specific host species here is defined as the proportion of host individuals the parasite infects (i.e., susceptible hosts) that live long enough for the parasite to complete its life cycle thus allowing transmission to a new host individual. The degree of susceptibility, i.e., the proportion of individuals in the population that can become infected, is affected by several factors such as whether the parasite has the ability to invade host tissues within the host and whether the parasite can evade the host immune defenses, whereas compatibility also includes the parasite-induced mortality of the hosts. Thus, if a parasite causes a high induced host mortality that reduces the transmission stages of the parasites, then the susceptibility is higher than the compatibility. When parasite prevalence (percentage of infected hosts in the population) in populations is measured, several other factors might influence the observed prevalence, such as possible competition between different parasite strains and birth and death rates of the host that might affect both the proportion of susceptible and compatible hosts in the population. However, taken together, prevalence of a parasite in a given host species is strongly influenced by the proportion of susceptible hosts in the population and the proportion of hosts that are encountering the parasite.

Specialist parasites have evolved adaptations to the few host species that they exploit (Sasal et al. 1999), but specialization to one or a few host species may result in

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trade-offs: reduced ability to infect and reach transmission stages in other host species (Futuyma and Moreno 1988, Pfennig 2001, Garamszegi 2006). An example of adaptation and coevolution between parasites and single host species that may prevent parasites from infecting other host species can be found in sympatric Blackcaps (*Sylvia atricapilla*) and Garden Warblers (*S. borin*). These two sister species share a diverse community of closely related avian blood parasites of the genera *Plasmodium* and *Haemoproteus*, yet each parasite lineage in the community exclusively infects either Blackcaps or Garden Warblers (Pérez-Tris et al. 2007).

Vector-borne parasites may be transmitted to suboptimal or incompatible host species. In these circumstances, a parasite's encounter rate of compatible hosts will be inversely proportional to the abundance of incompatible host species, which therefore act as "sinks" of parasite transmission. Both empirical and theoretical studies suggest that an increase in host species diversity may reduce the prevalence of a pathogen in single host species, due to dilution effects or "zooprophylactic effects" in which high species diversity reduces the chances for the parasite to be vectored into appropriate hosts (Daszak et al. 2000, Dobson 2004, Keesing et al. 2006). When the parasite can access, and be maintained in, a wide range of host species, even if some of them are suboptimal, total parasite prevalence may increase as a direct consequence of the increased abundance of both infected and susceptible individuals (Dobson 2004, Power and Mitchell 2004). Thus, in highly diverse parasite–host–vector communities, in which parasites may be vectored to suboptimal hosts, parasites with a wide host range may compensate for reduced compatibility to a single host species if broadening their host range increases their total host encounter rate. This has been called an "amplification effect" (Keesing et al. 2006).

Avian blood parasites (hemosporidians) of the genus *Plasmodium* (avian malaria) and the closely related genus *Haemoproteus* appear to follow such a pattern. As a group, avian hemosporidians are cosmopolitan and abundant in nearly all bird families (Valkiūnas 2005). They are transmitted by blood-feeding arthropod vectors with variable host preferences, and they exhibit large differences in bird host specificity and prevalence within single host species (Ricklefs and Fallon 2002, Waldenström et al. 2002, Bensch and Åkesson 2003, Malmqvist et al. 2004, Hellgren 2005, Ricklefs et al. 2005, Krizanauskiene et al. 2006, Reullier et al. 2006, Bensch et al. 2007). The infection status of individual hosts is normally determined by the presence of parasites in the blood. For *Haemoproteus,* presence of parasites in the blood represents the stage at which the parasites have formed transmittable gametocytes. For *Plasmodium* parasites, asexual stages may also be present in the blood, thus making it difficult to decide whether a host positive for *Plasmodium* necessarily harbors parasites in the transmission stage. Transmission of parasites is further influenced by parasitemia, the number of parasitized blood cells (Mackinnon and Read 2004), which may vary both within and between bird host species (Palinauskas et al. 2008, Zehtindjiev et al. 2008). Although parasitemia may be the most preferred measure of compatibility between host and parasites, it may cause interpretation problems in naturally infected hosts for which the time since infection is unknown. Further, parasitemia can range between 0.00001% and 10% infected erythrocytes during the course of an individual's infection (Zehtindjiev et al. 2008).

Plasmodium and *Haemoproteus* parasites with broad host range can be abundant within bird communities (Fallon et al. 2005), and their vectors (dipterans of the families Culicidae, Hippoboscidae, and Ceratopogonidae; Valkiūnas 2005) are likely to transfer parasites between different bird species, even if this could be reduced by host preference of the vectors (Malmqvist et al. 2004, Hellgren et al. 2008). The development success of parasite lineages may differ between vector species, thus affecting the probability that a certain host will be infected with a particular parasite lineage (Noden et al. 1995, Ghosh et al. 2000, Mackinnon and Read 2004). In this study we present and analyze empirical data of a multi-host and multi-parasite community of parasites that exhibit a broad difference in the ability to exploit several hosts. The specific hypothesis is that parasites with the ability to infect a wide range of host species may have higher total prevalence when all susceptible species are considered, compared to parasites with narrower host ranges. Thus, we predict that generalist parasites, on average, will be more prevalent in each of their single host species compared to host specialist parasites. To test this hypothesis, we examined 45 *Haemoproteus* and *Plasmodium* lineages obtained from 26 different bird species. These parasite lineages differ in both the number of recorded host species (1–10 hosts) and the prevalence that they reach in each host species.

Methods

Sampling and screening of parasites

We analyzed blood samples ($n = 3981$) collected in Europe between 1987 and 2005 from 26 bird species with at least 10 screened individuals (Appendices A and C). There may always be a trade-off in studies like this between being able to add more species vs. using as many individuals per species as possible. In our case the species that were of lower number, i.e., below 20 (which means $\geq$5% error in prevalence estimates) did not show any deviations in prevalence compared with the overall mean (Appendix A), except in the case of *Acrocephalus palustris*, which had a total prevalence of 0.08 (overall mean 0.40). However, this deviation is in the right direction (i.e., opposite of obtaining high parasite prevalence values that are based on few sampled individuals) and therefore should not influence the data set in the direction of the observed positive trends.

Blood samples were stored in SET-buffer (0.015 M NaCl, 0.05 M Tris, 0.001 M EDTA, pH 8.0) until DNA was extracted using either standard phenol-chloroform or ammonium acetate methods (Richardson et al. 2001, Sambrook et al. 2002). The extracted DNA was simultaneously screened for parasites of the genera *Haemoproteus* and *Plasmodium*, using nested-PCR (polymerase chain reaction) protocols described by Waldenström et al. (2004) and Hellgren et al. (2004). These two methods target the same region of the mitochondrial cytochrome *b* (cyt *b*) gene of the parasites, and show similar sensitivity (Hellgren et al. 2004). Parasites detected by a positive amplification were identified by sequencing 479 bp (base pairs) of the cyt *b*, and parasites with sequences differing by one nucleotide substitution were considered to represent evolutionarily independent lineages (Bensch et al. 2004, Ricklefs et al. 2005). Of the lineages used for analysis in this study, 37 have been found in more than one individual and the eight single individual lineages had all been amplified from more than one PCR run, thus minimizing the risks of assigning a lineage due to a PCR error.

All obtained lineages were used to construct a neighbor-joining tree with the program MEGA 3.1 (Kumar et al. 2004), using a Kimura two-parameter distance matrix under a gamma distribution with $\alpha = 0.874$ estimated using Modeltest 3.7 (Posada and Crandall 1998). The tree was rooted using 10 *Leucocytozoon* lineages. Parasite lineages formed two separate clusters representing the two genera, *Haemoproteus* and *Plasmodium* (Appendix B). Individual lineages were assigned to genera based on their phylogenetic relationships with previously identified lineages. All PCR experiments contained one negative control for every eight samples. In the very few cases in which negative controls showed signs of amplification (never other than faint bands in agarose gels), the whole PCR batch was run again to make sure that all positives were true.

Of the obtained parasite lineages, only lineages with confirmed transmission in Europe (Hellgren et al. 2007) were used in our analyses of the relationships between host range and parasite prevalence (Appendices B and C). We excluded parasites putatively transmitted in other geographic areas because the prevalence of many malaria parasites varies seasonally as a result of relapsing infections during major transmission periods (Valkiūnas 2005). As a consequence, tropically transmitted parasites found in European migratory birds may show much lower prevalence in Europe than in their African transmission areas, which makes their prevalence not comparable with the prevalence of parasites transmitted in Europe. Parasite transmission in Europe was confirmed for lineages that had been found either in a resident European bird species or in a juvenile migrant before autumn migration. It should be noted, however, that if all lineages were included in the analysis, the results did not change qualitatively. For identification of transmission area we also used information from species with fewer than 10 sampled individuals, including a total of 5569 sampled individuals in Europe. The host range of some parasite lineages would have increased slightly by using the whole data set, but including these did not change the main results of this study (for transmission areas of particular lineages, see also Hellgren et al. [2007]).

Host range vs. prevalence

The prevalence of each parasite lineage in each bird species was calculated as the proportion of individuals infected by the parasite. All prevalence comparisons were done separately for *Plasmodium* and *Haemoproteus* and all statistical analyses were done using arcsine square-root transformed values of prevalence.

Host range of the different parasite lineages was measured as the number of host species in which they were found. The host range of a parasite could also be measured as the taxonomic distance between identified susceptible hosts. One such measurement of taxonomic host range is the S_{TD} index, which measures the mean taxonomic distance among the identified host species (Clarke and Warwick 1998, Poulin and Mouillot 2003). However, using the S_{TD} index for our data would cause patterns in which a parasite that had been found in many species over a large taxonomic range could get a lower S_{TD} value than a parasite that had been found in only two host species that had the same maximum taxonomic distance as the parasite found in many host species (Fig. 1). To get a measure that included both the diversity of host species and the taxonomic distance between such species, we used a modified version of the host-range index S_{TD}. The modified index (S^*_{TD}) took into account not only the number of hosts species of the parasite and the taxonomic distance among them, but also the variance of the taxonomic distance among host species (Var S_{TD}; Poulin and Mouillot 2003). With this modification, the index reflects both the taxonomic distance between the hosts and the number of host species in which it has been observed (Fig. 1). The S^*_{TD} values were calculated as:

$$S^*_{\text{TD}} = S_{\text{TD}} + \frac{s-1}{1+\text{Var}\,S_{\text{TD}}}$$

$$S_{\text{TD}} = 2\frac{\sum\sum_{i<j}\omega_{ij}}{s(s-1)}$$

(see Clarke and Warwick 1998, Poulin and Mouillot 2003), and

$$\text{Var}\,S_{\text{TD}} = \frac{\sum\sum_{i\neq j}(\omega_{ij}-S_{\text{TD}})^2}{s(s-1)}$$

where ω_{ij} is the taxonomic distance between host species i and j (i.e., how many taxonomical steps need to be taken to get to their common ancestor, in our case species, families, and genera, because of the difficulty of

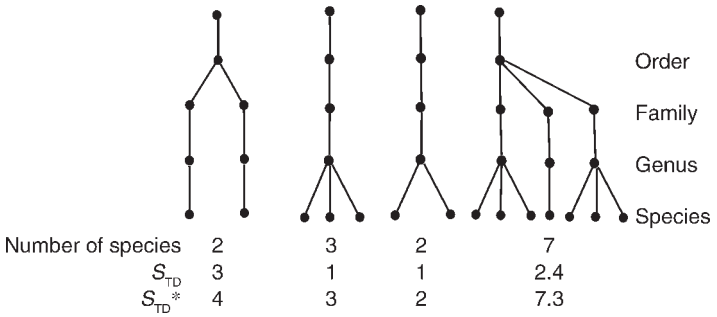

FIG. 1. Hypothetical example of how three different measurements of host range are related to each other. Note that S_{TD} (an index that measures the mean taxonomic distance among the identified host species) can produce a high value if the parasite is found in few species, although the maximum taxonomic distance is the same as when found in many different species. The modified index (S^*_{TD}) accounts for not only the number of hosts species of the parasite and the taxonomic distance among them, but also the variance of the taxonomic distance among host species (Var S_{TD}).

accurately determining branch lengths in a broad phylogenetic tree of birds; see Fig. 1 and Poulin and Mouillot 2003), and s is the number of host species exploited by the parasite.

Statistics

We tested whether a broad host range was associated with a high prevalence in single host species. To do this we used a sample in which all parasites identified were tested for presence or absence in all host species investigated. In a first analysis we used the maximum prevalence in a given host species found for each parasite. We are aware that parasites having one main host species, from which they "spill over" into other less preferred host species, could bias our sampling, e.g., if by chance we had sampled the main host for some parasite lineages, but had sampled only non-preferred host species for other parasite lineages. In order to avoid such bias, we repeated all analyses using the second-highest prevalence of the multi-host parasites. We further tested if patterns remained unchanged when we excluded both the highest prevalence found in multi-host parasites and the prevalence of parasites found in one species only, thereby removing potential bias caused by assigning an artificially narrow host range to parasites with low prevalence.

To test whether the different measurements of parasite prevalence were associated with host range (either the number of hosts or the S^*_{TD} index in different analyses), we used both standard regression methods and phylogenetically correct comparative methods based on standardized phylogenetically independent contrasts of both host range and prevalence. Phylogenetically correct methods account for the fact that closely related parasites are likely to share traits such as host range and prevalence due to common ancestry, thereby violating statistical independence assumptions. To calculate phylogenetically independent contrasts, we constructed a neighbor-joining (N-J) tree of the parasites included in the analysis, using PAUP* (Swofford 1993) and the best substitution model found by Modeltest 3.7 (Posada and Crandall 1998). We further constructed phylogenies using Bayesian inference using MrBayes 3.1 (Ronquist and Huelsenbeck 2003), based on 1 million iterations. The topology of both the Bayesian and the N-J trees were almost identical, with only small differences in some branches that were poorly supported in both phylogenies (data not shown). For the sake of simplicity we continued with the N-J trees when constructing the phylogenetic contrasts in PDAP (phenotypic diversity analysis programs). The trees were used in the software PDAP to calculate phylogenetically independent contrasts (Garland et al. 1993, Maddison and Maddison 2004, Midford et al. 2005). To have ultrametric phylogenies for calculations of independent contrasts, the lengths of the branches were manipulated according to Graffen's method (Garland et al. 1993). The analyses were repeated using unmanipulated trees and trees manipulated according to Nee's method (Garland et al. 1993) without any qualitative change in the results. The phylogenetic contrasts were calculated according to Felsenstein (1985) and regressions were calculated using the reduced major axis method forced through the origin using standardized contrasts values. All tests were done separately using lineages belonging to each of the two genera *Haemoproteus* and *Plasmodium*.

RESULTS

Out of 3981 investigated individuals, 1571 carried an infection of either *Haemoproteus* or *Plasmodium*. We found a total of 98 different lineages based on mtDNA sequences: 35 *Plasmodium* and 63 *Haemoproteus*. The total parasite prevalence, including lineages both with and without confirmed European transmission, ranged between 8% and 94% in each species, with a mean species prevalence of 40% (Appendix A). Of the infected individuals, 80 were found to be infected by more than one parasite. When calculating prevalence, we treated resolved multiple infections (for method see Pérez-Tris and Bensch 2005) as separate events (i.e., if two different parasite lineages were always found in double infection in all sampled individuals, then both parasites would have a prevalence of 1).

European transmission was confirmed for 34 *Haemoproteus* lineages and 11 *Plasmodium* lineages (Appendix B). For *Haemoproteus,* 26 of the 34 lineages were restricted to one host species. For *Plasmodium*, five of the 11 lineages were restricted to single host species. The maximum number of host species was nine for the *Plasmodium* lineage P-SGS1 and 10 for the *Haemoproteus* lineages H-PARUS1 and H-WW2.

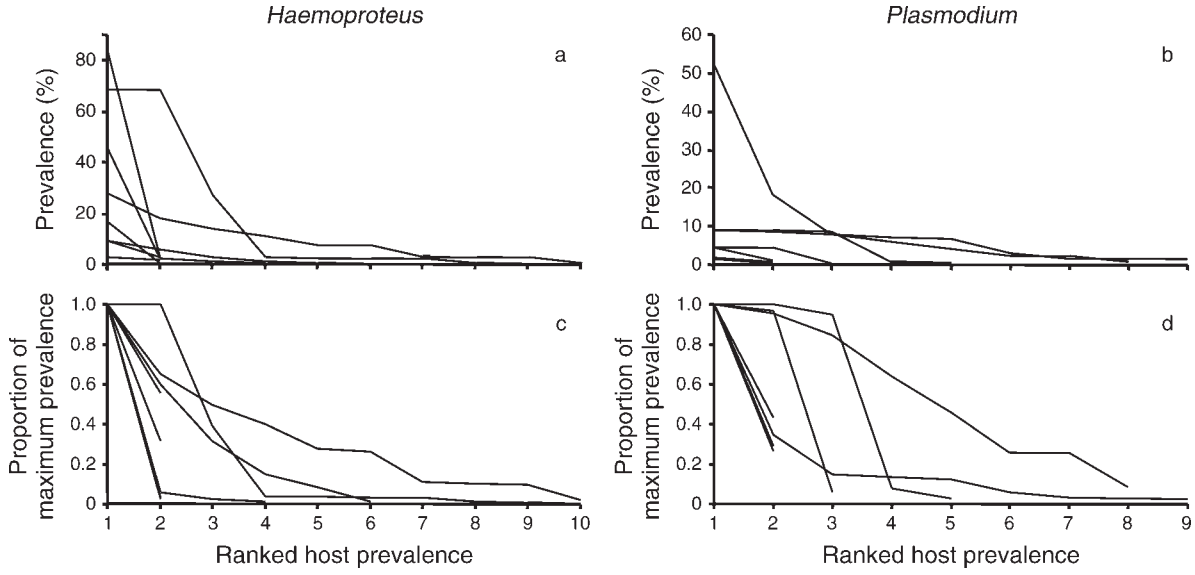

FIG. 2. Ranked prevalence of parasites found in multiple hosts: (a, c) *Haemoproteus* lineages (b, d) *Plasmodium* lineages. In order to visualize relative differences in prevalence, in panels (c) and (d) the highest prevalence in a single host is set to 1, and the proportion of such maximum prevalence is shown for additional hosts.

Host range and maximum prevalence

Multi-host parasites exhibited variable prevalence across their host range (Fig. 2a–d). The maximum prevalence of a parasite lineage in a single host species increased significantly with an increased host range (S^*_{TD}) of the parasite. This was observed for both *Plasmodium* (Table 1, Fig. 3a, b) and *Haemoproteus* lineages (Table 1, Fig. 4a, b). This pattern remained unchanged when we took the parasites' relatedness into account in phylogenetic regressions (Figs. 3b and 4b, Table 1; for all

TABLE 1. Statistical tests of associations between parasite host breadth and parasite prevalence.

Test, regression type, and host specificity	*Plasmodium* spp			*Haemoproteus* spp.		
	df	*t*	*P*	df	*t*	*P*
Maximum prevalence vs. host species						
Standard						
Nr. host	9	4.58	0.001	32	3.74	<0.001
S^*_{TD}	9	3.61	<0.01	32	3.83	<0.001
Phylogenetic						
Nr. host	9	6.86	<0.0001	32	4.99	<0.0001
S^*_{TD}	9	6.65	<0.0001	32	4.70	<0.0001
Second-highest prevalence vs. host species						
Standard						
Nr. host	9	2.15	<0.01	32	4.86	<0.0001
S^*_{TD}	9	3.32	<0.01	32	4.57	<0.0001
Phylogenetic						
Nr. host	9	5.66	<0.001	32	5.60	<0.0001
S^*_{TD}	9	5.58	<0.001	32	5.52	<0.0001
Second-highest prevalence, only unspecific parasites						
Standard						
Nr. host	4	4.7	<0.01	6	4.285	<0.01
S^*_{TD}	4	3.0	<0.05	6	3.824	<0.01
Phylogenetic						
Nr. host	4	3.4	<0.05	6	9.57	<0.0001
S^*_{TD}	4	3.1	<0.05	6	10.3	<0.0001

Notes: Regressions were calculated either using the total number of host species in which a parasite was found (without taxonomic relation of the host; Nr. host), or taking into account taxonomic distances between host species (S^*_{TD}). The table shows results obtained using standard (conventional) regression analyses and phylogenetic regressions. The same analyses were repeated taking away the extremes on both scales of the regression (i.e., second-highest prevalence vs. host species and second-highest prevalence, using only parasites with $n \geq 2$ hosts).

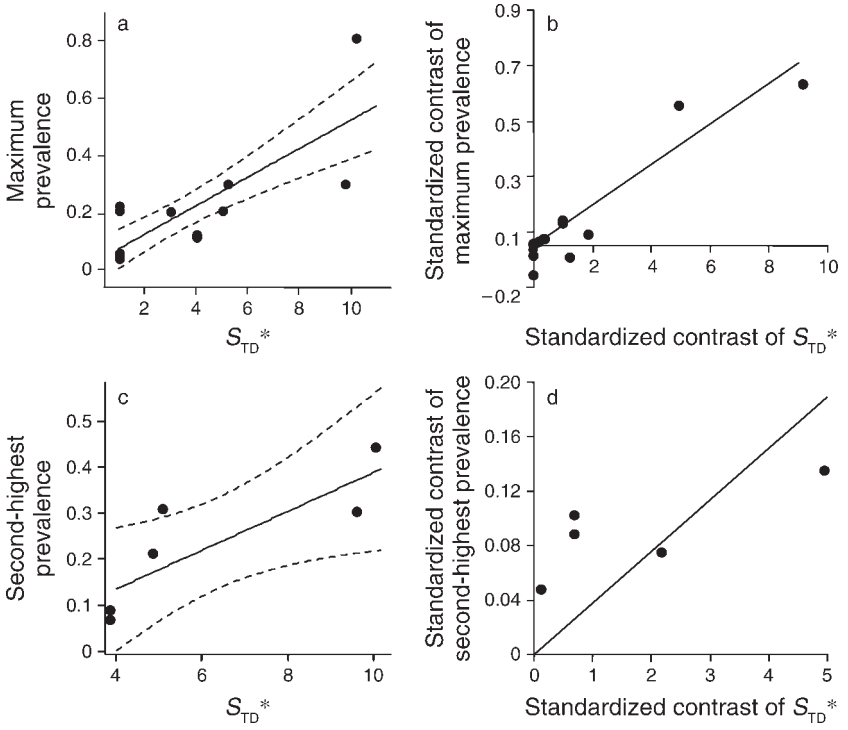

FIG. 3. Correlations between host range (S^*_{TD}) of *Plasmodium* lineages and host prevalence. (a) Maximum single-host prevalence (arcsine square-root transformed values) vs. host range. (b) Standardized phylogenetic contrasts of host range and maximum single-host prevalence. (c) Second-highest host prevalence without lineages found in single hosts vs. host range. (d) Standardized phylogenetic contrasts of host range and second-highest host prevalence without lineages found in single hosts. For statistics and values see Table 1.

regressions, $P < 0.001$). Using the number of host species as the host-range estimate produced similar results (Table 1). If parasites exhibited a "main host" in which the prevalence was high, a wide host range could potentially indicate that the parasite spilled over to other species, in which the prevalence should then be low. To examine whether the observed pattern could have resulted from spillovers, we conducted the same tests using the second-highest prevalence for the different multi-host lineages. Even if the host with the highest prevalence was removed, the results remained unchanged (for all regressions, $P < 0.001$; Table 1).

It should be noted, however, that spillover effects would still be seen if parasites had the ability to specialize on more than one host species, i.e., if they had several "main host species." Accounting for the potential bias of spillover, and that we may have failed to identify additional hosts for some of the lineages scored as single-host lineages, we restricted the analyses to lineages found in more than one host and regressed their second-highest prevalence against host range. The positive associations between prevalence and host range remained significant (Table 1), both when using S^*_{TD} and number of hosts (for *Haemoproteus* spp., for all measures of host range, $P < 0.01$; Fig. 4 c, d; for *Plasmodium* spp., for all measures of host range, $P < 0.05$; Fig. 3c, d). Even if no positive associations had been found between host range and prevalence, it is important to note that the data still show that parasites with broad host ranges have the ability to reach high prevalence in single host species. Thus, the *Plasmodium* lineage with the widest host range (P-SGS1: found in nine out of the 26 investigated bird species) was found in 53% of the sampled House Sparrows (*Passer domesticus*, $n = 232$), and the *Haemoproteus* lineage with the broadest host range (H-PARUS1: found in 10 of the 26 investigated bird species) reached a prevalence of 68% in both Great Tits (*Parus major*: $n = 28$) and Blue Tits (*Parus caereuleus*: $n = 35$).

DISCUSSION

In contrast to predictions from theories based on a trade-off between being a specialist and a generalist (Futuyma and Moreno 1988, Pfennig 2001, Garamszegi 2006), we found that parasites exhibiting the widest host range also exhibited the highest prevalence in single host species. This pattern was found within each of the two sister genera *Haemoproteus* and *Plasmodium*.

The parasite–host–vector system of avian *Haemoproteus* and *Plasmodium* is highly diverse with respect to all included parties. Globally this system contains roughly 10 000 potential avian host species and perhaps a similar number of unique lineages of *Haemoproteus* and *Plasmodium* (Bensch et al. 2004). The number of

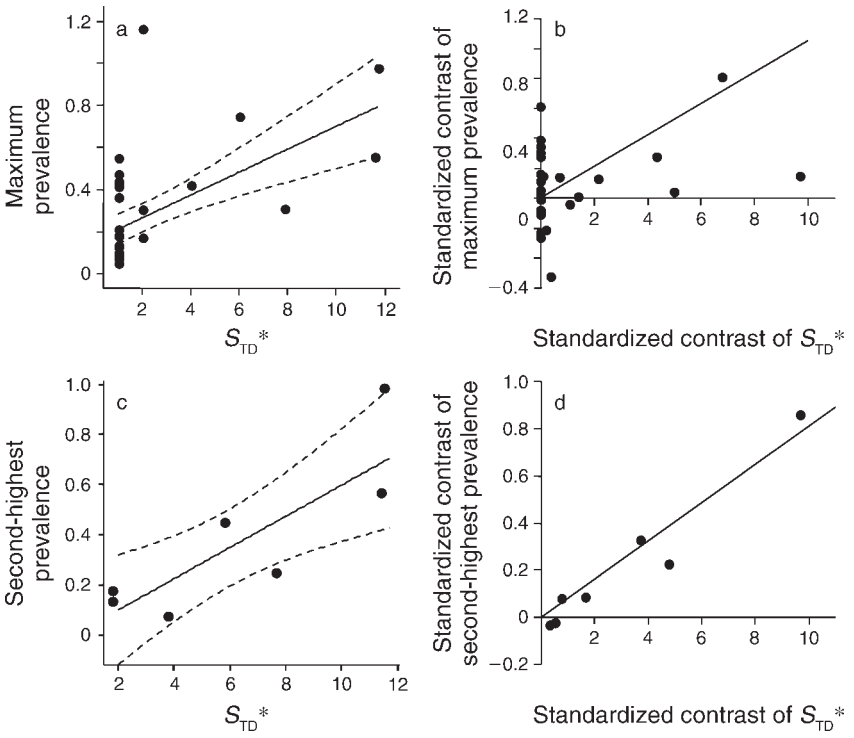

FIG. 4. Correlations between host range (S^*_{TD}) of *Haemoproteus* lineages and host prevalence. (a) Maximum single-host prevalence (arcsine square-root transformed values). (b) Standardized phylogenetic contrasts of host range and maximum single-host prevalence. (c) Second-highest host prevalence without lineages found in single hosts vs. host range. (d) Standardized phylogenetic contrasts of host range and second-highest host prevalence without lineages found in single hosts. For statistics and values see Table 1.

potential dipteran vectors is unknown, but the genera *Culicoides*, *Aedes*, *Culex*, and *Anopheles* are highly specious, exceeding 5000 named species (Capinera 2004). Because many vector species may take blood from several host species (Hellgren et al. 2008), the chance that a parasite ends up in a suboptimal host should be higher in species-rich vector–host systems than in species-poor host–vector systems (Dobson 2004, Keesing et al. 2006). The ability of a parasite to infect and complete its development in many different host species, even if it compromises specialization to one single host species, may boost parasite transmission success because of the increase in both the number of potential hosts and the probability of successful transmission, thereby increasing the overall prevalence of the parasite in the host community (Combes 1997).

Correlations between host range and prevalence

We applied several different methods for measuring prevalence and host range of the parasites to examine whether the observed correlation between prevalence and host range represents a biological rather than a statistical pattern resulting from biased sampling efforts. This included measurements that take into account the taxonomic relationship among the hosts (Clarke and Warwick 1998, Poulin and Mouillot 2003) and the relatedness of the parasites, to prevent biasing effects of phylogeny when estimating host range and prevalence of parasites. Regardless of the type of analysis chosen, the observed positive correlations between parasite host range and prevalence remained significant (Figs. 3a, b and 4a, b, Table 1). Such associations could be influenced by sampling effort if some parasites are always in low prevalence and therefore are missed when estimating host range. The result would be an underestimated host range for the less prevalent parasites. If all parasites have a "main host" then the chance of finding this host would increase with the number of hosts sampled for a particular parasite. However, even if we excluded parasites that were found in only one host species and considered the second-highest prevalence of multi-host parasites, the associations remained unchanged (although the significance levels were lowered, as expected due to strongly reduced sample size; Table 1, Figs. 3c, d and 4c, d). Our results remained consistent also when taking the phylogeny of the parasites into account, which shows that the traits responsible for the ability to have a wide host range and to be highly infective to particular host species have evolved several times among both *Plasmodium* and *Haemoproteus* parasites, a pattern also observed for both parasite genera by Fallon et al. (2005). Hence, we conclude that there must be a true biological pattern behind the positive association between host range and single-host

prevalence for both *Haemoproteus* and *Plasmodium* parasites.

Spillover or amplification effects?

Transmission of parasites to several host species may be driven from a major reservoir species in which the parasite reaches high prevalence in that host and spills over to other species that lack the ability to maintain the parasite in a viable population (Daszak et al. 2000, Power and Mitchell 2004). However, for both *Haemoproteus* spp. and *Plasmodium* spp., such a simple scenario seems unable to explain the observed data, because we found positive correlations between host range and maximum prevalence even when the main host (potential reservoir) was excluded from the analysis. Moreover, parasites with a broad host range reached high prevalence over a number of species (Fig. 2). Further research is needed to establish whether this system is driven solely by amplification effects or if there is a combination of spillover effects and amplification effects. Studying *Plasmodium* and *Haemoproteus* parasites in a bird community in Missouri, Ricklefs et al. (2005) found that the number of host species was positively correlated with the total number of infected individuals in the bird community. In other words, the more species in which a parasite lineage is found, the more individuals in the community are infected. As the infectiveness of the parasite in a specific host species is related to both the susceptibility of the host and the encounter rate (Combes 1997), a wide host range might increase the abundance of the parasite lineage in the total bird community. Vectors will be more likely to encounter infected hosts and, with several bird species functioning as hosts, a higher proportion of the vectors' blood meals will lead to successful transmission of the parasites, particularly in situations in which the vector is a host generalist. The encounter rate with that parasite would be increased for all species in the bird community leading to higher prevalence in all of the hosts. Hence, the prevalence in each host species is amplified due to a wide host range. This is, of course, a rather simplified hypothesis that does not take into account possible effects of parasite competition (Paul et al. 2002, de Roode et al. 2005, Råberg et al. 2006), parasite–host interactions due to fluctuating selection (Hamilton and Zuk 1982, Eshel and Hamilton 1984), or vector–host interactions (Malmqvist et al. 2004). Nevertheless, we suggest that it may partly explain why parasites with a broad host range often reach high prevalence in a range of host species.

It is also noteworthy that a similar case has been observed when studying the global assembly of parasites in a single species, the House Sparrow, *Passer domesticus*. In Europe the most prevalent *Plasmodium* lineage is SGS1, which also has been found in the largest number of host species. However, in House Sparrows in North America, the most abundant parasite lineage was GRW4, a lineage that has been found in an additional 38 bird species, globally (Alfonso Marzal, *unpublished data*). As we have shown here, avian hemosporidians may be a good model system for investigating the dynamics of multi-host/multi-parasite communities. Bird and parasite communities are often very diverse and could be suitable as a system in which to validate other theoretical work in natural populations, such as the extent to which prevalence is affected by differences in within- and between-species transmission in host communities with variable diversity (Holt et al. 2003, Kessing et al. 2006). However, the only known data on transmission of avian malaria-related parasites to date, i.e., based on the sister genus *Leucocytozoon* (which is vectored by simuliid flies), suggest that within-host-species transmission is higher than between-host-species transmission (Hellgren et al. 2008).

What determines the host specificity of avian malaria parasites?

Among both *Plasmodium* and *Haemoproteus* parasites it seems to be common that parasites are able to infect a broad range of hosts (Waldenström et al. 2002, Beadell et al. 2004, Ricklefs et al. 2004, 2005, Fallon et al. 2005, Ishtiaq et al. 2006, Krizanauskiene et al. 2006), a feature that has evolved repeatedly in the group (Fallon et al. 2005). Although parasite manipulation of the feeding behavior of the vector has been shown in human malaria (*P. falciparum*; Anderson et al. 1999, Lacroix et al. 2005) and vectors have been shown to exhibit different degrees of host specificity (Dekker et al. 2001, Pates et al. 2001, Malmqvist et al. 2004), avian hemosporidian parasites may often be transmitted between host species. As different host species probably provide different environments for the parasite, the between-generation environment might thus be rather unstable. Hence, it would be beneficial for the parasite to evolve physiological plasticity or abilities to survive in many different hosts (Richards et al. 2006).

Birds are exposed to a wide variety of parasites in this system, especially migratory species that are exposed to different parasite faunas in tropical and temperate regions. Frequent contact with various parasites may have promoted the evolution of generalist immune defense mechanisms, which may better protect the hosts against diverse parasite communities. Thus, if the host could not afford the trade-off of a specific immune response to a particular parasite lineage, then there would be no, or few, specific traits for the parasite to coevolve with, making it possible for the parasites to infect a variety of host species without reducing infectiveness to preferred host species. However, being a generalist in this system might take more than being able to overcome the acquired immune system of the host. For example, different host species may be fundamentally different habitats for the parasites if the chemical properties of the blood are species-specific and such variation can determine the ability of different parasites to invade host cells (Villegas et al. 2006). The system of avian hemosporidian parasites

is revealing itself as a complex, yet affordable system in which future research may help us to understand the interplay of host immunity and parasite ecology as possible determinants of the specificity of host–parasite relationships.

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APPENDIX A

Number of sampled host species, total parasite prevalence (sum of both *Haemoproteus* and *Plasmodium* infections), and number of different parasite lineages retrieved in each bird species (*Ecological Archives* E090-200-A1).

APPENDIX B

Phylogenetic relationship (neighbor-joining) of all *Haemoproteus* and *Plasmodium* lineages found in 26 different passerine bird species (*Ecological Archives* E090-200-A2).

APPENDIX C

Found lineages, GenBank number, the bird species wherein the lineages were found, the country where the samples were collected, and number of infected individuals (*Ecological Archives* E090-200-A3).