Host compatibility rather than vector-host-encounter rate determines the host range of avian Plasmodium parasites

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Blood-feeding arthropod vectors are responsible for transmitting many parasites between vertebrate hosts. While arthropod vectors often feed on limited subsets of potential host species, little is known about the extent to which this influences the distribution of vector-borne parasites in some systems. Here, we test the hypothesis that different vector species structure parasite-host relationships by restricting access of certain parasites to a subset of available hosts. Specifically, we investigate how the feeding patterns of Culex mosquito vectors relate to distributions of avian malaria parasites among hosts in suburban Chicago, IL, USA. We show that Plasmodium lineages, defined by cytochrome b haplotypes, are heterogeneously distributed across avian hosts. However, the feeding patterns of the dominant vectors (Culex restuans and Culex pipiens) are similar across these hosts, and do not explain the distributions of Plasmodium parasites. Phylogenetic similarity of avian hosts predicts similarity in their Plasmodium parasites. This effect was driven primarily by the general association of Plasmodium parasites with particular host superfamilies. Our results suggest that a mosquito-imposed encounter rate does not limit the distribution of avian Plasmodium parasites across hosts. This implies that compatibility between parasites and their avian hosts structure Plasmodium host range.

1. Introduction

Parasites are heterogeneously distributed across hosts [1]. This heterogeneity in host distribution can arise owing to (i) variability in the frequency of encounters between hosts and parasites and (ii) the ability of parasites to invade and persist on the hosts they encounter [2]. Combes [2] described these ecological drivers of host distribution as the encounter and host compatibility filters, respectively. Assessing the relative strength of these filters is a fundamental step in determining mechanisms that govern the distribution of a parasite across hosts. Understanding factors that modulate host range is important because changes in these factors alter transmission dynamics [3–5] and introduce novel parasites to naive hosts, sometimes with devastating consequences [6].

Previous studies have empirically demonstrated that both the encounter and host compatibility filter can be important obstacles for host infection. Studies commonly assess the strength of these filters by controlling for the encounter filter through experimental infection. These demonstrate that parasites differ in their compatibility with hosts [7–9], and that many are capable of infecting hosts outside their natural host range [10]. Infection probabilities on novel hosts can increase with phylogenetic relatedness with the original host, suggesting that the compatibility filter strengthens with increasing host phylogenetic distance [9,10]. Measuring the encounter filter directly in nature can be logistically difficult; however, studies that have done so reveal interesting patterns. Strong encounter filters can mask the influence of the host compatibility filter if less susceptible host species experience more encounters with parasites [11]. Strong encounter filters can exist in spite of high host–parasite sympatry. Non-parasitized host
species can occur in close proximity to highly parasitized host species [12], suggesting fine-tuning in the mechanisms of parasites to encounter hosts, and of hosts to evade them.

Vectors control host encounters for a diversity of parasites and provide a convenient way to measure encounter rates in nature. Many arthropod vectors transmit parasites between vertebrate hosts during blood-feeding activities. Thus, blood-feeding patterns effectively set the encounter rate between vector-borne parasites and hosts. Mosquitoes, which are important vectors for a diversity of pathogens, are known to feed heterogeneously across hosts by using some species disproportionately, relative to their abundance [13–15]. This heterogeneity in mosquito-feeding patterns can strongly influence disease transmission dynamics [3,4,13,16]. Mosquito-feeding networks may also be compartmentalized [17], with certain vector species using a distinct subset of available host species [14,18–21]. For instance, in the northeastern United States, *Culex restuans*, *Culex pipiens*, and *Culiseta melanura* obtain blood meals from birds, while the sympatric *Aedes vexans*, *Ochlerotatus* and *Anopheles* species rely primarily on mammals for blood meals [18,22,23].

Compartmentalization in vector-feeding patterns across hosts may serve as an ecological barrier to transmission, and limit access of vector-borne parasites to different suites of hosts [20]. In a community of hosts, vectors and vector-borne parasites, vector species can impose a limiting encounter filter for parasites by feeding on non-overlapping or weakly overlapping subsets of potential hosts [20,24]. These subsets form compartments in an interaction network that summarizes the feeding patterns of vectors on host species. If this network defines the routes parasites take to move between hosts, parasites would move more readily between hosts that exist within a compartment than between hosts that occupy different compartments. Accordingly, this would tend to homogenize parasite assemblages across host species that share the same compartments in the mosquito–host network. This model suggests an easily testable hypothesis, namely that host species fed upon by the same vector species harbour the same parasite species.

Avian malaria parasites of the genus *Plasmodium* provide a suitable system to investigate the impact of vector-feeding behaviour in delimiting the host range of a parasite. Avian *Plasmodium* parasites have complex life cycles, which include asexual stages of reproduction in a bird host and sexual stages of reproduction within a mosquito vector [25]. Briefly, the life cycle within the mosquito begins when gametocytes from an infectious bird are ingested during a blood meal. These gametocytes differentiate into gametes that fuse to form oocysts in the mosquito midgut. Ookinetes develop into oocytes that attach to the midgut wall. Sporozoites develop within oocytes. Once released, they selectively invade the mosquito’s salivary glands. Successful transmission between birds occurs when a mosquito survives long enough for the parasite to proceed through this life cycle and injects sporozoites into another bird upon taking a subsequent blood meal.

Despite the potential importance of vectors in structuring *Plasmodium*–host relationships, most studies have focused on characterizing the diversity of *Plasmodium* infections in avian hosts [26–32]. The identification of the vectors in these systems has lagged behind (but see [21,24,33–36]). Even fewer studies have investigated the role of vectors in the transmission process and in the evolutionary biology of these parasites (but see [21,24,37,38]). However, many studies hypothesize that vector dynamics may explain distributional patterns of these parasites [21,37,39,40].

Patterns of avian *Plasmodium* host range are highly idiosyncratic [26–29,38,41]. *Plasmodium* parasites are non-randomly distributed across host species, typically infecting only a subset of available hosts [26,28]. Some avian *Plasmodium* taxa are nearly restricted to a single host species [29,32]. In addition, these relationships can vary geographically, and *Plasmodium* parasites may occur on different hosts across their range [28,41]. These host–parasite relationships are not well preserved through time [42], and co-phylogenetic analyses of parasites and hosts reveal that host switching over evolutionary time-scales is pervasive [43,44]. These geographically variable relationships and host-switching events suggest that avian *Plasmodium* parasites have the ability to evolve the necessary machinery to exploit a broad range of hosts, despite their restricted host ranges at any given point in space and time. This raises the possibility that an encounter filter imposed by modular mosquito-feeding patterns could account for this apparent contradiction, by restricting access to only a subset of hosts that can be exploited by an avian *Plasmodium* parasite [24].

The topic has been approached before within the avian *Plasmodium* system. Gager et al. [24] integrated information on the distribution of *Plasmodium* lineages across vectors and the avian host *Turdus grayi* in central Panama. They discovered that two common *Plasmodium* lineages of *T. grayi* occurred in different vector species, demonstrating that the two species of vectors feed on *T. grayi*. In addition, the vectors carried many *Plasmodium* lineages that were not isolated from *T. grayi* despite access to this host. The study did not support the existence of a limiting encounter filter because *T. grayi* were exposed to both vectors and all the avian malaria lineages in the study area, but only a subset of *Plasmodium* lineages were found to infect *T. grayi* individuals. However, the study was limited to a single avian host, did not resolve the feeding patterns of vectors, and did not explore the hypothesis in a community context.

Here, we evaluate the influence of mosquito vectors in modulating the distribution of specific *Plasmodium* taxa across a community of avian hosts in suburban Chicago, IL, USA. Specifically, we identify local avian *Plasmodium* vectors and use a series of analyses to investigate whether their feeding patterns influence how *Plasmodium* parasites are distributed across avian hosts. We also investigate the potential for host compatibility to structure these relationships. Cumulatively, we assess the relative strength of a mosquito-imposed encounter filter and compatibility filter in delimiting the distribution of avian *Plasmodium* parasites across a host community in an effort to understand factors that influence parasite host range. We find mosquito-feeding patterns do not explain the heterogeneous distributions of *Plasmodium* parasites across avian hosts, suggesting that host compatibility issues dominate processes that structure parasite host range in this system.

2. Material and methods

(a) Study system and sampling

The study was conducted in 17 scattered suburban sites including parks, cemeteries and residential communities in Chicago, IL, USA ([45]; http://www.vetmed.wisc.edu/WNV). Avian blood samples
were collected from May through to September during 2006 and 2007. Mosquito samples were collected with canopy-level Centers of Disease Control light traps [46] from June through to September during the same years at 13 of the 17 sites in which birds were captured.

(b) Resolving mosquito-feeding patterns

Mosquito-feeding patterns were resolved by Hamer et al. [14]. The study identified the vertebrate source of 1043 blood meals of nine mosquito species in suburban Chicago. Six of the mosquito species were observed to feed on birds. However, only C. pipiens, C. restuans and A. vexans were well sampled, fed on birds and were abundant within the study area [46]. Avian blood meals were recovered from 488 C. pipiens, 172 C. restuans and 15 A. vexans individuals sampled from 2005 to 2007. An additional 75 C. pipiens and 77 C. restuans from 2008 to 2009 were added to the analysis presented here. Molecular procedures for identifying Culex blood meals may be found in Hamer et al. [14]. Engorged mosquitoes were sampled in the same study sites at which both avian hosts and mosquito vectors were surveyed for parasites. While C. pipiens represents a well-known species complex, previous study showed that introgression of molestus and quinquefasciatus forms is minimal in the Chicago population [47]. Thus, the numerous behavioural and physiological differences between these forms [48] are unlikely to influence the patterns presented here.

(c) Resolving parasite – bird and parasite – mosquito relationships

Avian hosts were sampled using standard mist netting protocols. Blood was obtained by jugular venepuncture and was stored in BA-1 diluent or Longmire’s lysis buffer at less than −20 °C. A sub-sample of 10 μl was used to extract DNA using an ammonium acetate protein precipitation procedure. Samples were purified through a standard isopropanol precipitation followed by two consecutive washes with 70 per cent ethanol. Samples were eluted in double-distilled polymerase chain reaction (PCR)-grade water for at least 3 days before further processing. DNA samples were screened for the presence of haemosporidian parasites through a PCR that targeted a small segment of the 16S rRNA gene [49]. Samples that screened positive with the 16S rRNA primers were used in a secondary nested PCR that targeted a 552 bp fragment of the haemosporidian cytochrome b gene. Details of this reaction are presented by Fecchio et al. [50]. The fragment was sequenced to identify the haemosporidian responsible for the infection.

The taxonomy of avian Haemosporida is controversial and currently unresolved. Traditionally, subtle morphological characters were used to distinguish taxa [25]. However, recent studies have demonstrated substantial genetic diversity within some morphospecies, and have raised the possibility of cryptic species in this system [31,51–53]. However, the status of most haemosporidian parasites as biological species remains untested. Thus, no species level of genetic divergence can be established. In addition, reliable independent nuclear markers are not available to identify isolated lineages by linkage disequilibrium criteria [51]. Here, we delimit evolutionary-independent parasite lineages based on the similarity of cytochrome b haplotypes in a manner similar to Ricklefs et al. [29]. Evolutionary-independent lineages are defined as the set of closely related (less than 1% sequence divergence) monophyletic parasite mitochondrial haplotypes recovered from the same host species or set of host species. Cytochrome b haplotypes of Plasmodium lineages identified in this manuscript are deposited in GenBank (accession no. KC789821–KC789828).

Three mosquito species (A. vexans, C. pipiens, and C. restuans) that were abundant [46] and observed to feed on birds in Chicago [14] were screened for the presence of Plasmodium parasites. Previous research has demonstrated that these Culex species are known avian malaria vectors [25] and are infected with many of the same avian Plasmodium lineages [36]. Little information exists on the vectormyia of A. vexans. This species was included in this parasite survey because it fed on birds and was abundant in the study site [46]. Individuals were pooled by species, site and date of capture. Pool sizes varied from 1 to 36 whole-bodied individuals. Culex pipiens and C. restuans are not reliably distinguished based on morphology [54]. Owing to the time and expense of the molecular diagnostics to distinguish these species [55], the Culex species were pooled together. DNA was extracted from mosquito pools using Qiagen blood and tissue kits following the manufacturer’s protocol. Mosquito DNA samples were screened and haemosporidian infections were identified using the same molecular procedures for bird hosts. Maximum-likelihood estimates of the infection rate in mosquitoes were calculated with the POOLINRATES (www.cdc.gov), v. 4.0 add-in for Microsoft EXCEL [56]. Because whole-bodied mosquitoes were used, we cannot distinguish the proportion of mosquitoes that had infectious sporozoites, which typically occupy the salivary glands in the thorax, from those that had oocysts or infections within the midgut [25]. We assume that the proportion of infected mosquitoes is correlated with the proportion of infectious mosquitoes across different Plasmodium lineages. This assumption is supported by Ishitia et al. [33], who demonstrated that Plasmodium prevalence from mosquito thorax isolations was statistically indistinguishable from abdominal isolations in wild mosquitoes collected across southwest Pacific Islands.

(d) Host phylogenetic relationship estimates

Phylogenetic distances between hosts were estimated with a phylogenetic tree based on a 656 bp fragment of the recombination-activating gene 1 (RAG1). A maximum-likelihood gene tree was constructed using the PHYLML plug-in in the program GENEIOUS [57]. The resulting topology was similar to that of Barker et al. [58]. See the electronic supplementary material, §2 for more information. Novel RAG1 sequences obtained for this study are deposited in GenBank (accession no. KC789829–KC789833).

(e) Statistical analyses

All analyses performed here focus on 10 commonly sampled avian host species with seven or more infections of one or more of seven commonly sampled Plasmodium lineages (summarized in table 1). Two Plasmodium cytochrome b haplotypes were identical to those of known Plasmodium morphospecies: Plasmodium cathemerium (AY377128, [59]) and Plasmodium elongatum (AY733085, [60]). These lineages are referred to by their scientific name. The mosquito-feeding patterns of the two Culex species across the 10 common avian Plasmodium hosts were compared with a G-test. One was added to each cell to avoid problems associated with zero cell values.

Mantel tests were used to assess whether (i) pairwise similarities in relationships between hosts and mosquitoes inferred from the blood-feeding patterns, and (ii) phylogenetic distance between host species were associated with pairwise similarity in the distribution of Plasmodium parasites across all pairwise combinations of host species. This statistical test measures the correlation between two equivalent distance matrices and assesses significance through a process of permutation. Each matrix used in the two Mantel tests placed the seven host species along rows and columns. The Morisita–Horn quantitative similarity index was used to estimate similarity in both the relationships with mosquitoes and Plasmodium parasites between host pairs. The Morisita–Horn quantitative similarity index was chosen because it best handled variation in the number of identified Plasmodium infections between hosts involved in a comparison. Morisita–Horn distances were computed using the vegan package in program R. Phylogenetic distance between host pairs was based on
Table 1. Number of Plasmodium infections of specific lineages across all 10 avian hosts and Culex mosquito vectors. (MLE$_{\text{C}_4}$ is a bias-corrected maximum-likelihood estimate of the number of infected mosquitoes per 1000 individuals for each Plasmodium lineage. Upper and lower 95% confidence limits are shown within parentheses. Abbreviations for host species include the first letter of the genus, and the first two letters of the species name respectively. APH, *Agelaius phoeniceus* (red-winged blackbird); CCA, *Cardinalis cardinalis* (northern cardinal); CME, *Carpodacus mexicanus* (house finch); DCA, *Dumetella carolinensis* (grey catbird); MAT, *Molothrus ater* (brown-headed cowbird); MME, *Melospiza melodia* (song sparrow); PDO, *Passer domesticus* (house sparrow); QQU, *Quiscalus quiscula* (common grackle); SVU, *Sturnus vulgaris*, (European starling); TMI, *Turdus migratorius* (American robin)).

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3. Results

All seven common *Plasmodium* lineages recovered from avian hosts were discovered in *Culex* mosquito pools. Maximum-likelihood estimates of mosquito infection rates for each *Plasmodium* lineage are presented in table 1. *Plasmodium* parasites were not detected among *A. vexans* pools. The mosquito-feeding patterns and the parasite screening results suggest *C. pipiens* and *C. restuans* are the major *Plasmodium* vectors in Chicago. Thus, *A. vexans* was not included in subsequent analyses.

Patterns of avian host use did not differ significantly between of *C. restuans* and *C. pipiens* (figures 1, 2; $G = 14.7$, d.f. = 9, $p = 0.10$, electronic supplementary material, table S1), suggesting that the two main vector species interact with a similar set of avian *Plasmodium* hosts. A Mantel test revealed no significant correlation between similarities in relationships with avian *Plasmodium* vectors and *Plasmodium* lineages across avian hosts ($r = -0.09$, $p = 0.58$), suggesting that host interactions with *Plasmodium* are not structured by the limited (and insignificant) variation in host utilization by mosquito vectors. This result remained unchanged when considering infections from hatch-year or after hatch-year birds independently (see the electronic supplementary material, §4).

By contrast, relationships between avian host species and *Plasmodium* lineages were strikingly heterogeneous (table 1; $G = 411$, d.f. = 54, $p < 0.001$). NMDS demonstrated relationships between *Plasmodium* lineages, avian hosts and *Culex* vectors (figure 2). The ordination splits hosts and parasites into two groups. Host species within the superfamiliy...
Muscipoidae (Turdus migratorius, Sturnus vulgaris, Dumetella carolinensis) overlap with the parasite lineages CHI02PL, CHI04PL, CHI07PL and CHI09PL, whereas those within the superfamily Passeroidea (Agelaius phoeniceus, Cardinalis cardinalis, Carpodacus mexicanus, Melospiza melodia, Molothrus ater, Passer domesticus, Quiscalus quiscula) group with P. cathemerium, P. elongatum, and CHI05PL. A Mantel test revealed a positive correlation (Mantel $r=0.58$, $p=0.006$) between phylogenetic similarity as indicated by branch lengths separating host species (see the electronic supplementary material, table S2) and the similarity of parasite relationships between host species pairs. The Plasmodium assemblage on Culex vectors grouped within the Muscipoidae cluster. CHI02PL, CHI04PL, CHI07PL and CHI09PL composed 64 per cent of the Plasmodium parasites in Culex vectors. Plasmodium cathemerium, P. elongatum, and CHI05PL composed 36 per cent of that parasite assemblage.

Pairwise G-tests offered a statistically explicit approach to assessing differences in Plasmodium assemblages across hosts and vectors. The tests, summarized in electronic supplementary material, figure S1, demonstrate that the Plasmodium assemblage of T. migratorius differed significantly from all other assemblages. This is associated with the high degree of association between T. migratorius and four of seven common Plasmodium lineages. Seven other pairwise comparisons differed significantly. Five of these pairs compared assemblages of Muscipoidae and Passeroidea hosts. Excluding T. migratorius, all comparisons between host pairs within Muscipoidae or the nine-primaried New World Passeroidea (all Passeroidea host here except P. domesticus) were statistically indistinguishable. Interestingly, eight of 10 comparisons between the Plasmodium assemblages on vectors and those of avian hosts exhibited significant differences.

Three separate Monte Carlo simulations, each with a unique set of assumptions (see the electronic supplementary material, §§3), revealed patterns consistent with the other analyses. The simulations suggest T. migratorius have more CHI02PL, CHI04PL and CHI07PL infections and less P. elongatum, P. cathemerium, and CHI05PL infections than expected (see the electronic supplementary material, tables S3a–c). Well-sampled Passeroidea hosts showed the opposite pattern. See electronic supplementary material, §3 for more information.

4. Discussion

Our original model of a limiting host-encounter filter for vector-borne parasites hinged on a key assumption: vectors feed on different subsets of hosts and these divergent feeding patterns structure parasite assemblages on hosts. This assumption was not supported by any of our analyses. Feeding patterns of the two dominant avian Plasmodium vectors were similar, highly connected, and provided different Plasmodium lineages the same relative access across host species. Moreover, the limited variation in the feeding patterns between C. restuans and C. pipiens did not explain variation in Plasmodium assemblages across hosts. Our data demonstrate that the feeding patterns of Culex mosquitoes in Chicago, IL, do not impose a compartmentalized encounter filter that structures the relationships between Plasmodium taxa and common avian host species.

Assemblages of Plasmodium parasites on avian host species were heterogeneous despite the similar feeding patterns of the two Culex species. This strongly suggests that compatibility...
issues that exist solely between the host and parasite structure these Plasmodium–bird relationships. This is corroborated by three important results of our analyses. (i) Significant differences exist between the Plasmodium assemblage on mosquito vectors and eight of 10 of the Plasmodium assemblages on hosts. In the absence of compartmentalized vector-feeding patterns, these differences must arise from differential compatibilities between host and parasite pairs. (ii) Monte Carlo simulations demonstrate that the frequency of infections of particular lineages in specific host species deviate from expectations. These comparisons reveal the presence of specific compatibility filters. (iii) Both the NMDS ordination and a Mantel test revealed that host relationships with Plasmodium parasites are phylogenetically structured in this system. Like other studies [9,10], this suggests that the compatibility filter strengthens with increasing phylogenetic distance.

Specific examples of both strong and porous host compatibility filters were evident within our data. Many hosts had fewer infections of specific Plasmodium lineages than expected by random assortment of hosts and parasites or the relative access provided by mosquito vectors. For instance, CHI02PL, CHI04PL, and CHI07PL were absent to rare in Passeroidea hosts despite these lineages making up 64 per cent of the infections in vectors. Perhaps the most striking example of parasite–host incompatibility is the near absence of P. elongatum and P. cathemerium from T. migratorius, despite these parasites being common in Culex mosquitoes, and the high frequency of contact between T. migratorius and these vectors. The apparent cases of incompatibility may arise through two distinct mechanisms. These Plasmodium lineages may have high virulence on these host species, and increase the probability of mortality before sampling [61]. Alternatively, these hosts may be resistant to the infection. This could be owing to adaptations of the immune system (such as those associated with major histocompatibility complex [62–64] or host cell surface proteins [65]), the lack of necessary machinery of the parasite to invade and persist in certain hosts, or both. Palinauskas et al. [7] demonstrated that experimentally challenged host species differed in their level of resistance towards Plasmodium relictum. Ultimately, experimental infection studies like this are necessary to discriminate between these hypotheses.

In addition, some Plasmodium lineages were more frequent in specific hosts than expected. Plasmodium cathemerium and P. elongatum occurred more frequently in some Passeroidea hosts. CHI05PL was recovered disproportionately from P. domestica. However, the most obvious example of this is the frequent recovery of CHI02PL, CHI04PL and CHI07PL from T. migratorius. These parasites were largely restricted to T. migratorius, and parasitized this host at rates that exceeded expectations generated by random association or the vector-imposed encounter rate. Indeed, our analyses suggest that CHI02PL, CHI04PL, and CHI07PL may be specialized on T. migratorius. Specialization on T. migratorius may not be coincidental. This host species accounts for more than 60 per cent of the blood meals of both Culex vector species, making it the most encountered host in the community for mosquito-borne Plasmodium parasites. The high probability of encounter for these Plasmodium parasites with T. migratorius probably mitigates a primary cost of specialization: the failure to find optimal hosts because they are infrequent in a multi-host community [66].

Expansions in host range can result when changes in vector–host contact rates introduce parasites to novel hosts [17]. However, numerous studies have revealed an important interplay between host compatibility and the encounter rate in driving pathogen transmission dynamics over time [3], space [5] and between ecological communities that differ in structure [4,67]. Indeed, host range expansions also depend on the compatibility of novel hosts toward those parasites, and will not proceed if new host–parasite combinations are incompatible. Traits that influence host compatibility, and its constituent properties of host susceptibility, parasite infectivity, and the virulence of infection, evolve over time [68,69]. In the West Indies, the same suite of avian hosts and malaria parasites assemble into different patterns of relationships across island replicates [27,28,41], and there is some evidence that these differences can arise over short time periods [42]. If host compatibility issues outweigh heterogeneity in the encounter rate in structuring these parasite–host relationships, such idiosyncratic patterns observed in the West Indies and elsewhere may suggest that compatibility mechanisms are highly labile, even when parasites with complex life cycles are involved.

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