Ecological Monographs, 75(4), 2005, pp. 543–559
© 2005 by the Ecological Society of America

COMMUNITY RELATIONSHIPS OF AVIAN MALARIA PARASITES IN SOUTHERN MISSOURI

Robert E. Ricklefs,[1,2] Bethany L. Swanson,[1,3] Sylvia M. Fallon,[1,4] Alejandro Martínez-Abraín,[1,5] Alexander Scheuerlein,[1] Julia Gray,[1] and Steven C. Latta[1,6]

Department of Biology, University of Missouri–St. Louis, 8001 Natural Bridge Road, St. Louis, Missouri 63121-4499 USA

Abstract. We studied the organization and temporal stability of an assemblage of malaria parasites (genera *Plasmodium* and *Haemoproteus*) and their passerine avian hosts in a forested study area in southern Missouri, USA, over four years. We detected parasite infections by polymerase chain reaction (PCR) of parasite DNA from host blood samples and identified parasite lineages by sequencing a part of the mitochondrial cytochrome *b* gene. We obtained 757 blood samples from 42 host species. Prevalence of malaria parasitism judged by PCR averaged 38.6% and varied in parallel in the three most abundant host species over the four years of the study. Parasite prevalence bore a U-shaped relationship to host sample size. Prevalence was weakly positively associated with host body mass, but not with foraging stratum, nest height, nest type, plumage brightness, or sexual dichromatism. Over the sample as a whole, parasite prevalence did not vary between males and females or between hatch-year and older individuals.

We differentiated 34 parasite lineages. The number of host species per lineage varied from one to eight and increased with sample size. We recovered up to 14 lineages of parasite from a single host. Three relatively common lineages in the Ozarks were found nowhere else; four others were recovered from other sites in eastern North America; and six additional well-sampled lineages were distributed in the Greater Antilles among resident island host species. Parasites that are endemic among native species of hosts on the tropical wintering grounds of Ozark birds were recovered from hatch-year birds in the Ozarks, indicating that transmission takes place on the summer breeding grounds, and consequently, that suitable vectors are present in both the temperate and tropical portions of the parasite lineage distributions.

We estimate that the number of parasite lineages within a local area will approximate the number of host species and that our perception of host breadth and parasite diversity will increase for most lineages and hosts with increased sampling. Thus, host–parasite relationships in a local area, including the role of parasites in sexual selection and the evolutionary maintenance of sex, are likely to be complex, with population and evolutionary dynamics involving many actors.

Key words: avian malaria; birds; Haemoproteus; *host distribution; host–parasite relationships; Missouri; Ozarks; parasitemia; parasite diversity; parasite prevalence;* Plasmodium; *polymerase chain reaction (PCR).*

Introduction

Community relationships of parasites and their hosts are poorly understood, particularly where the taxonomy of the parasites is not settled. Although parasitologists widely believe that most parasites are host specialists, recent quantitative studies of communities of ectoparasites and endoparasites (Poulin 1995, 1999, Cumming 1998, Johnson et al. 2002, Fallon et al. 2003*a,* Beadell et al. 2004) show considerable variation in host breadth. Prevalence of infection also varies among host species and with respect to time and space (Dobson and Hudson 1995, Bensch and Akesson 2003). Although some parasite–host interactions have been characterized in detail (Van Riper et al. 1986, Dybdahl and Lively 1995, Hudson and Dobson 1997, Hudson et al. 1998, Atkinson et al. 2001*b,* Jarvi et al. 2001, Lively and Jokela 2002, Krist et al. 2004), little is known about the dynamics or even the patterns of parasites at the community level. This is particularly true for microparasites, such as trypanosomes and the organisms that cause malaria (Apanius et al. 2000, Sehgal et al. 2001, Fallon et al. 2004), where the identities of the parasite populations infecting most host species are unknown.

Manuscript received 2 December 2004; revised 28 March 2005; accepted 8 April 2005. Corresponding Editor: L. P. Lounibos.

[2] E-mail: ricklefs@umsl.edu

[3] Present address: Department of Biological Sciences, Bowling Green State University, Bowling Green, Ohio 43403 USA.

[4] Present address: AAAS Environmental Fellow, US EPA, National Center for Environmental Assessment, 1200 Pennsylvania Avenue NW, Washington D.C. 20460 USA.

[5] Present address: IMEDEA (CSIC-UIB), Avenida de los Pinares 106, 46012 El Saler, Valencia, Spain.

[6] Present address: PRBO Conservation Science, 4990 Shoreline Highway 1, Stinson Beach, California 94970 USA.

Recent application of polymerase chain reaction (PCR) and sequencing to parasite presence and identity has, however, opened the door to a new level of understanding of host–parasite interactions in many parasite communities (Bensch et al. 2000, Perkins 2000, Jarvi et al. 2002, 2003, Perkins and Schall 2002, Richard et al. 2002, Ricklefs and Fallon 2002, Waldenström et al. 2002, Fallon et al. 2003*b,* Beadell et al. 2004, Bensch et al. 2004).

A first step to understanding the evolution and dynamics of parasite–host communities is to characterize their structure. In the present study, we describe the avian malaria parasites of a community of forest birds in southern Missouri, USA, sampled over four years, based on PCR screening of parasites in the peripheral circulation and sequencing of the parasite mitochondrial cytochrome *b* gene. Our objectives were to quantify variation in parasite prevalence among species and among years, and to determine the degree to which this variation reflects the composition of parasite communities as opposed to changes in the abundance of individual parasite lineages on particular host species. Our data also allow us to describe the community relationships of a single, complex host–parasite community based on distinction of parasite lineages by genetic sequences.

Community relationships among blood parasites and their hosts have not been described before. Nonetheless, the degree of host specialization and variation in parasite abundance over time have played important roles in the development of theories concerning host–parasite coevolution, the evolution of sex, sexual selection, and the evolution of virulence (Hamilton and Zuk 1982, Bull 1994, Ewald 1994, Lively and Dybdahl 2000, Lively and Jokela 2002, Dybdahl and Storfer 2003). Few empirical data are available to evaluate assumptions and predictions of these theories. This primarily empirical study fills some of these gaps.

Background

Avian malaria is caused by related parasites in two genera, *Plasmodium* and *Haemoproteus*, in the phylum Apicomplexa, class Haemosporida (Atkinson and Van Riper 1991, Valkiunas 2005). The two genera differ in their life cycles and primary vectors. Many workers refer to the disease caused by *Haemoproteus* as haemoproteosis, reserving the term ''malaria'' for *Plasmodium* infections (Valkiunas 2005). Because our study was not concerned with disease symptoms, we use ''malaria'' as an adjective for both types of infections, reflecting the phylogenetic position of *Haemoproteus* imbedded within *Plasmodium* (see next paragraph). The infective stages of both parasites in their vertebrate hosts are differentiated sex cells (gametocytes) inside red blood cells in the peripheral circulation. *Haemoproteus* undergoes asexual reproduction in various tissues of the vertebrate host, including the liver, before invading the blood circulation. *Plasmodium* undergoes additional cycles of asexual reproduction in the peripheral blood, hence *Plasmodium* infections are thought to be more dangerous to their hosts. Both parasites undergo sexual union in their dipteran primary hosts, which are mostly *Culex* or other culicid mosquitoes (Culicidae) for avian *Plasmodium*, and *Culicoides* midges (Ceratopogonidae) for avian *Haemoproteus* (Atkinson and Van Riper 1991).

The phylogenetic relationships among many lineages of avian malaria parasites have been worked out recently by DNA sequencing (Bensch et al. 2000, Perkins and Schall 2002, Ricklefs and Fallon 2002, Beadell et al. 2004). Avian and reptilian *Plasmodium* taxa are either sister to, or paraphyletic with respect to, avian *Haemoproteus*. Avian and reptilian malaria parasites as a whole are sister to *Plasmodium* parasites of mammals, and together these are related to *Leucocytozoon* (Perkins and Schall 2002, Hellgren et al. 2004), another common apicomplexan blood parasite of birds, whose dipteran vectors are black flies (Simuliidae; Valkiunas 2005). Avian malaria lineages defined by cytochrome *b* sequences are about as diverse as their hosts (Ricklefs and Fallon 2002, Ricklefs et al. 2004). Host specificity ranges broadly from a single species of host to distribution across a wide range of species, sometimes distantly related (Bensch et al. 2000, Ricklefs and Fallon 2002, Waldenström et al. 2002, Fallon et al. 2003*a,* Beadell et al. 2004, Ricklefs et al. 2004). Although closely related parasite lineages tend to occur in related hosts, abundant evidence points to host switching in the history of avian malaria parasites with little support for close cospeciation (Bensch et al. 2000, Ricklefs and Fallon 2002, Waldenström et al. 2002, Ricklefs et al. 2004). Several cases of closely related lineages occurring in the same host species have been identified (Bensch et al. 2000, Ricklefs and Fallon 2002, Beadell et al. 2004). It is not known whether these represent genetic variation within a single parasite species, speciation within a single host population, or reinvasion of a former host following species formation in an alternative host species. However, recent analyses by Bensch and his colleagues, using both mitochondrial and nuclear markers (Bensch et al. 2004), indicate that lineages based on sequence variation in cytochrome *b* represent evolutionarily independent populations or species.

Materials and Methods

Field localities

The study area consisted of several clearcuts with regenerating forest within sites established by the long-term Missouri Ozark Forest Ecosystem Project (MOFEP) in Shannon County in the Ozark region of southern Missouri (37°14′ N, 90°58′ W; more information *available online*).[7] The study was conducted each summer from 1999 through 2002 during early July, with

[7] ⟨http://mofep.conservation.state.mo.us/⟩

limited supplemental sampling in May 2000. The sampling in early July corresponded to a period after the peak of avian reproduction when populations included a mix of adults and independent birds of the year. Vegetation within the clearcuts was dense and reached 2–4 m in height. The clearcuts were surrounded by black oak (*Quercus velutina*), scarlet oak (*Q. coccinea*), and white oak (*Q. alba*) forest with scattered shortleaf pines (*Pinus echinata*) and hickories (*Carya texana*, *C. glabra*, *C. tomentosa*). Due to the permeable nature of the sandstone/dolomite bedrock, streams seldom carry running water on the surface during mid-summer, but many springs are present in the area.

Mist nets were set and run by field workers from MOFEP. Generally, 12 nets were set in each clearcut and these were moved every two days. All birds were sexed, aged (HY, hatch-year birds; AHY, after-hatch-year birds) based on criteria in Pyle (1997), and given a U.S. Fish and Wildlife Service numbered metal band. We obtained blood samples from most of the birds caught by MOFEP personnel at one site per day over a period of several days each summer. Birds were banded and released after a blood smear and a blood sample were collected.

Blood sampling

We obtained 5–10 μL of blood by venipuncture from the ulnar vein in the wing. Smears were prepared from a drop (∼2–3 μL) of blood. The slides were then air dried, fixed within 1 h for 1 min in absolute methanol, and stained within 2 wk with 0.4% w/v Modified Giemsa Stain Solution in buffered methanol (Sigma-Aldrich Corporation, St. Louis, Missouri, USA). Slides were placed in Giemsa for 30 s, followed by two consecutive baths of 30 s each in deionized water to reduce staining artifacts, before being rinsed thoroughly in deionized water and air dried.

The remainder of the blood was immediately placed in lysis buffer or ethanol. Samples collected in 1999 were stored in either Queen's Lysis Buffer (Seutin et al. 1991) or Puregene Cell Lysis Buffer (Gentra Systems, Minneapolis, Minnesota, USA). Samples collected in 2000 were stored in Puregene Cell Lysis Buffer or absolute ethanol. Samples from 2001 and 2002 were stored in Puregene Cell Lysis Buffer.

Additional samples were obtained from Alabama, Michigan, and Connecticut in the eastern United States, and from Puerto Rico, the Dominican Republic, Jamaica, and the Cayman Islands in the Greater Antilles. All fieldwork was carried out under appropriate collecting permits, and animal care and use protocols approved by the Institutional Animal Care and Use Committee at the University of Missouri-St. Louis, USA.

Examination of blood smears

Slides made during 1999 were examined by one of us (A. Martínez-Abraín) to determine prevalence of malaria parasites. We ascertained presence or absence of parasites by scanning 100 fields under a 100× oil-immersion objective. The 100 fields, containing ∼100 erythrocytes each, were chosen in a line from one end of the slide to the other in order to compensate for differences in thickness of the blood smear (Weatherhead and Bennett 1992). We did not distinguish *Plasmodium* and *Haemoproteus*, and we did not attempt to identify species of parasites from blood smears.

Molecular analysis

DNA extraction.—DNA from blood samples in Puregene Lysis Buffer was extracted by salt precipitation following the manufacturer's protocol. DNA from samples in Queen's Lysis Buffer was extracted using a standard phenol-chloroform extraction (Seutin et al. 1991, 1993) with ethanol precipitation and without a final dialysis step. Blood samples stored in ethanol were centrifuged, all ethanol was removed by pipette and evaporation, and the blood was suspended in Puregene Cell Lysis Buffer plus 20 μg of Proteinase K and incubated for 12–24 h at 37°C. We then followed the manufacturer's protocol for extraction.

Screening.—All DNA samples were initially screened for parasites using polymerase chain reaction (PCR). We used screening primers designed to amplify a 154 nucleotide segment of RNA-coding mitochondrial DNA (Fallon et al. 2003*b*): 343F 5′-GCT CAC GCA TCG CTT CT-3′ and 496R 5′-GAC CGG TCA TTT TCT TTG-3′. PCR reactions were run in 10 μL volumes that contained the following final concentrations: 1X QIAGEN buffer, 2.5 mmol/L $MgCl_2$, 0.2 mmol/L of each dNTP, 0.5 μmol/L of each primer, and 0.25 units Taq polymerase. One μL of the extracted DNA was used in each reaction for amplification. Thermal cycling conditions were as follows: initial denaturation of 2 min at 94°C followed by 35 cycles with 50 s denaturation at 94°C, 50 s annealing at 55°C, and extension at 72°C for 1 min 10 s. This was followed by a final extension of 3 min at 72°C.

Cytochrome b *amplification*.—We attempted to amplify a segment of the cytochrome *b* gene for all samples obtained in 1999 and for those samples obtained in other years and determined to be infected by PCR screening. We used one or more of three sets of primer pairs designed from published sequences of the cytochrome *b* gene for *Plasmodium* (Escalante et al. 1998). The primer pairs were: 543F 5′-AAA AAT ACC CTT CTA TCC AAA TCT-3′ and 926R 5′-CAT CCA ATC CAT AAT AAA GCA T-3′, 413F 5′-GTG CAA CYG TTA TTA CTA A-′ and 1162R 5′-TTG TTC YGC TCA ATA CTY AGA-3′ and 413F with 926R. PCR reactions were run in 25 μL volumes with the following final concentrations: 1X QIAGEN buffer, 2.75 mmol/L $MgCl_2$, 0.2 mmol/L of each dNTP, 0.5 μmol/L of each primer, and 0.625 units Taq polymerase. We used 1.2 μL of extracted DNA in each reaction. Thermal cycling conditions were as follows: initial denaturation of 2 min at 94°C followed by 35 cycles with denaturation

for 50 s at 94°C, annealing for 50 s at 48°C (543F-926R) or 46°C (413F-1162R and 413F-926R), 1 min 10 s extension at 72°C. This was followed by a final extension of 3 min at 72°C. The amplified product was purified by gel extraction using the Geneclean III kit and protocol (Qbiogene, Montreal, Quebec, Canada).

Sequencing.—We purified the amplified product by gel extraction. Sequencing of cytochrome *b* gene fragments was carried out on an automated ABI Prim 377 sequencer (Applied Biosystems, Foster City, California, USA) according to the manufacturer's protocol. The sequences were edited and aligned using Chromas (Technelysium, Tewantin, Australia) and DNASTAR software (Madison, Wisconsin, USA) and are available through GenBank (accession numbers AF465554, AF465559, AF465563, AF465576, AF465580, AF465582, AF-540207, AY167241, AV167242, AY455658, AY455661, AY540206, AY540208-AY540222, AY540222, AY-817747-AY817756). Multiple infections, identified by double peaks in sequence chromatograms, were rare in this study. In cases in which lineage identity was unclear from a multiple infection, the sequence was removed from further analyses.

Phylogenetic analysis.—We constructed a phylogenetic hypothesis for the malaria parasites with the neighbor joining algorithm implemented in PAUP* 4.0b10 (Swofford 1998) using HKY85 genetic distances (Hasegawa et al. 1985) based on about 350 base pairs (bp) of cytochrome *b* sequence.

Statistical analyses

All statistical analyses are based on likelihood-ratio χ^2 values for contingency tables comparing numbers of infected and noninfected individuals in different samples. Other analyses are described in the *Results* section.

Results

Overall malaria prevalence

We obtained 757 samples from 42 host species (Appendix A). To determine the relative efficiencies of detecting malaria parasites by visual inspection of blood smears and PCR, we used both methods to examine the 1999 samples for which we obtained both smears and blood samples. The results showed that visual scanning of blood smears is ineffective (Appendix B), as other studies have demonstrated (Jarvi et al. 2002, Richard et al. 2002, Fallon et al. 2003*b*).

Over all four years of the study, 271 of 726 (37.3%) samples were screened as positive by PCR; of these, we obtained sequences from 155 (57.2%). An additional nine samples that had been screened as negative by PCR, but from which we obtained sequence, brought the total number of confirmed infections to 280 (38.6%). Based on our visual examination of smears in 1999 (Appendix B), PCR screening is likely to have missed an additional 2% (4 out of 188) of individuals that had visible infections, but for which PCR screens were negative. Thus, because we attempted to sequence relatively few PCR-negative samples in 2000–2002, we likely underestimated infections detectable by all three methods by ~2%.

Table 1. Proportion of potential host individuals that were positive by screening PCR (polymerase chain reaction) for malaria parasites by year.

Year	PCR screens			Proportion positive
	Negative	Positive	Total	
1999	87	101	188	0.537
2000	71	63	134	0.470
2001	153	55	208	0.264
2002	144	52	196	0.265
Total	455	271	726	0.373

Variation in overall malaria prevalence among years

Malaria prevalence determined by PCR screening varied significantly among years (G_{adj} = 47.2, df = 3, $P < 10^{-9}$), being higher during the first two years of the study (50.9%) than during the last two years (26.5%; Table 1). We have no information concerning the abundance of vectors during the study period, and variation in parasite prevalence was not obviously related to variation in precipitation or temperature between years during the study period.

Variation in malaria prevalence among species

Prevalence of parasites in 12 species for which we screened 17 or more samples is presented in Table 2. Prevalence varied from a high of 0.579 (44 out of 76) in the Yellow-breasted Chat *Icteria virens* to a low of 0.105 (2 out of 19) in the Blue-winged Warbler *Vermivora pinus*. Malaria prevalence was significantly heterogeneous among species (G_{adj} = 48.8, df = 12, $P < 10^{-5}$) due to the high proportion of infected individuals in two species, the Yellow-breasted Chat and the Red-eyed Vireo (*Vireo olivaceus*). Prevalence was statistically homogeneous (107/383 = 0.279) among the remaining 10 species (G_{adj} = 9.14, df = 9, P = 0.42). The proportion of positive samples from which we obtained sequence also varied significantly among species (excluding *V. pinus* [0 sequences], G_{adj} = 27.4, df = 10, P = 0.0022). The highest sequencing success pertained to the two species with the highest malaria prevalence, *Icteria virens* and *Vireo olivaceus* (80/107 = 0.748). Among the remaining nine species, sequencing success was homogeneous (G_{adj} = 8.50, df = 8, P = 0.39), averaging 0.412 (44 out of 105).

To determine whether parasitism rates varied in parallel or independently among individual species between years, we performed a likelihood ratio test (Proc CATMOD; SAS Institute 2000) on the relationship between presence or absence of infection and year and species for the three most common species in the study: *Vireo olivaceus*, the White-eyed Vireo *Vireo griseus*,

TABLE 2. Proportion of blood samples screened as positive by host species over four years.

Species	PCR screen: Negative	Positive	Total	Proportion screened positive	Sequences	Proportion sequenced
Icteria virens	32	44	76	0.579	29	0.659
Vireo olivaceus	56	63	119	0.529	51	0.810
Passerina cyanea	28	17	45	0.378	7	0.412
Dendroica discolor	11	6	17	0.353	2	0.333
Parula americana	21	11	33	0.344	5	0.455
Helmitheros vermivora	25	13	38	0.342	4	0.308
Wilsonia citrina	40	16	56	0.286	6	0.375
Vireo griseus	63	22	85	0.259	11	0.500
Seiurus aurocapillus	15	5	20	0.250	2	0.400
Mniotilta varia	39	11	50	0.220	5	0.455
Oporornis formosus	17	4	21	0.190	2	0.400
Vermivora pinus	17	2	19	0.105	0	0.000
Total	364	214	578	0.370	124	0.579

and *Icteria virens*. Both species ($\chi^2 = 17.8$, df = 2, $P = 0.0001$) and year ($\chi^2 = 20.13$, df = 3, $P = 0.0002$) were significant effects, but the interaction between the two ($\chi^2 = 4.8$, df = 6, $P = 0.56$) was not. Therefore, among these three species, we could not reject the hypothesis that parasite prevalence varied in parallel between years, despite the fact that the relative abundances of the host species changed over the study period. Because the clearcut areas used for netting grew up considerably in three years, Yellow-breasted Chats decreased in abundance and Red-eyed Vireos increased, while numbers of White-eyed Vireos showed no trend (Appendix A).

Variation in parasite prevalence with respect to host abundance

We asked whether less common species had different levels of prevalence than more common species by examining prevalence (I) as a function of the sample size of hosts, assuming that species are captured in mist nets in proportion to their relative abundance. This is probably a reasonable assumption for the regenerating clearcuts within which the study was conducted because vegetation was generally <3 m and birds rarely flew above net height.

We divided species into four categories based on the number of individuals screened (<10, 13–22, 30–60, >80) and found that the proportion of infected hosts was highly heterogeneous among these abundance categories ($G_{adj} = 32.8$, df = 3, $P < 10^{-6}$; Table 3). The least and most abundantly sampled species had the highest prevalence of malaria (0.51 and 0.46 vs. 0.23 and 0.31 for the middle abundance classes). When species represented by fewer than four individuals (mostly species that are not characteristic of forested environments) were excluded from the lowest abundance class, the nine remaining species included 34 infected and 22 uninfected individuals ($I = 0.61$).

The relationship between host abundance (sample size, N) and malaria infection was also examined by a quadratic regression of the arcsine-transformed prevalence for each species vs. its sample size, weighted by the square root of the sample size (Fig. 1). We evaluated the model arcsin(square root(I)) = $\log_{10}$(sample) + $\log_{10}$(sample)^2 by Proc GLM (SAS Institute 2000). Species with $N < 4$ were not included. The quadratic relationship was significant and concave upward, with the highest prevalence in the least and most common species: arcsin(square root(I)) = 2.26 [SE = 0.42, $t = 5.4$, $P < 0.0001$]− 2.45 [SE = 0.64, $t = -3.8$, $P = 0.0008$] log(N) + 0.85 [SE = 0.23, $t = 3.8$, $P = 0.0010$] log(N)^2 [model $F = 7.4$, df = 2, 23, $P = 0.0032$, $R^2 = 0.40$].

Variation in parasite prevalence among localities

Comparisons of malaria parasite prevalence among different locations in North America based on reports in the literature are set out in Appendix C.

TABLE 3. Prevalence of malaria parasites in species divided into four abundance classes based on number of samples per species.

Variable	No. samples screened per species: <10	13–22	30–60	>80	Total
Number of species	25	9	5	3	42
Infected	40	34	68	129	271
Not infected	38	113	153	151	455
Total	78	147	221	280	726
Proportion infected	0.513	0.231	0.308	0.461	0.373

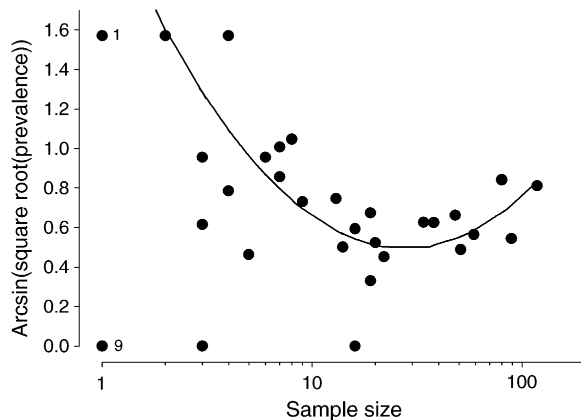

FIG. 1. Relationship between malaria prevalence and sample size among species of birds sampled in the Missouri Ozarks, USA. The quadratic regression does not include species with sample sizes of <4 individuals.

Malaria prevalence and host traits

To determine whether the prevalence of malaria parasites was related to other characteristics of the host species, we compiled data on body size, foraging stratum, sexual dichromatism, nest type, and nest height from a variety of ornithological sources, including Harrison (1978), and personal experience (Appendix D). Mass (in grams) of both sexes averaged together was obtained from Dunning (1993). Foraging stratum was treated as an ordinal variable with 0 = ground; 1 = ground, herb/shrub; 2 = herb/shrub; 3 = herb/shrub, subcanopy; 4 = subcanopy; 5 = subcanopy, canopy; and 6 = canopy. Nest height was similarly treated with 0 = ground; 1 = mostly 1–5 feet (0.305–1.524 m); 2 = mostly 1–10 feet (0.305–3.05 m); 3 = mostly 6–20 feet (1.8288–6.096 m); and 4 = mostly >20 feet (>6.096 m). Nest type was categorized as O = open, C = cavity, D = domed, P = pensile; and sexual dimorphism was treated as an ordinal variable with 0 = sexes alike, 1 = moderate dimorphism, 2 = striking dimorphism.

Analysis of variance (Proc GLM; SAS Institute 2000) weighted by the square root of sample size ($N > 3$) revealed that only log-transformed host mass ($F = 14.3$, df = 1, 23, $P = 0.0010$) and nest height ($F = 5.4$, df = 1, 23, $P = 0.030$) were significant (overall model $F = 9.8$, df = 2, 23, $P = 0.0008$, $R^2 = 0.46$) and that both were positively related to arcsine-transformed prevalence. When the analysis was run with $\log(N)$ and $\log(N)^2$, nest height was no longer significant ($t = 1.14$, df = 1, 21, $P = 0.27$). With nest height deleted from the model, the logarithm of mass remained a significant, albeit weaker effect ($t = 2.29$, df = 1, 22, $P = 0.032$), and sample size maintained its highly significant quadratic effect on arcsine-transformed prevalence ($\log(N)$, $F = 9.2$, df = 1, 22, $P = 0.0060$; $\log(N)^2$, $F = 8.0$, df = 1, 22, $P = 0.0096$; model $F = 8.3$, df = 3, 22, $P = 0.0007$, $R^2 = 0.53$).

Age and sex

Among all sexed AHY individuals of all species, parasite prevalence did not differ ($G_{adj} = 0.277$, $P = 0.60$) between females ($I = 0.446$, $N = 92$) and males ($I = 0.484$, $N = 95$).

Among all individuals that could be aged, parasite prevalence was significantly higher ($G_{adj} = 6.237$, $P = 0.013$) in AHY individuals ($I = 0.428$, $N = 311$) than in HY individuals ($I = 0.332$, $N = 337$). However, substantial heterogeneity in both parasite prevalence and proportion of HY birds among species might have caused this apparent difference.

To remove the effect of this potential heterogeneity, parasite prevalence was compared between AHY and HY individuals in eight species with 10 or more individuals in each age class (HVE, *Helmithoros vermivora*; IVI, *Icteria virens*; MVA, *Mniotilta varia*; PAM, *Parula americana*; PCY, *Passerina cyanea*; VGR, *Vireo griseus*; VOL, *Vireo olivaceus*; WCI, *Wilsonia citrea*). Adult prevalence exceeded hatch-year prevalence in four of the eight species, and differed significantly among age classes only in the Worm-eating Warbler (HVE; $I_{AHY} = 0.533$, $N = 15$, $I_{HY} = 0.167$, $N = 18$; $G = 4.80$, $P = 0.028$). Based on this analysis, we conclude that infection rates in first-year and older birds did not differ significantly in our study.

Parasite lineages

We distinguished parasite lineages based on ~350 nucleotides of cytochrome *b* sequence. Parasite sequences differing by only a single nucleotide were checked carefully against the original chromatograms from the sequencing gel and were considered the same lineage when they were recovered from the same host species. Numbered lineages differed by two or more nucleotide substitutions, or ~0.6% genetic distance. There is no general agreement on the amount of genetic variation in mtDNA that exists within "species" of malaria parasite. Fallon et al. (2003*a*) distinguished malaria lineages differing by >1%, and Perkins (2000) suggested that 3% was a reasonable level of specific differentiation, based primarily on differentiation among vertebrate species. Named species of malaria parasite that infect primates differ by as little as 1% sequence divergence (Escalante et al. 1998). Multiple sequences of the mitochondrial genome from *Plasmodium falciparum* recovered from humans differ by no more than a single nucleotide over the cytochrome *b* region used in this study (Joy et al. 2003). Species limits have been explored in avian malaria using multiple genetic markers only by Bensch et al. (2004), who confirmed complete linkage disequilibrium among lineages with cytochrome *b* genetic distances as small as 0.5%.

Besides genetic markers, the only other data that we can bring to bear on the species issue is host distribution. We presume that parasite lineages from the

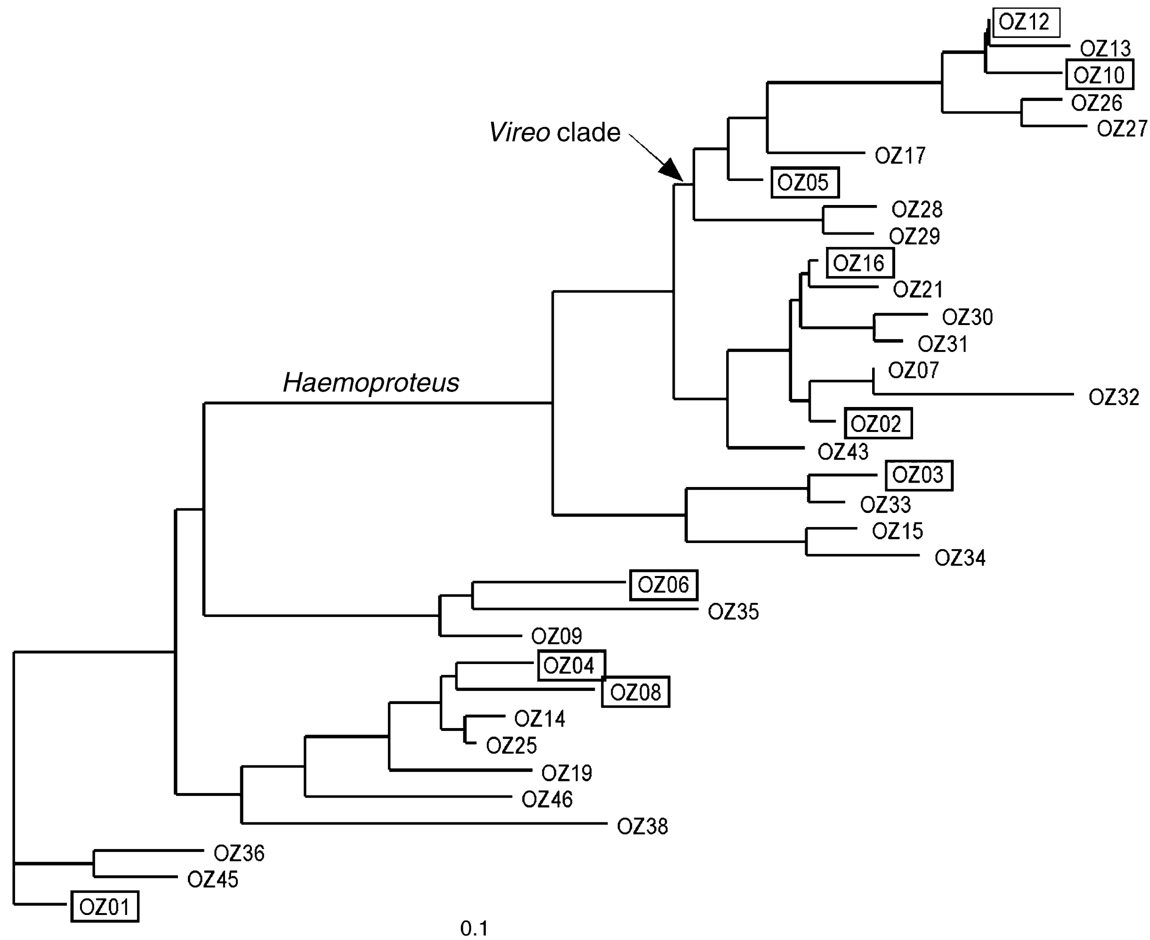

FIG. 2. Phylogenetic relationships among malaria parasite lineages recovered from bird hosts in the Missouri Ozarks. The tree was produced by a neighbor-joining algorithm using HKY85 genetic distances. Names of common lineages are enclosed in boxes. *Haemoproteus* parasites and a clade of *Haemoproteus* recovered exclusively from species of *Vireo* are also indicated. The distance bar represents 10% nucleotide substitution.

same locality that are restricted to different hosts likely represent separate species. By this criterion, some lineages differing by as few as two substitutions (0.6%) would be considered separate species, although in several cases, more divergent lineages have overlapping host distributions (Appendix E).

The avian malaria genus *Leucocytozoon*, which is transmitted by blackflies (Simuliidae) and is common in much of northern North America (Greiner et al. 1975), was not identified by visual inspection of smears in our Missouri Ozark sample or through PCR and sequencing (Perkins and Schall 2002, Hellgren et al. 2004). The absence of *Leucocytozoon* from the Ozarks likely is related to the scarcity of suitable vectors.

Phylogenetic relationships of parasite lineages

A neighbor-joining reconstruction of phylogenetic relationships of parasite lineages based on cytochrome *b* sequences is shown in Fig. 2. Support for many of the nodes, judged by Bayesian analysis, is strong in spite of the small number of nucleotides used in the analysis (Ricklefs et al. 2004). As shown in several previous analyses (Bensch et al. 2000, Perkins and Schall 2002, Ricklefs and Fallon 2002, Waldenström et al. 2002, Beadell et al. 2004, Ricklefs et al. 2004), host and parasite phylogenies correspond rather poorly. Nonetheless, there is significant host conservatism close to the tips of the phylogeny (Ricklefs and Fallon 2002, Beadell et al. 2004, Ricklefs et al. 2004), and parasites recovered from vireos (Vireonidae) are phylogenetically distinct from those from most other oscine passerines, as indicated in Fig. 2. *Plasmodium* lineages are paraphyletic to a distinct and well-supported clade of *Haemoproteus* lineages. Relatively common lineages, represented by five or more infections in our sample, are about evenly distributed between *Plasmodium* and *Haemoproteus*.

The joint distribution of parasite lineages and host species is shown in Table 4, where each is arranged in order of descending occurrence in the sample. Includ-

Table 4. Distribution of parasite lineages over hosts, with parasites arranged from left to right, and hosts from top to bottom, in decreasing order of frequency in infections.

Species code	Lineage number (all lineages designated by prefix OZ)																				Total	
	10	**08**	**01**	02	**06**	05	03	16	**04**	12	**14**	**09**	15	**19**	07	13	17	21	**25**	30	H	**P**
VOL	26			1		7				4		**1**	2	**1**	1	2	2	1		1	49	**2**
IVI		**23**	**2**	1				1	**3**												2	**28**
VGR	1	**1**	**1**	2		1		2				**1**						1			8	**4**
PCY			**5**	1									1								2	**5**
CCA			**3**						**1**		**1**										1	**5**
DDO			**1**		**1**		2			1											4	**2**
WCI		**1**	**3**		**2**																0	**6**
MVA					**2**	1			**1**		**1**										1	**4**
PAM				1			4														5	**0**
HVE					**1**			2						**1**							2	**2**
POL	1			1																1	3	**1**
BBI					**1**														**2**		0	**3**
PRU				2																	3	**0**
TLU			**2**				1														1	**2**
DDI											**1**										1	**1**
HMU												**1**									0	**2**
OFO				1	**1**																1	**1**
PER			**1**																		1	**1**
SAU							**1**				1										1	**1**
VFL																					2	**0**
Total	28	**25**	**18**	10	**9**	9	8	6	**5**	5	**4**	**3**	3	**3**	2	2	2	2	**2**	2		

Notes: This table includes hosts and parasites with more than one entry. Lineages of *Haemoproteus* (H) are shown in normal type, and lineages of *Plasmodium* (P) are shown in boldface.

Additional singleton parasite lineages are: 28, **36** on VOL; 26, **38** on VGR; 33 on CCA; 43 on DDO; **45** on MVA; **46** on POL; 31 on PRU; 34 on DDI; **35** on HMU; 32 on PER; 27 and 29 on VFL.

Additional singleton hosts are: CTR, 16; DMA, **6**; DPE, 7; PCA, **19**.

A complete list of species abbreviations is available in Appendix A. Acronyms not mentioned in the text are: CCA, *Cardinalis cardinalis*; CTR, *Carduelis tristis*; DDI, *Dendroica discolor*; DDO, *Dendroica dominica*; DMA, *Dendroica magnolia*; OFO, *Oporornis formosus*; PCA, *Polioptila caerulea*; PER, *Pipilo erythrophthalmus*; POL, *Piranga olivacea*; PRU, *Piranga rubra*; SAU, *Seiurus aurocapillus*.

ing uniquely sampled lineages and hosts, we recovered 21 lineages of *Haemoproteus* (hereafter in normal type) and 13 lineages of *Plasmodium* (hereafter in boldface). The most abundant parasites were lineages 10 and **8**, which were recovered almost exclusively from *Vireo olivaceus* (VOL) and *Icteria virens* (IVI), respectively. Other specialized parasites in the sample (lineages 5 and 12) were also recovered primarily from these two species. At the other extreme, lineages **1**, 2, and **6**, which were also relatively abundant in our sample, were recovered from eight, eight, and seven hosts, respectively. Other lineages were sparsely sampled, and it is difficult to draw conclusions about host breadth. Specialization on the Red-eyed Vireo (VOL) is understandable in that few other species in the study area are closely related to this species. The White-eyed Vireo (*Vireo griseus*, VGR) comes from a different section of the genus (Cicero and Johnson 2001) and had relatively low parasite prevalence. Several parasites of *V. griseus* also were recovered from *V. olivaceus* (lineages 10, 2, 5, **9**, and 21). Specialization of lineage **8** on the Yellow-breasted Chat (*Icteria virens*) makes less sense because the chat belongs to the group of nine-primaried oscine passerines (Klicka et al. 2000, Lovette and Bermingham 2002) that are abundantly represented in our sample, including many species of warbler, tanager, and finch. Equally interesting are many parasite lineages recovered only once in our sample (see Table 4, footnote), even though apparently suitable hosts were abundant (e.g., lineages 28 and **36** on *Vireo olivaceus*).

Number of hosts per parasite lineage

Within our study area in the Missouri Ozarks, the number of hosts from which each parasite lineage was recovered varied from one to eight (Table 4). As one would expect, the number of hosts increased with the number of recoveries of a given parasite lineage, as the number of hosts is ultimately limited by the number of individuals sampled (Fig. 3). For many lineages, the number of hosts approached or equaled the number of individuals recovered, suggesting that these parasites are broadly distributed across a wide variety of hosts, but not common on any one of them (e.g., lineages 2 and **6** in Table 4).

The most host-specific parasite lineages have the lowest ratio of hosts (H) to individual parasite infections (N): lineage 10 (28, 3, VOL; where $N = 28$, $H = 3$, and primary host = VOL), 5 (8, 2,VOL), 12 (5, 2, VOL), and **8** (25, 3, IVI). Lineages 10, 5, and 12 occur within a clade of *Haemoproteus* parasites that was recovered primarily from vireos and includes lineages 13 (VOL), 17 (VOL), 26 (VGR), 27 (*Vireo flavifrons*, VFL), 28 (VOL), and 29 (VFL) (Fig. 2). Thus, extreme host specificity in our data might reflect the

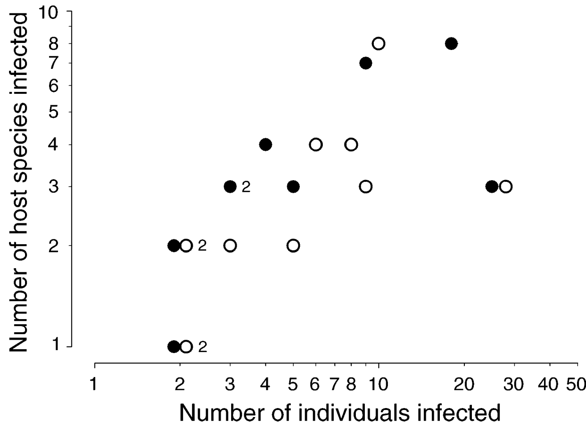

FIG. 3. Number of host species as a function of the number of each type of parasite lineage recovered from the Missouri Ozarks. *Plasmodium* lineages are indicated by solid symbols. Small numerals indicate the number of cases making up each symbol. The two abundant lineages with only three hosts are 10 and **8**, which are nearly restricted to the Red-eyed Vireo (VOL) and Yellow-breasted Chat (IVI), respectively.

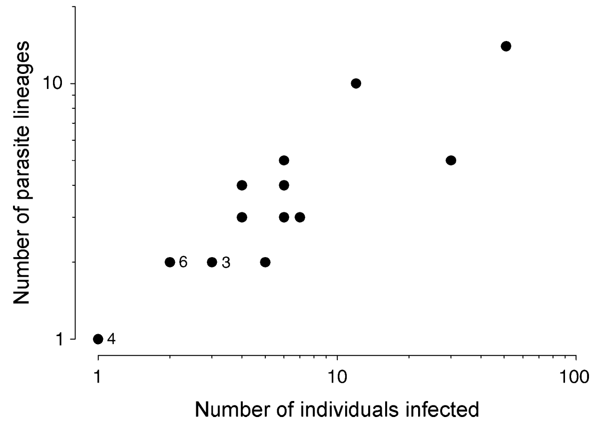

FIG. 4. Number of lineages of parasites as a function of the number of infections recorded for each host species in the Missouri Ozarks. Small numerals indicate the number of cases making up each symbol.

taxonomic isolation of particular groups of hosts. The two *Plasmodium* lineages (**4** and **8**) recovered primarily from *Icteria virens* (IVI) are also sister taxa on the tree in Fig. 2.

Number of parasite lineages per host

Our data suggest that most hosts can be infected by a wide variety of parasite lineages (Fig. 4). The number of parasite lineages increased as approximately the square root of the number of individuals infected. To some extent, the variety of parasite lineages might represent genetic variation within individual parasite species; however, the lineages recovered from a particular host mostly were not closely related. For example, the 10 lineages recovered from the White-eyed Vireo (VGR) (**1**, 2, 5, **8**, **9**, 10, 16, 21, 26, **38**) span the entire parasite phylogeny, with only 16 and 21 being closely positioned in the parasite phylogeny (differing by three nucleotide substitutions).

Temporal variation in frequency of lineages

To determine whether lineages varied independently in frequency between years, we analyzed a year-times-lineage contingency table for 100 cases of seven lineages (10, **8**, **1**, 2, **6**, 3, 5) recovered from eight or more host individuals. Because three of the cells in the table were empty (one in each of three years), we added 1 to the numbers of parasites recovered in each year. A contingency test showed that the distribution of lineages among years was not significantly heterogeneous (G_{adj} = 9.4, df = 18, P = 0.95). Indeed, the distribution was more homogeneous than one would expect by chance. Thus, within the power of our test, the abundances of lineages appear to vary in parallel over three years, just as we found for overall prevalence of malaria parasitism in different host species.

Age, sex, and lineage

As we have seen, parasite prevalence did not vary between male and female host individuals. The only host species from which we recovered a large number of parasites from sexed individuals was the Yellow-breasted Chat (*Icteria virens*), for which lineages **1**, **4**, and **8** were recovered in one, two, and seven cases from females and one, zero, and ten cases from males. With 0.5 added to each of the cells, the test for heterogeneity was not significant (G_{adj} = 1.64, df = 2, P = 0.44).

Evidence for differences in parasite prevalence between first year (HY) and older (AHY) birds was weak, although older birds had higher parasite prevalence overall in the entire sample. The only host species for which it was possible to compare lineages from HY and AHY birds was the Red-eyed Vireo (VOL, *Vireo olivaceus*; Table 5). The two common lineages (5 and 10) were recovered in similar numbers from both age

TABLE 5. Number of each of 13 parasite lineages recovered from known-aged individuals of the Red-eyed Vireo over four years in the Missouri Ozarks, USA.

	Lineage												
Age	2	5	7	9	10	12	13	15	17	19	21	28	30
AHY	1	3	0	0	10	3	0	0	0	0	1	0	1
HY	0	4	1	1	13	1	2	2	2	1	0	1	0

Note: Ages are designated as HY (hatch year) and AHY (after hatch year).

classes, as was the less common lineage 12. However, 10 HY birds were infected by seven lineages not recovered from AHY individuals. If our sample of parasite lineages in adults had been drawn from the HY + AHY pool with replacement (N = 47), the probability that none of the unique HY lineages would have been recovered from adults would have been $(1–10/47)^{19}$ = 0.011. Thus, it is somewhat unlikely that adults would have lacked HY lineages 7, **9**, 13, 15, 17, **19**, and 28 by chance. Turning this argument around, three lineages recovered from each of one AHY individual were not found in HY individuals. The probability of this occurring by chance when drawn from the HY+AHY pool with replacement is $(1–3/47)^{28}$ = 0.158. Thus, the absence of these lineages from HY individuals is likely. These probabilities indicate that AHY and HY birds harbor a somewhat different, albeit largely overlapping, array of lineages. Three of the seven lineages recovered uniquely from HY individuals (13, 17, 28) were not found in other species. The other four were uncommon in our sample: lineage 7 on DPE (*Dendroica petechica*, N = 1), **9** on VGR (N = 1) and HMU (*Hylocichla mustelina*, N = 1), 15 on PCY (N = 1) and **19** on HVE (N = 1) and PCA (*Polioptila caerulea*, N = 1); the three lineages recovered uniquely from AHY birds were all recovered from at least one other species, and from many others in the case of lineage 2. This raises the possibility that some parasite lineages are specialized to infect young individuals with naïve immune systems.

Local lineages found elsewhere

We have sampled malaria parasites at other locations within eastern North America and in the Greater Antilles. Data presently available show that many of the parasite lineages recovered in the Missouri Ozarks are widely distributed (see Appendix F).

In the case of parasite lineages distributed in both the Greater Antilles and North America, one may ask whether infections are transmitted to migrants only in the tropics and are carried northward to the breeding grounds, or whether they are also, or exclusively, transmitted to northern hosts on the breeding grounds. We found that each of the geographically widespread lineages, except for lineage **4**, of which only two were recovered from known-age hosts, was present in HY birds (Table 6) and must, therefore, have been transmitted on the breeding grounds. Thus, although these lineages are abundant in different hosts in the wintering areas, they are evidently endemic to migratory host populations and infect naïve individuals during the summer in North America. Their presence in species endemic to the Greater Antilles (Appendix F) demonstrates that they are also transmitted there as well.

Discussion

Use of PCR and sequencing to screen parasite communities

Most of the parasite infections identified in this study would have been missed had we relied only on microscopic reading of blood smears. All 188 smears obtained in 1999 were scanned visually for at least 15 minutes per slide, yielding 17% positive infections. PCR of the blood samples from the same individuals revealed 73 additional infections to give a total prevalence of 56%. This value probably also underestimates prevalence because we can only detect infections active in the peripheral blood circulation (Jarvi et al. 2002).

Table 6. Ages of hosts infected in the Missouri Ozarks with malaria parasites whose center of abundance is in the Greater Antilles.

Lineage	HY individuals	AHY individuals
2	3	6
4	0	2
12	1	4
17	2	0
19	2	1
21	1	1
Total	7	14

PCR of mitochondrial ribosomal RNA genes using our primary screening primers (Fallon et al. 2003*b*) failed to detect four infections found on blood smears, which would have reduced the parasite prevalence observed solely by this method by 2%. However, these infections were picked up by subsequent PCR of the mitochondrial cytochrome *b* gene, which we used to identify parasite lineages. PCR of conservative RNA sequence occasionally fails because of DNA quality or quantity, and perhaps occasionally owing to nucleotide substitutions in the primer binding sequences. The problem is worse for the less conservative cytochrome *b* gene, for which a single primer pair was able to amplify only 57% of infections identified by initial screening in our study. Use of multiple primer pairs and nested PCR would increase the rate of detecting infections and the identification of lineages (Perkins and Schall 2002, Fallon et al. 2003*b,* Hellgren et al. 2004). However, returns diminish quickly with increased effort, and one must balance the two according to the objectives of a particular study.

Clearly, studies of parasite prevalence relying on visual screening are inadequate. Many infections occur at intensities below one cell in 10 000, which is the general lower limit for visual screening without substantially increased effort. PCR screening probably can detect infections whose presence in the peripheral blood is at least an order of magnitude lower (Fallon et al. 2003*b,* Hellgren et al. 2004). A general caveat about working with either visual inspection of smears or PCR screening is that these techniques detect only those infections that are present in peripheral blood. Avian malaria can persist in individuals for long periods; certainly several years (Atkinson et al. 2001*a,* Jarvi et al. 2002), and perhaps a lifetime. Atkinson and his colleagues (Atkinson et al. 2001*a, b,* Jarvi et al. 2002) advocate the use of serology to identify infections by means of the antibody response to malaria

parasites. The advantage of serology is that it can detect chronic infections not actively present in peripheral blood. The disadvantages are that antibodies can be detected for some period after an infection has been cleared from the body, and that the technique is parasite lineage specific (Jarvi et al. 2003). That is, serological screens have to be developed for each ''species'' of parasite individually, at least at present. For the near future, PCR using a variety of primer pairs and including nested PCR (Perkins and Schall 2002, Fallon et al. 2003*b,* Jarvi et al. 2003, Hellgren et al. 2004, Waldenström et al. 2004) provides the most effective method of screening the prevalence of active malaria infections within bird populations.

Overall parasite prevalence

Prevalence of parasites varied significantly between years over our four-year study, being higher during the first two years than during the second two years. This change might have been associated with variation in climate conditions, but any association of this type was not obvious in our study, and we did not sample vector abundance. The prevalence of parasites generally is related to the abundance of vectors. This is evident in the association of *Leucocytozoon* with the distribution of simuliid flies in North America (Greiner et al. 1975) and Europe (Scheuerlein and Ricklefs 2004). Moreover, infection of particular parasites varied in parallel with spatial and temporal variation in vector abundance in several studies (Van Riper et al. 1986, Atkinson et al. 1988, Super and Van Riper 1995, Sol et al. 2000).

If climate variation were important, one might expect different temporal changes in the prevalence of *Plasmodium* (primary vector *Culex* mosquitoes) and *Haemoproteus* (primary vector *Culicoides* midges). We tested this by comparing the prevalence of the two malaria genera during the years 1999–2002. A contingency test on the complete data was marginally significant (G_{adj} = 7.46, df = 3, P = 0.059), with *Haemoproteus* holding rather steady in numbers, while the prevalence of *Plasmodium* decreased. This trend might have been caused by a decrease in the abundance of the most common *Plasmodium* lineage (**8**), which was recovered almost entirely from *Icteria virens*. Because of succession of the clearcut sites, the frequency of Yellow-breasted Chats decreased over the study period from 36 captures in 1999 to 12 captures in 2002. When the contingency test was run with lineage **8** excluded, the result was no longer significant (G_{adj} = 4.32, df = 3, P = 0.23). Thus, relative changes in parasite genera in our sample appear to reflect the mix of potential host species rather than changes in the parasite community or the relative abundance of vectors.

Distribution of prevalence among hosts

Parasite prevalence was highest in the Red-eyed Vireo *Vireo olivaceus* (VOL) and the Yellow-breasted Chat *Icteria virens* (IVI) (Table 2). In both these hosts, single parasite lineages (10 and **8**, respectively) accounted for most of the infections. When these lineages were removed from the analysis, the prevalence of infection became homogeneous (165/529 = 0.312) among all 12 species with more than 17 records (G_{adj} = 15.7, df = 11, P = 0.152). Because lineages **8** and 10 were the only common ones that exhibited a high degree of host restriction, this raises the possibility that host specialization might increase the infectiveness of parasites.

In this study, parasite prevalence assumed a U-shaped distribution with respect to host abundance (Table 3 and Fig. 1). Greater prevalence among abundant hosts is consistent with high transmission rates in dense populations (Brown et al. 2001, Galvani 2003, Wonham et al. 2004). The high prevalence of parasites among the least common hosts is more puzzling, unless these parasites are selected for high virulence to offset the difficulty of transmission at low host density. This seems unlikely, however, because parasites recovered from the less common hosts are a mixture of common parasite lineages with multiple hosts and unique lineages that may or may not be host specialists. Alternatively, low host abundance and high parasite prevalence might be directly related through general poor quality of the host immune system or a role of specialized blood parasites in limiting population densities of their hosts. In the case that less common host populations pick up parasite lineages from other, more abundant host populations as ''spillover,'' they might not have evolved resistance to these lineages and suffer from them in proportion to their susceptibility. Finally, hosts that were relatively uncommon in our study areas might sustain denser populations elsewhere and our data for these species could simply represent abundance-prevalence relationships in their core environments. Increased spatial sampling should help to resolve the nature of these patterns.

Because vectors likely contribute to the prevalence of malaria parasites, several investigators have tried to relate prevalence to habitat characteristics that might influence vector abundance (see Valkiunas 2005:169). For example, Garvin and Remsen (1997) found that parasite prevalence increased with nest height in a community of birds in southern Louisiana. Super and Van Riper (1995) related the lack of transmission of blood parasites in a coastal scrub community on the Channel Islands of southern California to the absence of suitable vectors (see also Freeman-Gallant et al. 2001). In the present study, neither foraging height nor nesting height accounted for variation in parasite prevalence.

Other intrinsic characteristics of host individuals might be associated with variation in infection. The presence of parasites was positively related to body mass (Valkiunas 2005), as found in an analysis of prevalence in European passerine birds (Scheuerlein and Ricklefs 2004), but the effect was weak. Other variables, such as plumage brightness, sexual dimorphism,

and song repertoires (presumably sexually selected traits) have been related to parasite prevalence (Hamilton and Zuk 1982, Read 1987, Read and Harvey 1989, Zuk 1991, Korpimaki et al. 1995, Buchanan et al. 1999, Møller et al. 1999). In this study, however, neither plumage brightness nor sexual dimorphism was significantly related to parasite prevalence. This is not particularly surprising because taxonomically nested analysis of variance has shown that prevalence of parasites varies at a low taxonomic level, primarily among species within genera (Scheuerlein and Ricklefs 2004). Thus, prevalence evidently is more labile than many of the life-history traits with which it has been associated, and it would not be expected to show close correlation with them. Habitat distribution and nest placement tend to be more flexible and might provide a basis for variation in parasite prevalence; however, our study did not address these factors.

Prevalence did not vary between the sexes in our study, although gender bias in parasite infection has been reported in several species. McCurdy et al. (1998) summarized several studies that showed no difference in overall parasitism between the sexes, but significantly higher prevalence of *Haemoproteus* infections in females compared to males. High testosterone levels in males during the breeding season might suppress the immune system and lead to higher parasite prevalence (Weatherhead et al. 1993, Zuk 1996, Zuk and McKean 1996, Hughes and Randolph 2001); however, the greater stress of reproduction in females might also weaken immune defenses (Møller and Saino 1994).

Parasite prevalence also did not vary between hatch-year (HY) and older (AHY) individuals. Our samples were collected primarily in early July, after most breeding was finished and populations included large numbers of birds of the year. Dale et al. (1996) noted lower parasite prevalence among first year males of Pied Flycatchers *Ficedula hypoleuca* (see also Merila and Andersson 1999, Sol et al. 2000). Many individuals are infected as nestlings, although the infection might not appear in the peripheral circulation for several weeks (Atkinson and Van Riper 1991). Many birds brought into the St. Louis Bird Rehabilitation Center as nestlings develop malaria infections (R. E. Ricklefs and B. L. Swanson, *unpublished data*), indicating transmission during this vulnerable stage. If malaria infections are persistent in birds (Garnham 1966, Fallis et al. 1974, Atkinson et al. 2000, Valkiunas 2005:169), then prevalence of infection, whether patent or not, should increase with age and accumulated exposure. This might be balanced by increasing ability of older birds to suppress the expression of infections in the circulating blood. Relatively few empirical studies have addressed age effects on prevalence of blood parasites (e.g., Dale et al. 1996, Sol et al. 2000), and the accumulation and loss of infections in natural host populations remains an important problem.

Parasite lineages

We used ~350 nucleotides of the mitochondrial cytochrome *b* gene to type parasite lineages. It is likely that sequences differing by as few as one or two nucleotides, that is, ~0.5%, represent evolutionarily independent lineages that can be considered as separate species (Bensch et al. 2004). This is much less than the genetic distance typically associated with different species of birds or mammals, which averages ~5% for mitochondrial DNA (Klicka and Zink 1997, Avise and Walker 1998, Johns and Avise 1998, Zink et al. 2004). However, synonymous substitution occurs in malaria mitochondrial DNA at a much lower relative rate than in vertebrate hosts, and so less genetic divergence may represent more adaptive evolutionary change (Ricklefs and Fallon 2002, R. E. Ricklefs, *unpublished data*).

Bensch et al. (2004) observed complete linkage disequilibrium between mitochrondrial sequences differing by as little as 0.5% and the nuclear dihydrofolate reductase-thymidylate synthase (DHFR-TS) gene, indicating clearly that small mitochondrial DNA variants represented genetically isolated populations of parasites. Further work with multiple independent markers from different parts of the malaria genome will clarify species limits in the future. At present, we feel confident that differences of 1–2 nucleotides out of 350 bp represent different lineages.

If there is error in this judgment, it is likely to be on the side of conservatism; namely, that each of our lineages might include significant evolutionary variety, over its range if not locally. We have seen in this study how the same parasite lineage may infect a nonoverlapping set of hosts in different locations, particularly in comparisons between Missouri and the Greater Antilles. Fallon et al. (2003*a,* 2005) found dramatic variation in the prevalence of particular parasite lineages in the same hosts on different islands in the Lesser Antilles. Because local parasite prevalence is relatively stable over time (Fallon et al. 2004), this variation in prevalence, indeed presence or absence, probably represents significant shifts in the balance between host and parasite evolution within each island community. It is as difficult to decide whether these different island populations should be regarded as species as it is to decide the species status of allopatric populations in general. This will become a problem when analyses of host–parasite relationships are conducted on a regional basis, but it does not affect the interpretation of patterns observed locally.

Phylogenetic relationships among hosts and parasites

Although cospeciation is prominent in some types of host–parasite relationships (Moran and Baumann 1994, Page and Hafner 1996), others, including malaria parasites of birds do not exhibit close association between the diversification of parasite and host (Johnson

et al. 2002, Ricklefs and Fallon 2002, Ricklefs et al. 2004). Some associations between parasite and host lineages are evident at a rather high level (Ricklefs et al. 2004). For example, in the phylogeny of Ozark malaria parasites depicted in Fig. 2, parasites in one relatively large clade of *Haemoproteus* lineages infect almost exclusively hosts of the genus *Vireo*, although parasites outside this clade also have been recovered from vireo hosts. At a lower phylogenetic level, Ricklefs and Fallon (2002) identified significant conservatism of host distribution among closely related lineages of parasites. For example, in Fig. 2, the two most prominent parasites of the Yellow-breasted Chat *Icteria virens* (lineages **4** and **8**) are sister lineages in the genus *Plasmodium*. Beyond this evidence of structure in the phylogenetic relationships of parasites and their hosts, the distribution of hosts across the parasite phylogeny, and vice versa, reflects substantial host switching. Such switching sometimes occurs across considerable taxonomic distance. For example, the close sister lineages **14** and **25** infect strictly Emberizidae and strictly Paridae, respectively, in our North American samples. Many parasite lineages are recovered from hosts ranging across most of the passerine phylogeny, for example from emberizids to vireos. Presumably, such distributions represent the jumping of parasite lineages to new hosts, and it would not be surprising if these formed new host-specific lineages over time, thus completing the switch to a new host.

The organization of host–parasite assemblages

Our survey of malaria parasites in southern Missouri revealed virtually every possible pattern of co-occurrence from extreme specialization to general distribution with respect to both parasites on hosts and vice versa (Table 4). If a generalization is to be made, parasites are more likely to specialize on a small number of host species than are hosts likely to be infected by one or a few parasite lineages. At one extreme, we found two common parasite lineages (**8** and 10) nearly restricted to single host species. Inspection of Fig. 3 suggests, however, that these two lineages are exceptional and that host breadth in the other lineages increases regularly with the number of infections of that lineage sampled. Thus, although parasite lineages might be specialized at some level of host taxonomy, most seem capable of infecting a broad range of species within the Emberizidae or Vireonidae, for example. As shown in Fig. 4, the number of parasite lineages recovered from a given host increases steadily, as approximately the square root of the number of infections sequenced. The evolution of host breadth, reflecting a balance between the advantage of population size and contagion and the disadvantage of host specialization, is poorly understood (Woolhouse et al. 2001). At this point, we cannot sort out whether apparent host breadth and its associated parasite abundance (Fig. 3) reflect intrinsic properties of the parasite or the vagaries of sampling.

Overall, 42 host species yielded 34 lineages of parasite, 14 of them observed only once (Table 4). The number of parasite lineages would no doubt increase if sampling were to increase, and it will likely turn out to be true that lineages of avian malaria parasites will approximate the number of their host species (Ricklefs and Fallon 2002).

Temporal change in the prevalence in parasite lineages

Considerable interest in the dynamics of parasite lineages arises from the hypothesis that sexual reproduction in hosts evolved, and is maintained, by the requirement to keep pace with the evolution of parasites, the so-called ''Red Queen'' hypothesis (Dybdahl and Lively 1995, Ebert and Hamilton 1996, Poulin et al. 2000, Lively and Jokela 2002, Dybdahl and Storfer 2003). One prediction of Red Queen dynamics is that lineages of parasites on a host should vary temporally in response to the rapid evolution of immune responses to particular lineages (Dybdahl and Lively 1995, Lively and Dybdahl 2000, Lively and Jokela 2002). Bensch and Akesson (2003) tested this hypothesis in Willow Warblers *Phylloscopus trochilus* in Sweden and found the prevalence of two *Haemoproteus* lineages to vary both spatially and temporally, as predicted. Although our study did not have the geographic scope of Bensch and Akesson's, we did not find evidence for temporal variation over several parasite lineages and hosts within a local study area over four years. Samples of avian malaria parasites from the West Indian islands of Puerto Rico and St. Lucia separated by almost a decade did, however, pick up some new lineages and lose others, representing significant changes in the frequencies of lineages within these islands (Fallon et al. 2004). We cannot judge whether this is an appropriate time scale for Red Queen dynamics.

Age and sex of the host

We detected no differences overall in the prevalence of malaria parasites infecting males and females or HY and AHY individuals. Our ability to determine whether the spectrum of parasite lineages differed between males and females was limited to a comparison in the Yellow-breasted Chat *Icteria virens* that was negative. We were able to compare HY and AHY individuals only in the Red-eyed Vireo *Vireo olivaceus*. Younger and older birds were infected by the most common parasite lineages (5, 10, and 12; Table 5), but seven uncommon lineages uniquely appeared in birds of the year, whereas only three uncommon lineages were recovered from adults. The array of parasite lineages in HY and AHY birds differed significantly and suggested that juveniles might be infected by a broader and somewhat different array of parasite lineages than adults, perhaps owing to their naïve immune systems. Presum-

ably, initial infection by these unusual parasites leads to effective immune defense against them later in life, with the result that they no longer appear in AHY individuals.

The geography of avian malaria

Among common parasites, three *Haemoproteus* lineages were restricted to the Missouri Ozarks and did not appear in samples from Alabama, Connecticut, and Michigan, USA. Four were recovered elsewhere in North America, and six others were common in the Greater Antilles, although generally on different hosts from those in North America (Appendix F). Because avian blood parasites presumably can spread rapidly, as in the recent case of West Nile virus (Bernard and Kramer 2001, Dobson and Foufopoulos 2001, Kramer and Bernard 2001, Marfin and Gubler 2001, Marra et al. 2004, O'Leary et al. 2004), we were surprised to find localized parasites within eastern North America. Two of the localized lineages (5 and 10) were recovered primarily from *Vireo olivaceus*, which was not well sampled elsewhere. However, eight infections of the third lineage (3) were recovered from four non-vireo hosts. Of the lineages restricted to North America, two were specialized on hosts that do not migrate to the Greater Antilles (**25**, Tufted Titmouse *Baeolophus bicolor*, BBI; **35**, Wood Thrush *Hylocichla mustelina*, HMU); one was specialized on a host that commonly winters in the Greater Antilles (7, Yellow-rumped Warbler *Dendroica coronata*, DCO), while the fourth (**1**) is a common, generalized parasite.

Six common parasite lineages occur in both North America and the Greater Antilles. Because these are transmitted to HY birds on the breeding grounds, these lineages must be considered as endemic to North America. In this case, one does not have to postulate transmission from resident tropical species to migrants on the wintering grounds, as Waldenström et al. (2002) have inferred for some lineages of parasites of sylviid warblers that migrate from Europe to Africa. Whether the parasite lineages in North American migrants represent the same genetic populations as the same lineages in the Greater Antilles, and whether they are transmitted to North American migrants on the wintering grounds, cannot be determined without more variable genetic markers. Because no naïve individuals enter populations of migrants on the wintering grounds, direct evidence of local parasite transmission, in contrast to the expression of parasite infections contracted on the breeding grounds, would be difficult to ascertain. Without a larger phylogenetic framework for the parasites, one cannot also determine whether these lineages originated in the West Indies or in North America (e.g., Waldenström et al. 2002).

Conclusions

Sequence-based studies of avian malaria parasites are beginning to reveal the diversity of parasite lineages and complexity of host–parasite relationships in natural communities. Phylogenetic analysis additionally holds out the prospect of untangling the evolutionary histories of parasites with respect to host and geographic distribution. It is clear that the picture provided by a marker like the mitochondrial cytochrome *b* gene can reveal only coarse aspects of parasite diversity. Multiple and more rapidly evolving genetic markers will undoubtedly unveil other layers of complexity. It is not yet evident what generalizations will come from this first pass at the molecular natural history of avian malaria parasites other than to provide a rich phenomenology against which previous conceptions of parasite–host relationships can be tested. Already, the notion of host specialization by avian malaria parasites should be set aside as a general premise, although some parasite lineages do appear to be restricted to few hosts, or even a single host. An outcome of these investigations may be that no generalizations can be formed about the distribution of parasites with respect to hosts and geography. If this turns out to be the case, we may have to content ourselves with models of population and evolutionary dynamics that produce a wide variety of outcomes.

One of the lessons to be derived from this study is that sample size severely constrains our ability to discern pattern in parasite communities of any complexity. Even with a sample approaching 800 host individuals in this study, the number of host and parasite lineages was large enough that sample sizes were limiting with respect to the establishment of statistically valid interpretations of the distribution of parasites among hosts and variation among years, for example.

An additional challenge is to characterize the distributions of parasite lineages over host species in a way that is suitable for comparing community properties between samples. Rarefaction of samples with respect to both hosts and lineages might be a fruitful approach, but large samples from host–parasite communities will be required to validate any particular index of parasite distribution. Regardless, it is clear that the relative abundance of avian malaria parasites and the development of molecular tools to characterize parasite lineages make this an excellent model system for the study of parasite–host relationships.

Acknowledgments

This study was supported by National Science Foundation grant DEB-0089226, National Institutes of Health/National Institute on Aging grant AG20263-01, and a grant from the University of Missouri Research Board. A. Martínez-Abraín was supported by a grant from the Fulbright Foundation. The Missouri Ozark Forest Ecosystem Project generously provided support for logistics and accommodation. John Faaborg and Paul Porneluzi coordinated the able field crews who conducted the mist netting. Michele Schutzenhofer, Hilary Schlickman, and Emily Mohl assisted with blood sampling. Rich and Brenda Keith, of the Kalamazoo Nature Center, Vicksburg, Michigan, and Judy Richardson, of the Connecticut Audubon Birdcraft Museum, Fairfield, Connecticut, provided blood samples from their netting operations. Scott So-

mershoe helped with sampling in Alabama. Many people have provided invaluable help for our fieldwork in the West Indies, particularly John Faaborg and Joseph Wunderle in Puerto Rico, Peter Marra in Jamaica, and Patricia Bradley in the Cayman Islands.

Literature Cited

Apanius, V., N. Yorinks, E. Bermingham, and R. E. Ricklefs. 2000. Island and taxon effects in parasitism and resistance of Lesser Antillean birds. Ecology **81**:1959–1969.

Atkinson, C. T., R. J. Dusek, and J. K. Lease. 2001*a*. Serological responses and immunity to superinfection with avian malaria in experimentally-infected Hawaii Amakihi. Journal of Wildlife Diseases **37**:20–27.

Atkinson, C. T., R. J. Dusek, K. L. Woods, and W. M. Iko. 2000. Pathogenicity of avian malaria in experimentally-infected Hawaii Amakihi. Journal of Wildlife Diseases **36**: 197–204.

Atkinson, C. T., D. J. Forrester, and E. C. Greiner. 1988. Epizootiology of *Haemoproteus meleagridis* (Protozoa: Haemosporina) in Florida: seasonal transmission and vector abundance. Journal of Medical Entomology **25**:45–51.

Atkinson, C. T., J. K. Lease, B. M. Drake, and N. P. Shema. 2001*b*. Pathogenicity, serological responses, and diagnosis of experimental and natural malarial infections in native Hawaiian thrushes. Condor **103**:209–218.

Atkinson, C. T., and C. Van Riper III. 1991. Pathogenecity and epizootiology of avian haematozoa: *Plasmodium*, *Leucocytozoon*, and *Haemoproteus*. Pages 19–48 *in* M. Zuk, editor. Bird–parasite interactions. Ecology, evolution, and behavior. Oxford University Press, New York, New York, USA.

Avise, J. C., and D. Walker. 1998. Pleistocene phylogeographic effects on avian populations and the speciation process. Proceedings of the Royal Society of London, Series B **265**:457–463.

Beadell, J. S., E. Gering, J. Austin, J. P. Dumbacher, M. A. Peirce, T. K. Pratt, C. T. Atkinson, and R. C. Fleischer. 2004. Prevalence and differential host-specificity of two avian blood parasite genera in the Australo-Papuan region. Molecular Ecology **13**:3829–3844.

Bensch, S., and A. Akesson. 2003. Temporal and spatial variation of hematozoans in Scandinavian willow warblers. Journal of Parasitology **89**:388–391.

Bensch, S., J. Pérez-Tris, J. Waldenström, and O. Hellgren. 2004. Linkage between nuclear and mitochondrial DNA sequences in avian malaria parasites: multiple cases of cryptic speciation? Evolution **58**:1617–1621.

Bensch, S., M. Stjernman, D. Hasselquist, O. Ostman, B. Hansson, H. Westerdahl, and R. T. Pinheiro. 2000. Host specificity in avian blood parasites: a study of *Plasmodium* and *Haemoproteus* mitochondrial DNA amplified from birds. Proceedings of the Royal Society of London, Series B **267**:1583–1589.

Bernard, K. A., and L. D. Kramer. 2001. West Nile virus activity in the United States, 2001. Viral Immunology **14**: 319–338.

Brown, C. R., N. Komar, S. B. Quick, R. A. Sethi, N. A. Panella, M. B. Brown, and M. Pfeffer. 2001. Arbovirus infection increases with group size. Proceedings of the Royal Society of London, Series B **268**:1833–1840.

Buchanan, K. L., C. K. Catchpole, J. W. Lewis, and A. Lodge. 1999. Song as an indicator of parasitism in the sedge warbler. Animal Behaviour **57**:307–314.

Bull, J. J. 1994. Perspective: virulence. Evolution **48**:1423–1437.

Cicero, C., and N. K. Johnson. 2001. Higher-level phylogeny of new world vireos (Aves: Vireonidae) based on sequences of multiple mitochondrial DNA genes. Molecular Phylogenetics and Evolution **20**:27–40.

Cumming, G. S. 1998. Host preference in African ticks (Acari, Ixodida): a quantitative data set. Bulletin of Entomological Research **88**:379–406.

Dale, S., A. Kruszewicz, and T. Slagsvold. 1996. Effects of blood parasites on sexual and natural selection in the pied flycatcher. Journal of Zoology **238**:373–393.

Dobson, A., and J. Foufopoulos. 2001. Emerging infectious pathogens of wildlife. Philosophical Transactions of the Royal Society of London, Series B **356**:1001–1012.

Dobson, A., and P. Hudson. 1995. The interaction between the parasites and predators of red grouse *Lagopus lagopus scoticus*. Ibis **137**:S87–S96.

Dunning, J. B.,Jr. 1993. CRC handbook of avian body masses. CRC Press, Boca Raton, Florida, USA.

Dybdahl, M. F., and C. M. Lively. 1995. Host–parasite interactions; infection of common clones in natural populations of a freshwater snail (*Potamopyrgus antipodarum*). Proceedings of the Royal Society of London, Series B **260**: 99–103.

Dybdahl, M. F., and A. Storfer. 2003. Parasite local adaptation: Red Queen versus Suicide King. Trends in Ecology and Evolution **18**:523–530.

Ebert, D., and W. D. Hamilton. 1996. Sex against virulence: the coevolution of parasitic diseases. Trends in Ecology and Evolution **11**:79–82.

Escalante, A. A., D. E. Freeland, W. E. Collins, and A. A. Lal. 1998. The evolution of primate malaria parasites based on the gene encoding cytochrome *b* from the linear mitochondrial genome. Proceedings of the National Academy of Sciences USA **95**:8124–8129.

Ewald, P. W. 1994. Evolution of infectious disease. Oxford University Press, Oxford, UK.

Fallis, A. M., S. S. Desser, and R. A. Khan. 1974. On species of *Leucocytozoon*. Advances in Parasitology **12**:1–67.

Fallon, S. M., E. Bermingham, and R. E. Ricklefs. 2003*a*. Island and taxon effects in parasitism revisited: avian malaria in the Lesser Antilles. Evolution **57**:606–615.

Fallon, S. M., E. Bermingham, and R. E. Ricklefs. 2005. Host specialization and geographic localization of avian malaria parasites: a regional analysis in the Lesser Antilles. American Naturalist **165**:466–480.

Fallon, S. M., R. E. Ricklefs, S. Latta, and E. Bermingham. 2004. Temporal stability of insular avian malarial parasite communities. Proceedings of the Royal Society of London, Series B **271**:493–500.

Fallon, S. M., R. E. Ricklefs, B. L. Swanson, and E. Bermingham. 2003*b*. Detecting avian malaria: an improved polymerase chain reaction diagnostic. Journal of Parasitology **89**:1044–1047.

Freeman-Gallant, C. R., K. D. O'Connor, and M. E. Breuer. 2001. Sexual selection and the geography of *Plasmodium* infection in Savannah sparrows (*Passerculus sandwichensis*). Oecologia **127**:517–521.

Galvani, A. P. 2003. Epidemiology meets evolutionary ecology. Trends in Ecology and Evolution **18**:132–139.

Garnham, P. C. C. 1966. Malaria parasites and other Haemosporidia. Blackwell Scientific Publications, Oxford, UK.

Garvin, M. C., and J. V. Remsen. 1997. An alternative hypothesis for heavier parasite loads of brightly colored birds: exposure at the nest. Auk **114**:179–191.

Greiner, E. C., G. F. Bennett, E. M. White, and R. F. Coombs. 1975. Distribution of the avian hematozoa of North America. Canadian Journal of Zoology **53**:1762–1787.

Hamilton, W. D., and M. Zuk. 1982. Heritable true fitness and bright birds: a role for parasites. Science **218**:384–387.

Harrison, C. 1978. A field guide to the nests, eggs and nestlings of North American birds. Collins, Glasgow, UK.

Hasegawa, M., H. Kishino, and T. Yano. 1985. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. Journal of Molecular Evolution **22**:160–174.

Hellgren, O., J. Waldenstrom, and S. Bensch. 2004. A new PCR assay for simultaneous studies of Leucocytozoon, Plasmodium, and Haemoproteus from avian blood. Journal of Parasitology **90**:797–802.

Hudson, P. J., and A. P. Dobson. 1997. Transmission dynamics and host–parasite interactions of *Trichostrongylus tenuis* in Red Grouse (*Lagopus lagopus scoticus*). Journal of Parasitology **83**:194–202.

Hudson, P. J., A. P. Dobson, and D. Newborn. 1998. Prevention of population cycles by parasite removal. Science **282**:2256–2258.

Hughes, V. L., and S. E. Randolph. 2001. Testosterone depresses innate and acquired resistance to ticks in natural rodent hosts: a force for aggregated distributions of parasites. Journal of Parasitology **87**:49–54.

Jarvi, S. I., C. T. Atkinson, and R. C. Fleischer. 2001. Immunogenetics and resistance to avian malaria in Hawaiian honeycreepers (Drepanidinae). Studies in Avian Biology **22**:254–263.

Jarvi, S. I., M. E. M. Farias, H. Baker, H. B. Freifeld, P. E. Baker, E. Van Gelder, J. G. Massey, and C. T. Atkinson. 2003. Detection of avian malaria (*Plasmodium* spp.) in native land birds of American Samoa. Conservation Genetics **4**:629–637.

Jarvi, S. I., J. J. Schultz, and C. T. Atkinson. 2002. PCR diagnostics underestimate the prevalence of avian malaria (*Plasmodium relictum*) in experimentally-infected passerines. Journal of Parasitology **88**:153–188.

Johns, G. C., and J. C. Avise. 1998. A comparative summary of genetic distances in the vertebrates from the mitochondrial cytochrome *b* gene. Molecular Biology and Evolution **15**:1481–1490.

Johnson, K. P., R. J. Adams, and D. H. Clayton. 2002. The phylogeny of the louse genus *Brueelia* does not reflect host phylogeny. Biological Journal of the Linnean Society **77**: 233–247.

Joy, D. A., X. R. Feng, J. B. Mu, T. Furuya, K. Chotivanich, A. U. Krettli, M. Ho, A. Wang, N. J. White, E. Suh, P. Beerli, and X. Z. Su. 2003. Early origin and recent expansion of *Plasmodium falciparum*. Science **300**:318–321.

Klicka, J., K. P. Johnson, and S. M. Lanyon. 2000. New World nine-primaried oscine relationships: constructing a mitochondrial DNA framework. Auk **117**:321–336.

Klicka, J., and R. M. Zink. 1997. The importance of recent ice ages in speciation: a failed paradigm. Science **277**: 1666–1669.

Korpimaki, E., P. Tolonen, and G. F. Bennett. 1995. Blood parasites, sexual selection and reproductive success of European kestrels. Ecoscience **2**:335–343.

Kramer, L. D., and K. A. Bernard. 2001. West Nile virus in the western hemisphere. Current Opinion in Infectious Diseases **14**:519–525.

Krist, A. C., J. Jokela, J. Wiehn, and C. M. Lively. 2004. Effects of host condition on susceptibility to infection, parasite developmental rate, and parasite transmission in a snail–trematode interaction. Journal of Evolutionary Biology **17**:33–40.

Lively, C. M., and M. F. Dybdahl. 2000. Parasite adaptation to locally common host genotypes. Nature **405**:679–681.

Lively, C. M., and J. Jokela. 2002. Temporal and spatial distributions of parasites and sex in a freshwater snail. Evolutionary Ecology Research **4**:219–226.

Lovette, I. J., and E. Bermingham. 2002. What is a wood-warbler? Molecular characterization of a monophyletic parulidae. Auk **119**:695–714.

Marfin, A. A., and D. J. Gubler. 2001. West Nile encephalitis: an emerging disease in the United States. Clinical Infectious Diseases **33**:1713–1719.

Marra, P. P., S. Griffing, C. Caffrey, A. M. Kilpatrick, R. McLean, C. Brand, E. Saito, A. P. Dupuis, L. Kramer, and R. Novak. 2004. West Nile virus and wildlife. Bioscience **54**:393–402.

McCurdy, D. G., D. Shutler, A. Mullie, and M. R. Forbes. 1998. Sex-biased parasitism of avian hosts: relations to blood parasite taxon and mating system. Oikos **82**:303–312.

Merila, J., and M. Andersson. 1999. Reproductive effort and success are related to haematozoan infections in blue tits. Ecoscience **6**:421–428.

Møller, A. P., P. Christe, and E. Lux. 1999. Parasitism, host immune function, and sexual selection. Quarterly Review of Biology **74**:3–20.

Møller, A. P., and N. Saino. 1994. Parasites, immunology of hosts, and host sexual selection. Journal of Parasitology **80**:850–858.

Moran, N., and P. Baumann. 1994. Phylogenetics of cytoplasmically inherited microorganisms of arthropods. Trends in Ecology and Evolution **9**:15–20.

O'Leary, D. R., A. A. Marfin, S. P. Montgomery, A. M. Kipp, J. A. Lehman, B. J. Biggerstaff, V. L. Elko, P. D. Collins, J. E. Jones, and G. L. Campbell. 2004. The epidemic of West Nile virus in the United States, 2002. Vector Borne and Zoonotic Diseases **4**:61–70.

Page, R. D. M., and M. S. Hafner. 1996. Molecular phylogenies and host–parasite cospeciation: gophers and lice as a model system. Pages 255–270 *in* P. H. Harvey, A. J. L. Brown, J. M. Smith, and S. Nee, editors. New uses for new phylogenies. Oxford University Press, Oxford, UK.

Perkins, S. L. 2000. Species concepts and malaria parasites: detecting a cryptic species of *Plasmodium*. Proceedings of the Royal Society of London, Series B **267**:2345–2350.

Perkins, S. L., and J. J. Schall. 2002. A molecular phylogeny of malarial parasites recovered from cytochrome *b* gene sequences. Journal of Parasitology **88**:972–978.

Poulin, R. 1995. Phylogeny, ecology, and the richness of parasite communities in vertebrates. Ecological Monographs **65**:283–302.

Poulin, R. 1999. Speciation and diversification of parasite lineages: an analysis of congeneric parasite species in vertebrates. Evolutionary Ecology **13**:455–467.

Poulin, R., L. J. Marshall, and H. G. Spencer. 2000. Genetic variation and prevalence of blood parasites do not correlate among bird species. Journal of Zoology **252**:381–388.

Pyle, P. 1997. Identification guide to North American birds. Part 1. Slate Creek Press, Bolinas, California, USA.

Read, A. F. 1987. Comparative evidence supports the Hamilton and Zuk hypothesis on parasites and sexual selection. Nature **328**:68–70.

Read, A. F., and P. H. Harvey. 1989. Reassessment of comparative evidence for Hamilton and Zuk theory on the evolution of secondary sexual characters. Nature **339**:618–619.

Richard, F. A., R. N. M. Sehgal, H. I. Jones, and T. B. Smith. 2002. A comparative analysis of PCR-based detection methods for avian malaria. Journal of Parasitology **88**:819–822.

Ricklefs, R. E., and S. M. Fallon. 2002. Diversification and host switching in avian malaria parasites. Proceedings of the Royal Society of London, Series B **269**:885–892.

Ricklefs, R. E., S. M. Fallon, and E. Bermingham. 2004. Evolutionary relationships, cospeciation, and host switching in avian malaria parasites. Systematic Biology **52**:111–119.

SAS Institute. 2000. SAS OnlineDoc. Version 8. SAS Institute, Cary, North Carolina, USA.

Scheuerlein, A., and R. E. Ricklefs. 2004. Prevalence of blood parasites in European passeriform birds. Proceedings of the Royal Society of London, Series B **271**:1363–1370.

Sehgal, R. N. M., H. I. Jones, and T. B. Smith. 2001. Host specificity and incidence of *Trypanosoma* in some African

rainforest birds: a molecular approach. Molecular Ecology **10**:2319–2327.

Seutin, G., J. Brawn, R. E. Ricklefs, and E. Bermingham. 1993. Genetic divergence among populations of a tropical passerine, the streaked saltator (*Saltator albicollis*). Auk **110**:117–126.

Seutin, G., B. N. White, and P. T. Boag. 1991. Preservation of avian blood and tissue samples for DNA analyses. Canadian Journal of Zoology **69**:82–90.

Sol, D., R. Jovani, and J. Torres. 2000. Geographical variation in blood parasites in feral pigeons: the role of vectors. Ecography **23**:307–314.

Super, P. E., and C. Van Riper III. 1995. A comparison of avian hematozoan epizootiology in two California coastal scrub communities. Journal of Wildlife Diseases **31**:447–461.

Swofford, D. L. 1998. PAUP*. Phylogenetic analysis using parsimony (*and other methods). Version 4. Sinauer Associates, Sunderland, Massachusetts, USA. ⟨http://paup.csit.fsu.edu/⟩

Valkiunas, G. 2005. Avian malaria parasites and other Haemosporidia. CRC Press, Boca Raton, Florida, USA.

Van Riper, C., III, S. G. Van Riper, M. L. Goff, and M. Laird. 1986. The epizootiology and ecological significance of malaria in Hawaiian land birds. Ecological Monographs **56**: 327–344.

Waldenström, J., S. Bensch, D. Hasselquist, and O. Ostman. 2004. A new nested polymerase chain reaction method very efficient in detecting *Plasmodium* and *Haemoproteus* infections from avian blood. Journal of Parasitology **90**: 191–194.

Waldenström, J., S. Bensch, S. Kiboi, D. Hasselquist, and U. Ottosson. 2002. Cross-species infection of blood parasites between resident and migratory songbirds in Africa. Molecular Ecology **11**:1545–1554.

Weatherhead, P. J., and G. F. Bennett. 1992. Ecology of parasitism of brown-headed cowbirds by haematozoa. Canadian Journal of Zoology **70**:1–7.

Weatherhead, P. J., K. J. Metz, G. F. Bennett, and R. E. Irwin. 1993. Parasite faunas, testosterone and secondary sexual traits in male red-winged blackbirds. Behavioral Ecology and Sociobiology **33**:13–23.

Wonham, M. J., T. de-Camino-Beck, and M. A. Lewis. 2004. An epidemiological model for West Nile virus: invasion analysis and control applications. Proceedings of the Royal Society of London, Series B **271**:501–507.

Woolhouse, M. E. J., L. H. Taylor, and D. T. Haydon. 2001. Population biology of multihost pathogens. Science **292**: 1109–1112.

Zink, R. M., J. Klicka, and B. R. Barber. 2004. The tempo of avian diversification during the Quaternary. Philosophical Transactions of the Royal Society of London, Series B **359**:215–219.

Zuk, M. 1991. Parasites and bright birds: new data and a new prediction. Pages 317–327 *in* M. Zuk, editor. Bird–parasite interactions. Ecology, evolution, and behaviour. Oxford University Press, Oxford, UK.

Zuk, M. 1996. Disease, endocrine–immune interactions, and sexual selection. Ecology **77**:1037–1042.

Zuk, M., and K. A. McKean. 1996. Sex differences in parasite infections: patterns and processes. International Journal for Parasitology **26**:1009–1023.

APPENDIX A

A table presenting sample sizes of host species in each year of the study is available in ESA's Electronic Data Archive: *Ecological Archives* M075-022-A1.

APPENDIX B

A comparison of infection prevalence estimated by visual examination of blood smears and PCR analysis of DNA extracted from host blood samples is available in ESA's Electronic Data Archive: *Ecological Archives* M075-022-A2.

APPENDIX C

A comparison of malaria parasite prevalence among different locations in North America based on reports in the literature is available in ESA's Electronic Data Archive: *Ecological Archives* M075-022-A3.

APPENDIX D

A table of host characteristics used in analysis is available in ESA's Electronic Data Archive: *Ecological Archives* M075-022-A4.

APPENDIX E

Evidence for species distinction in malaria parasites from host distribution is available in ESA's Electronic Data Archive: *Ecological Archives* M075-022-A5.

APPENDIX F

Tables and a discussion of the distribution of parasite lineages within eastern North America and the Caribbean Basin are available in ESA's Electronic Data Archive: *Ecological Archives* M075-022-A6.