Ecology Letters, (2005) **8**: 838–845 doi: 10.1111/j.1461-0248.2005.00788.x

LETTER

Dispersal increases local transmission of avian malarial parasites

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Abstract

The relationships between dispersal and local transmission rate of parasites are essential to understanding host–parasite coevolution and the emergence and spread of novel disease threats. Here we show that year-round transmission, as opposed to summer transmission, has repeatedly evolved in malarial parasites (genera *Plasmodium* and *Haemoproteus*) of a migratory bird. Year-round transmission allows parasites to spread in sympatric host's wintering areas, and hence to colonize distantly located host's breeding areas connected by host-migration movements. Widespread parasites had higher local prevalence, revealing increased transmission, than geographically restricted parasites. Our results show a positive relationship between dispersal and local transmission of malarial parasites that is apparently mediated by frequent evolutionary changes in parasite transmission dynamics, which has important implications for the ecology and evolution of infectious diseases.

Keywords

Avian malaria, comparative methods, cytochrome *b* sequences, dispersal potential, geographical range size, *Haemoproteus*, parasite prevalence, parasite transmission, *Plasmodium*, *Sylvia atricapilla*.

Ecology Letters (2005) 8: 838–845

INTRODUCTION

Parasites are powerful selective agents that influence virtually all aspects of their hosts' life (e.g. Clayton & Moore 1997; Dronamraju 2004). The Red Queen hypothesis suggests that hosts and parasites are engaged in a continuous coevolutionary arms race, in which host resistance and parasite infectivity are under intense reciprocal selection (Jaenike 1978). The dynamics of this coevolutionary arms race determine the outcome of the host–parasite interaction, in terms of host resistance and parasite infection success (Ebert 1994; Ebert & Herre 1996), which in turn affect parasite transmission rate and virulence (Bull 1994). When several horizontally transmitted parasite species infect one host species in the same locality, variation in parasite transmission rate may lead to large differences among parasites in the proportion of hosts being infected in the population, or local prevalence (Ebert 1994; Ebert & Herre 1996).

An important factor underlying variation in parasite local prevalence may be the ability of parasites to disperse among host populations. For example, parasite dispersal might affect local host–parasite co-adaptation processes. If parasite infection success depends on host–parasite genetic interactions that make each individual host susceptible to some parasites but resistant to others (Borghans *et al.* 2004), immigrant parasite genotypes can be either in advantage or in disadvantage to track local host's resistance genotypes (Thompson 1999; Lenormand 2002; Dybdahl & Storfer 2003), which should increase or decrease parasite transmission rates, respectively. Besides, even if all hosts are equally susceptible, a variable epidemiology of parasites, involving variable opportunities for transmission, can also cause variation in parasite prevalence. Widespread parasites might attain higher local prevalence if they are successfully transmitted in a wide range of environmental conditions. For example, widespread parasites are expected to be generalist with respect to transmission needs (such as intermediate hosts, e.g. Krasnov *et al.* 2005), because they face more variable environmental conditions across their range than geographically restricted parasites.

When it comes to natural host–parasite interactions, very little is known about the potential of parasites to evolve different dispersal rates and how ecological mechanisms are underlying variation in parasite dispersal potential. As a consequence, it remains largely unknown whether dispersal reduces or increases local transmission of parasites (Gandon

2002; Dybdahl & Storfer 2003). This uncertainty is worrying, because the occurrence of parasites that are both easily dispersed and highly infectious would pose serious threats of disease spread and emergence in new populations.

We tested these two alternative hypotheses taking advantage of the possibility to distinguish two groups of malarial parasites with different potential to disperse between populations of a migratory passerine bird, the blackcap *Sylvia atricapilla* (L.). Blackcaps are widely distributed in Europe, where they are structured into populations with different migratory behaviours (Pérez-Tris *et al.* 2004), but birds breeding in distantly located regions winter together in geographically restricted warm Mediterranean areas (Pérez-Tris & Tellería 2002). Avian malarial parasites (protozoans of the genera *Plasmodium* and *Haemoproteus*) are transmitted by blood-feeding insect vectors. Transmission often takes place only during the birds' breeding season (Valkiūnas 2005), that is in periods of population allopatry, which should hamper parasite dispersal between distantly located host populations. During non-breeding periods, summer-transmitted parasites retreat from birds' blood to spleen and liver tissue, which blocks transmission to vectors (Valkiūnas 2005). However, some parasites are transmitted all year around (Waldenström *et al.* 2002), and hence could make use of the coexistence of different host populations in sympatric wintering grounds to spread across the host's range, taking advantage of bird migration movements. We identified frequent evolutionary changes in transmission dynamics of blackcap parasites, from summer transmission to year-round transmission. Year-round transmitted parasites were widespread and had a high local prevalence, supporting the idea that dispersal increases local transmission of malarial parasites.

MATERIALS AND METHODS

Bird sampling and parasite screening

Between May and August from 1999 to 2003, we captured 97 blackcaps at six breeding localities (Fig. 1). These

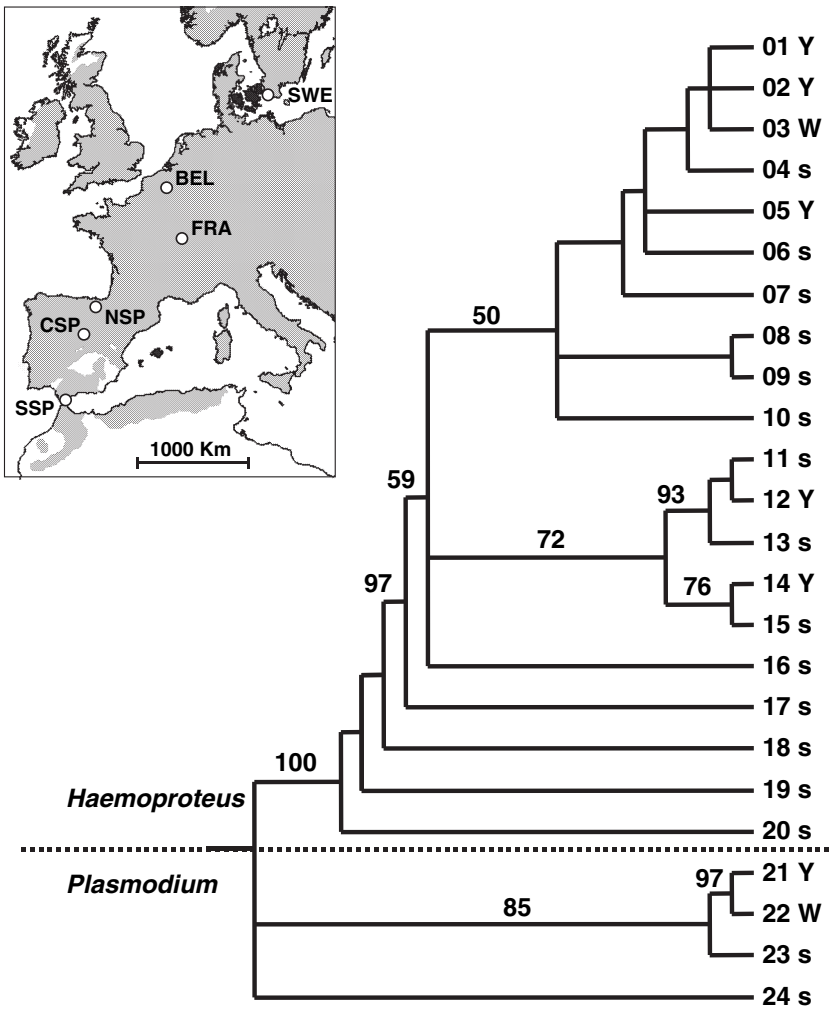

Figure 1 Phylogeny and phenotype of 24 malarial parasites of blackcaps from six European localities. The map shows the location of sites (SSP, southern Spain; CSP, central Spain; NSP, northern Spain; FRA, France; BEL, Belgium; SWE, Sweden) in the blackcaps' range (shaded). The tree shows bootstrap support to internal branches (numbers on branches, values <50% are not shown). Each parasite lineage has been assigned a code indicating its phenotype with respect to transmission time, in summer (s), in winter (W) or all year around (Y).

populations span the whole breeding range of Western European blackcaps and spend the winter mainly in southern Spain (Pérez-Tris *et al.* 2004), where birds from different origins concentrate in high numbers in the same habitats (Pérez-Tris & Tellería 2002). During four winters (December to January, from 1999 to 2003), we captured another 264 blackcaps in southern Spain, at the moment when resident blackcaps occur in sympatry with blackcaps from western European areas. Over-wintering migrants and resident locals were distinguished by morphology (Pérez-Tris *et al.* 1999). We sexed and aged all birds by plumage (distinguishing between first-year and older birds; Svensson 1992). We took blood samples of all birds and marked them with rings to avoid repetition.

We extracted total DNA from blood, and used a highly efficient nested polymerase chain reaction (PCR) method (Waldenström *et al.* 2004) to detect parasite infections by amplification of 479 bp of the parasite cytochrome *b* gene. We used DNA sequences to distinguish parasite lineages, and resolved all multiple infections using TA-cloning (Pérez-Tris & Bensch 2005). All negative results were confirmed by repeated PCR, and sample quality was confirmed by amplification of birds mitochondrial DNA (Pérez-Tris *et al.* 2004).

We determined whether the parasites we found were transmitted only in summer or all year around by studying parasite occurrence in the blood of blackcaps captured during four winters in southern Spain. A benign climate in winter and the presence of suitable vectors in this area make parasite transmission possible during periods of blackcap sympatric occurrence (Blackwell 1997; Valkiūnas & Iezhova 2004). It is important to note that, while *Haemoproteus* species only cast infective gametocytes in the bloodstream, *Plasmodium* species can also be found in blood as non-infective asexual stages (Valkiūnas 2005). This somewhat limits the usefulness of parasite presence in blood as a surrogate of transmission potential in *Plasmodium* species. However, these parasites typically present only asexual stages during a short period immediately following the infection event, but regularly produce gametocytes later in the course of an infection (Valkiūnas 2005). Although we cannot demonstrate that *Plasmodium* parasites found in blood all year around were always infective, it is very unlikely that these parasites subsisted in blood for extended periods without producing gametocytes (Valkiūnas 2005).

To make sure that seasonal occurrence of parasites was not a sampling artefact reflecting overall variation in parasite abundance, we compared the observed parasite community in winter with the expected community given the null hypothesis that the relative abundances of parasites in winter were equal to their summer abundances. To generate the null hypothesis, we used a simulated population of 10 000 infected blackcaps, in which each parasite lineage occurred with the same frequency as observed in summer. From this simulated population, we randomly selected 5000 groups of 100 parasite infections (because we found 100 parasite infections in winter, see Results), and measured the prevalence of each parasite in each random trial. We used these simulations to estimate (i) the richness of parasite lineages expected in winter from random effects, and (ii) the probability of year-round occurrence of each individual parasite lineage, should winter presence of each parasite be dependent on its abundance in summer. We used 5000 simulations to attain high confidence in significance tests, and set the size of the hypothetical population to 10 000 infected birds to prevent repetition of random groups, given the number of simulations and the size of the groups. These simulations were repeated twice, firstly assuming winter prevalences proportional to the average summer prevalences of all parasites found in summer (in all breeding areas, Table 1), and secondly assuming winter prevalences proportional to the prevalences of the parasites found in the population breeding in southern Spain (in the same area as sampled in winter, Table 1). We considered the most conservative outcome of both analyses, suggesting the highest possible sampling bias.

We did not find differences in parasite prevalence (all parasites combined) among blackcap populations (Table 1), sexes (88% in males and 85% in females) or age classes (84% in adults and 92% in juveniles; all $P > 0.32$). The frequency of multiple infections (mean ± SD: 36 ± 24%) and the number of parasites mixing in multiple infections (mean ± SD: 2.4 ± 0.62) were also similar among populations, sexes and age classes (all $P > 0.29$). Therefore, we pooled sexes and age classes in our analyses. Differences in sampling effort were not correlated with number of parasites found ($P = 0.92$) or variance of parasite prevalence ($P = 0.35$) in each sample ($n = 10$, six breeding populations and four winters), even if such effects were tested using 5000 Monte Carlo simulations ($P = 0.457$ and $P = 0.176$ respectively). Therefore, restricted sampling did not bias our estimates of parasite phenotype, geographical range or local prevalence.

Half of our summer sample (51% of all blackcaps) was composed of juvenile birds. Given that infected juveniles acquired parasites locally and short before our sampling (because they were born in the area and had never abandoned it), we could evaluate the equivalence between summer parasite prevalence and local transmission rate of parasites in our study. A very high repeatability of the local prevalence of each parasite lineage in both age classes ($r_i = 0.91$, $F_{21,22} = 2.41$, $P < 0.0001$) demonstrated such an equivalence.

Statistical analyses

We measured the summer geographical range of parasites as the number of host-breeding populations infected, which

Table 1 Description of the 24 parasite lineages found in blackcaps

Lineage	Parasite name	GenBank accession no.	Transmission time	GR (*n*)	WP (%)	Summer prevalence (%)						
						SSP	CSP	NSP	FRA	BEL	SWE	Mean
1	*Haemoproteus* sp. (strain SYAT01)	AY831750	All year around	6	18.2	37.5	16.7	54.5	5.6	33.3	14.3	27.0
2	*Haemoproteus* sp. (strain SYAT10)	AY831757	All year around	1	1.8	–	–	–	–	5.6	–	5.6
3	*Haemoproteus* sp. (strain SYAT26)	AY831767	Winter	–	1.1	–	–	–	–	–	–	–
4	*Haemoproteus* sp. (strain SYAT17)	AY831763	Summer	3	–	4.2	–	9.1	–	5.6	–	6.3
5	*Haemoproteus* sp. (strain SYAT02)	AY831751	All year around	6	7.0	33.3	66.7	54.5	61.1	33.3	50.0	49.8
6	*Haemoproteus* sp. (strain SYAT04)	AY831753	Summer	1	–	–	–	9.1	–	–	–	9.1
7	*Haemoproteus* sp. (strain SYAT28)	AY831768	Summer	2	–	–	–	–	–	5.6	7.1	6.3
8	*Haemoproteus* sp. (strain SYAT14)	AY831761	Summer	2	–	4.2	–	9.1	–	–	–	6.6
9	*Haemoproteus* sp. (strain SYAT19)	AY831765	Summer	1	–	4.2	–	–	–	–	–	4.2
10	*Haemoproteus* sp. (strain SYAT11)	AY831758	Summer	1	–	–	–	9.1	–	–	–	9.1
11	*Haemoproteus* sp. (strain SYAT09)	AY831756	Summer	2	–	4.2	8.3	–	–	–	–	6.3
12	*Haemoproteus* sp. (strain SYAT13)	AY831760	All year around	5	0.9	12.5	16.7	18.2	5.6	16.7	–	13.9
13	*Haemoproteus* sp. (strain SYAT21)	AY831766	Summer	2	–	4.2	–	–	–	–	7.1	5.7
14	*Haemoproteus* sp. (strain SYAT07)	AY831754	All year around	1	5.3	–	–	–	–	–	7.1	7.1
15	*Haemoproteus* sp. (strain SYAT16)	AY831762	Summer	4	–	4.2	8.3	9.1	5.6	–	–	6.8
16	*Haemoproteus* sp. (strain SYAT12)	AY831759	Summer	1	–	4.2	–	–	–	–	–	4.2
17	*Haemoproteus* sp. (strain SYAT29)	AY831769	Summer	1	–	–	–	–	–	5.6	–	5.6
18	*Haemoproteus* sp. (strain SYAT18)	AY831764	Summer	1	–	–	8.3	–	–	–	–	8.3
19	*Haemoproteus* sp. (strain SYAT03)	AY831752	Summer	3	–	4.2	16.7	–	–	16.7	–	12.5
20	*Haemoproteus* sp. (strain WW2)	AY831755	Summer	1	–	–	–	–	–	–	28.6	28.6
21	*Plasmodium* sp. (strain SGS1)	AF495571	All year around	3	6.9	12.5	33.3	–	5.6	–	–	17.1
22	*Plasmodium* sp. (strain GRW11)	AY831748	Winter	–	1.9	–	–	–	–	–	–	–
23	*Plasmodium* sp. (strain COLL1)	AY831747	Summer	1	–	4.2	–	–	–	–	–	4.2
24	*Plasmodium* sp. (strain SYAT24)	AY831749	Summer	1	–	4.2	–	–	–	–	–	4.2

SSP, southern Spain; CSP, central Spain; NSP, northern Spain; FRA, France; BEL, Belgium; SWE, Sweden.

Strain names and GenBank accession numbers of the cytochrome *b* sequences are shown for reference. The table shows transmission dynamics, geographical range size (GR, number of breeding populations in which each parasite was found) and prevalence of each parasite. Winter prevalences (WP) are given as annual averages, and summer prevalences are shown both as per-population local prevalences (population names as in Fig. 1) and average local prevalences. Parasite lineages 3 and 22 (found only in winter) were excluded from comparative analyses.

reflects the realised ability of parasites to disperse between host-breeding populations. We used the proportion of hosts being infected in each population (local prevalence) as a measure of local transmission rate of parasites (see also Ebert 1994; Ebert & Herre 1996).

We used PAUP*4.0 (Swofford 1998) to build an unrooted neighbour-joining phylogenetic tree based on parasite sequences. We used a General Time Reversible model of nucleotide substitution under a gamma distribution ($\alpha = 0.73$) and an assumed proportion of invariable sites equalling 0.47. This was the best of 56 models according to the Akaike information criterion implemented in Modeltest 3.6 (Posada & Crandall 1998). We used maximum likelihood as the optimality criterion, and followed a heuristic search with random addition of sequences, keeping best trees only, and using the tree-bisection-reconnection algorithm for branch swapping. Support to internal branches was based on a heuristic bootstrap analysis with 1000 replicates (Fig. 1). Given the difficulty to define species limits among avian malarial parasite mtDNA lineages (Bensch *et al.* 2004), we treated all lineages as independent. Nevertheless, we took into account the fact that closely related parasites may show similar phenotypes because of shared ancestry, by conducting phylogenetically based analyses (Felsenstein 1985; Garland *et al.* 1993).

We used phylogenetic ANOVA (Garland *et al.* 1993) to assess whether year-round transmitted parasites had a broader geographical range and a higher local transmission rate than summer transmitted parasites. We tested *F*-statistics against 10 000 evolutionary simulations of character evolution along the phylogeny, assuming a gradual Brownian motion model of evolution (Garland *et al.* 1993), and setting branch lengths proportional to genetic distances. Nevertheless, setting all branch lengths to unity produced the same results, showing the robustness of the pattern.

We calculated phylogenetically independent contrasts Felsenstein (1985) of geographical range size and local prevalence of parasites, which were standardized by dividing them by their standard deviations. For this analysis, we used raw data values, but we transformed branch lengths using Nee's method (Garland *et al.* 1993) because it provided the best standardization of contrasts. However, using different transformations of branch lengths or data values did not change our results quantitatively or qualitatively.

RESULTS

Of 361 blackcaps screened, 184 were infected with one to four different parasites, which means 126 parasite infections of breeding birds (southern Spain, $n = 33$; central Spain, $n = 21$; northern Spain, $n = 19$; France, $n = 15$; Belgium, $n = 22$; Sweden, $n = 16$), and 100 infections of birds wintering in southern Spain. We found 24 parasite mtDNA lineages (Table 1) showing between 0.2 and 12% sequence divergence, all of them qualifying as *Plasmodium* spp. or *Haemoproteus* spp. according to similarity with parasite sequences published in GenBank (Fig. 1).

Among the 22 parasite lineages found in summer, only six were found also in wintering birds, thus qualifying as year-round transmitted. The remaining 16 parasites were classified as being summer transmitted. Another two parasites that were only found in winter could not be assigned meaningful values for geographical range or local prevalence, and therefore they were excluded from the analyses (Table 1). We compared our results with the results expected from random sampling effects. Our simulations showed that at least 12 parasites (lower 95% percentile value) were expected to occur in both seasons, but only six were observed in the winter sample. Individual probabilities of parasite occurrence in winter (given summer prevalence) were always above 50% (>95% if local summer prevalences were considered; Fig. 2), so that all the 16 missing parasites were not found in winter because of random sampling effects was very unlikely ($P = 2.6 \times 10^{-12}$). In fact, finding two new parasites in winter that are not found in summer was particularly unexpected if overall abundance determined the probability of winter occurrence of parasites, because any of the 16 parasites observed only in summer should, in principle, be more abundant than the parasites not found in summer. Importantly, the parasites found both in summer and in winter had very different prevalence between seasons

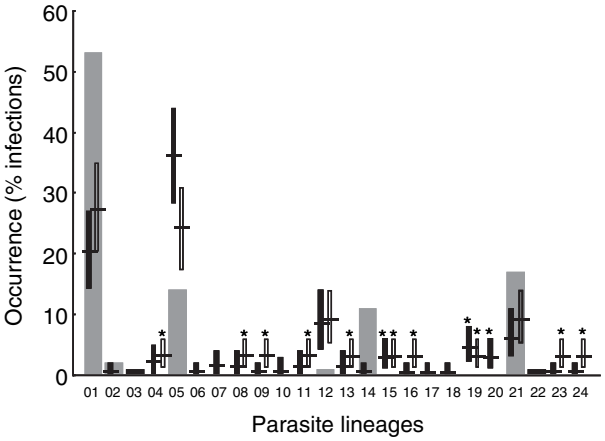

Figure 2 Prevalence of malarial parasites observed in winter (bars), in relation to the expected prevalence (mean and 5–95% percentile range) given the structure of summer parasite communities in all breeding populations (filled plots) and in the local population breeding in southern Spain (open plots). Parasites with plots marked with asterisks were expected in 100 winter infections (with $P > 0.95$), given the corresponding summer prevalences, yet we did not find them. Two parasites found only in winter (black bars) are distinguished from year-round transmitted parasites (grey bars).

(Fig. 2), occurring during winter, on average, at 5% higher prevalence than the expected from summer abundance. Such a pattern further contributes to discard sampling bias as the cause for the absence in winter of most parasites found in summer.

Remarkably, changes in seasonal dynamics of infection could be directly observed in the resident population in southern Spain. Summer prevalence among resident blackcaps was 87.5%, which was similar to the average prevalence in migratory populations (mean ± SE: 87.9 ± 5.6). In winter, 42.9% of local resident birds were infected, which was not significantly different from the prevalence in migrants (36.9%, $n = 264$, Fisher exact $P = 0.49$). Parasites infecting the local population in winter were the same as found among migrants, despite summer parasite richness was higher in the sedentary population (14 lineages) than in the migratory populations (mean ± SE: 7 ± 0.63). Therefore, variation in season of appearance of different parasites revealed different parasite phenotypes. The phylogenetic distribution of these phenotypes showed that year-round transmission has evolved repeatedly in malarial parasites of blackcaps (Fig. 1).

We found a strong association between the ability of parasites to be transmitted in the winter period of host sympatry and their dispersal potential among distantly located breeding populations of hosts, which was revealed by both standard and phylogenetically based analyses. Thus, year-round transmitted parasites had a broader geographical range than summer transmitted parasites ($F_{1,20} = 8.38$, standard $P = 0.0089$, phylogenetically correct $P = 0.0026$; Fig. 3a). We also found a clear association between dispersal potential and local prevalence of parasites. Year-round transmitted parasites had a higher average local prevalence in the host-breeding populations where they occurred ($F_{1,20} = 6.78$, standard $P = 0.017$, phylogenetically correct $P = 0.0073$; Fig. 3b). According to these results, the most widespread parasites showed the highest local prevalence, as revealed both by standard regression analysis ($R = 0.66$, $F_{1,20} = 15.37$, $P = 0.0008$) and regression on standardized phylogenetically independent contrasts ($R = 0.78$, slope of the 'reduced major axis' regression forced through the origin = 0.087, $F_{1,20} = 69.48$, $P < 0.0001$; Fig. 3c).

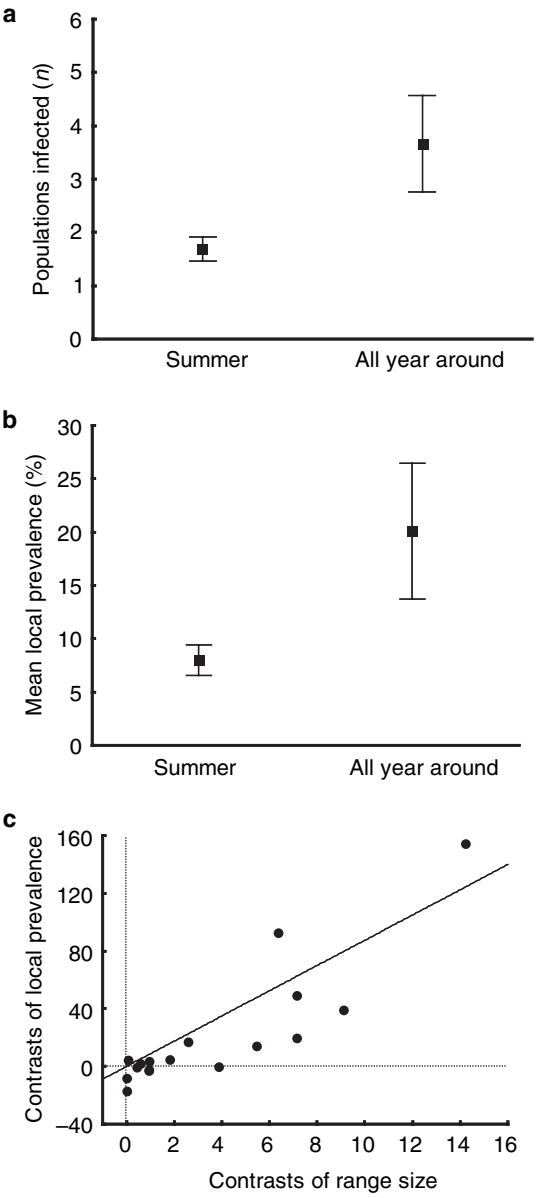

Figure 3 Relationships between seasonal transmission, dispersal potential and local transmission rate of parasites. The graphs show differences between summer transmitted parasites and year-round transmitted parasites in geographical range size (a) and mean local prevalence (b); mean values ± SE. The association between dispersal potential and local transmission of parasites was illustrated by a positive correlation between phylogenetically independent contrasts of geographical range size and local prevalence (c). The graph shows standardized contrasts, which were all given positive value for range size.

DISCUSSION

Positive relationships between range size and local abundance have been found in many organisms, yet the causes are generally unknown and the biological mechanisms that have been suggested are hardly applicable to vector-borne parasites (Gaston *et al.* 1997). Our results confirmed a positive relationship between range size and local prevalence in malarial parasites, supporting the evolutionary change of seasonal transmission dynamics as the mechanism. Thus, we conclude that year-round transmission opportunities increased the potential of parasites to disperse among local host-breeding populations, and this phenotypic change

increased parasite local prevalence in this host–parasite interaction.

Local prevalence is a fair correlate of local infection success in horizontally transmitted parasites (Ebert 1994; Ebert & Herre 1996). In our study, highly repeatable prevalence of each parasite in adults and in juveniles (locally infected) demonstrates the correspondence between prevalence and transmission rate. However, the mechanisms that might increase local transmission of widespread parasites remain to be investigated. For example, an increased capacity to disperse among host populations may enhance local infectivity of parasites if added genetic variation because of immigration helps them to track variable frequencies of host's resistance alleles, a process that otherwise would be entirely dependent on parasite mutation and recombination (Lively & Dybdahl 2000; Gandon 2002; Dybdahl & Storfer 2003). By revealing extensive phenotypic divergence among mtDNA lineages of avian malarial parasites, our results add up to recent studies supporting the view that such malarial lineages are evolutionarily independent entities (Bensch *et al.* 2004). Evolutionary independence allows for extensive genetic variation at infectivity loci in otherwise genetically similar malarial lineages (Cui *et al.* 2003; Leclerc *et al.* 2004), which is a necessary condition for parasite local transmission to be enhanced by immigration (Lively & Dybdahl 2000; Gandon 2002; Dybdahl & Storfer 2003). In the future, reciprocal experimental infections of blackcaps (e.g. Ebert 1994) should help to determine whether or not local adaptation processes lie behind the correlation between parasite dispersal and local prevalence revealed in this study.

The association between seasonal transmission dynamics, geographical range and local prevalence of parasites can be governed by mechanisms other than host–parasite co-adaptation. Our results show that the ability to be transmitted during extended time, reaching periods of host sympatry, evolved in different independent occasions in blackcap parasites. Seasonal transmission of malarial parasites may evolve in relation to their capacity to evade the host's immune system. Parasites that are easily recognised by the host immune system may be selected for transmission during periods of reduced host immunocompetence, for example, during the birds' breeding season, when host resources that could be used for immunity are devoted to reproduction instead (Sheldon & Verhulst 1996). Such seasonal relapses in blood, clearly demonstrated by most parasites in our study, are common among avian *Haemoproteus* and *Plasmodium* parasites (Valkiūnas 2005). However, some parasites could evade the host-immune system, being free to cast infective gametocytes in the bloodstream in larger numbers and during extended periods of time (Mackinnon & Read 1999). An extended gametocyte production, eventually reaching periods of host sympatry in wintering grounds, would favour parasite dispersal. Besides, an abundant production of gametocytes would boost parasite transmission (Mackinnon & Read 1999), thus generating the evolutionary association between non-seasonal transmission, geographical range size and local prevalence observed in this study.

Whether a higher prevalence of widespread parasites is governed by host–parasite interaction genes, epidemiology of parasites or a combination of both processes, our study reveals a strikingly high genetic and ecological diversity of malarial parasites in a migratory bird, which increases the complexity of both host–parasite and parasite–parasite interactions. For example, multiple parasite infections probably entail in-host parasite competition, which may increase the virulence of some parasites (Read & Taylor 2001) and compromise transmission of others (Paul *et al.* 2002). Besides, the existence of malarial parasites with variable transmission rates has important implications for disease evolution (Clayton & Moore 1997; Dybdahl & Storfer 2003; Dronamraju 2004), as both theoretical models and empirical research are consistent in showing a relationship between parasite transmission rate and virulence (Ebert & Herre 1996; Mackinnon & Read 1999). We do not know whether different blackcap parasites have different in-host replication rates, or if replication rates of particular parasites change in presence of other parasites, but a higher transmission success of year-round transmitted parasites suggest that these parasites may infect their hosts with higher intensity than summer transmitted parasites, and therefore may be more damaging (Mackinnon & Read 1999). Higher virulence could select for increased immunity of hosts against parasites with an extended period of transmission, which would explain the apparently short evolutionary persistence of this phenotypic trait (all year-round transmitted parasites are recently derived lineages, Fig. 1). Last but not least, phenotypic diversity increases the versatility of parasite faunas to adapt to different seasonal cycles of insect vector activity, which poses a serious threat to biodiversity by increasing the opportunities for disease to emerge and spread in natural populations (Altizer *et al.* 2003). Importantly, all these features of parasites are often hidden to the eye, demonstrating the importance of molecular methods to assess genetic and phenotypic diversity of parasites, which will certainly contribute to a better future understanding of the ecology and evolution of host–parasite interactions.

ACKNOWLEDGEMENTS

We thank Sylvain Gandon, Trevor Price, Robert E. Ricklefs and three anonymous referees for insightful comments. This study was funded by the European Community (J.P. Marie Curie grant HPMF-CT-2002-02096), the Swedish Research Council and Carl Trygger Foundation (SB).

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Editor, Jacob Koella
Manuscript received 14 April 2005
Manuscript accepted 9 May 2005