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Diversity, distribution and exchange of blood parasites meeting at an avian moving contact zone

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Abstract

Research on contact zones has paid relatively little attention to host–parasite interactions, although these situations have important but different implications depending on whether one considers the host or the parasite's perspective. We investigated both the role of a host contact zone in parasite expansion and whether parasites could influence contact zone dynamics. We studied the diversity and the patterns of parasite exchange (genera *Haemoproteus* and *Plasmodium*) infecting two parapatric sibling passerines meeting at a moving contact zone in western Europe. We amplified and sequenced a fragment of the parasite cytochrome *b* gene. The expanding host harboured more diverse parasites, which might indicate a superior ability to face a diverse parasite fauna than the receding host. Prevalence was very high in both hosts, due to the frequent occurrence of two sister *Haemoproteus* lineages. Despite the recent movement of the contact zone, these two parasites fitted almost perfectly to the geographic range of their main host species. Yet, we found several cases of cross-species infection in sympatric areas and evidences of asymmetrical spreading of parasites from the expanding host towards the receding host. Altogether, our results suggest that the host contact zone mainly acts as a barrier to parasite expansion even if recurrent host shifts are observed. Besides, they also support the idea that parasite-mediated competition might contribute to the displacement of hosts' contact zones, thereby emphasizing the role of parasitism on the population dynamics of sympatric species.

Keywords: contact zone dynamics, cross-species infection, *Hippolais icterina*, *Hippolais polyglotta*, prevalence, sympatry

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Introduction

The areas where two parapatric sibling species meet, known as contact zones, are often remarkably narrow, yet their geographic position may fluctuate or even move directionally (Barton & Hewitt 1985; Dasmahapatra *et al*. 2002). Contact-zone dynamics have been interpreted as the consequence of different biotic and abiotic factors, such as interspecific competition (Bull & Burzacott 2001), hybridization (Rhymer & Simberloff 1996; Ellstrand & Schierenbeck 2000; Moody & Les 2002), varying impact of predation (Bull 1991), or climate change (Dukes & Mooney 1999). However, the reasons why some contact zones are stationary while others move, and the mechanisms explaining such movements, are rarely known. Because parasites are considered to be a major factor affecting sympatric populations (Hudson & Greenman 1998; Prenter *et al*. 2004), it is remarkable that their role in contact-zone dynamics has not been studied more frequently (Strauss 1994).

There are several reasons to focus on host–parasite interactions in contact zones. The most obvious one is that two species that come into contact also bring their parasites together, thereby creating opportunities for parasite exchange, which may have consequences for both hosts and parasites (Hafner *et al*. 1998; Tompkins *et al*. 2003). From the hosts' perspective, contact with new parasites can have dramatic consequences in terms of disease. For example, the introduction of vectors and reservoirs of avian malaria in Hawaii caused the extinction of many

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native Hawaiian birds and the retreat of many others to vector-free habitats (van Riper *et al.* 1986), but it also caused strong selection for increased parasite resistance in some host species, which have recently recolonized habitats with high malaria transmission (Woodworth *et al*. 2005). Interspecific parasite interchange is particularly expected in contact zones because host species are usually closely related. Besides, some host species could free themselves of parasites in newly colonized areas, due to the inability of their parasites to complete their life cycles (Torchin *et al*. 2003). Both the contact with new parasites and parasite release in recently colonized areas can cause competitive asymmetries between sympatric hosts, favouring the expansion of one species into its counterpart's range (Shea & Chesson 2002).

Contact zones are not only important from the perspective of hosts, but also from the perspective of parasites, because hosts' contact zones can be either barriers or conducts for parasite expansion into a new host species. The opportunities for parasites to expand across hosts' contact zones will depend on the degree of specialization of the host–parasite relationships brought into contact (Bensch *et al*. 2000; Ricklefs & Fallon 2002; Ricklefs *et al*. 2004), the availability of suitable intermediate hosts, or the occurrence of environmental conditions allowing parasite development (Osta *et al*. 2004; Valkiûnas 2005). Therefore, the analysis of host–parasite interactions at contact zones is not only interesting in the context of the configuration of species ranges of neighbouring sibling species, but it can also help us to understand the evolution of multihost parasitism (Gandon 2004).

We studied the diversity, geographic distribution and patterns of between-host occurrence of blood parasites (genera *Haemoproteus* and *Plasmodium*) infecting two sibling passerine species whose breeding ranges overlap in western Europe: the melodious warbler, *Hippolais polyglotta*, and the icterine warbler, *Hippolais icterina*. Both species are summer visitors to Europe. Melodious warblers breed in southwestern Europe and migrate mostly to western sub-Saharan Africa, whereas icterine warblers breed in north and central Europe and migrate to south-central Africa. It remains unclear whether the two wintering ranges are completely separated or overlap to some extent (Cramp 1992). The contact zone between both species has moved towards northeast during the last century. This movement results from the expansion of the melodious warbler and the synchronous receding of the icterine warbler on the southwestern border of its range (Jouard 1935; Yeatman-Berthelot & Jarry 1994; Faivre *et al*. 1999; Secondi *et al*. 2003). Previous studies have investigated the role of interspecific interactions on the displacement of the contact zone, but none of them could convincingly explain why icterine warblers are receding on the entire western border of their range. Interspecific competition seems not to drive the expansion of the melodious warbler. First, icterine warblers are larger than melodious warblers, and therefore they are expected to be dominant in social contests (Alatalo & Moreno 1987; Cassey 2001). Furthermore, these species show little trophic overlap, and therefore they are unlikely to compete for food resources (Faivre 1993). Hybridization seems not to account for the general displacement of the zone either, because hybridization rate is heterogeneous between sites and is generally low in sympatric populations (Faivre *et al*. 1999; J. Secondi, unpublished). If the displacement of the contact zone is mediated by natural enemies, blood parasites of the genera *Plasmodium* and *Haemoproteus* are good candidates to take part in such a process, as they are the agents of infectious diseases (including avian malaria), which are known to cause high mortality and morbidity in wild bird populations (Atkinson *et al*. 2000; Merino *et al*. 2000; Sol *et al*. 2003; Valkiûnas 2005). Moreover, avian malarial parasites are horizontally transmitted by insect vectors, which opens opportunities for cross-species transmission of parasites in sympatric areas.

The recent development of molecular tools for the detection of avian blood parasites has greatly improved our understanding of the ecology and evolution of bird–parasite interactions (Bensch *et al*. 2000; Ricklefs & Fallon 2002; Fallon *et al*. 2004; Ricklefs *et al*. 2004). We used molecular methods to detect and identify parasite lineages infecting *Hippolais* warblers, both at their contact zone and beyond, including a large representation of sympatric and allopatric areas. The lack of recombination between mitochondrial and nuclear DNA in avian *Plasmodium* and *Haemoproteus* suggests that mitochondrial lineages represent cryptic species (Bensch *et al*. 2004), and such an idea is further supported by ecological differentiation of parasites with nearly identical mitochondrial DNA sequences (Pérez-Tris & Bensch 2005a). Our goals were to investigate (i) whether the contact zone between these two warbler species acts as a barrier or as a channel for the geographic expansion of their parasites, and (ii) if there is any evidence of parasite release or spillover, which may explain the expansion of melodious warblers into the range of icterine warblers. The displacement of the contact zone could be caused by asymmetric costs or different rates of cross-species transmission of parasites. Given that parasites are generally more virulent when infecting a novel host (Atkinson *et al*. 2000), we expected to find an asymmetric interchange of parasites, with a higher frequency of cross-species infections from the expanding melodious warbler towards the receding icterine warbler than in the opposite way. To answer these questions, we analysed the phylogenetic diversity of parasites infecting both warbler species, the degree of host specificity exhibited by parasites, and the distribution of parasite sharing at the contact zone and across the geographic range of the bird hosts.

Materials and methods

Bird sampling

We studied 179 melodious warblers and 106 icterine warblers from 22 populations distributed between Spain and Russia (Fig. 1, Table 1), which includes the whole breeding range of melodious warblers and the western part of the distribution of icterine warblers. Populations were classified as allopatric, recent allopatric or sympatric according to their location with respect to the contact zone (Fig. 1). Allopatric populations have birds of only one species. Recent allopatric populations of the expanding melodious warbler are located in the wake of the moving contact zone, and became allopatric during the last century due to the retreat of icterine warblers. Finally, sympatric populations define the current configuration of the contact zone, and include areas where individuals of both species coexist (Fig. 1).

Birds were caught using mist nets during the breeding seasons of 2001 and 2002. Captures occurred between early May and early July, except for two populations (Magdeburg and Rybachy), which were sampled in late July and late August in 2001, respectively. Blood samples (20–40 μL) were taken from the brachial vein of birds, and were stored in preservation buffer until analysis. All birds were identified as melodious or icterine warblers using morphological characteristics (Faivre *et al*. 1999), song (Secondi *et al*. 2003), and genetic markers [cytochrome *b* sequences and amplified fragment length polymorphism (AFLP) profile; J. Secondi, unpublished]. Genetic analyses allowed us to identify a hybrid bird (found in Mulhouse, one of the sympatric areas; J. Secondi, unpublished), which had been scored in the field as an adult melodious warbler, and was sexed as male in the laboratory. All birds were aged in the field as juveniles or older birds (adults hereafter), based on plumage and bare parts (Svensson 1992). Given that most birds were captured early in the breeding season, our sample included very few juveniles (13 melodious and 11 icterine warblers). Analyses of parasite infections of juvenile birds are nonetheless important, as they have spent their whole life in their natal area and therefore provide information on local transmission of parasites. Birds were sexed using molecular markers (Griffiths *et al*. 1998). Two melodious warblers were not sexed, and one icterine warbler was not aged.

Parasite screening

We extracted total DNA from birds' blood using a standard phenol–chlorophorm protocol. All samples were quantified and diluted to a working concentration of 25 ng/μL. We used a nested polymerase chain reaction (PCR) to detect parasite infections (Hellgren *et al*. 2004). This method consists of a preamplification step (20 cycles) with primers HaemNFI and HaemNR3, followed by a final amplification (35 cycles) with primers HaemF and HaemR2 designed to target a 479-bp fragment of the cytochrome *b*

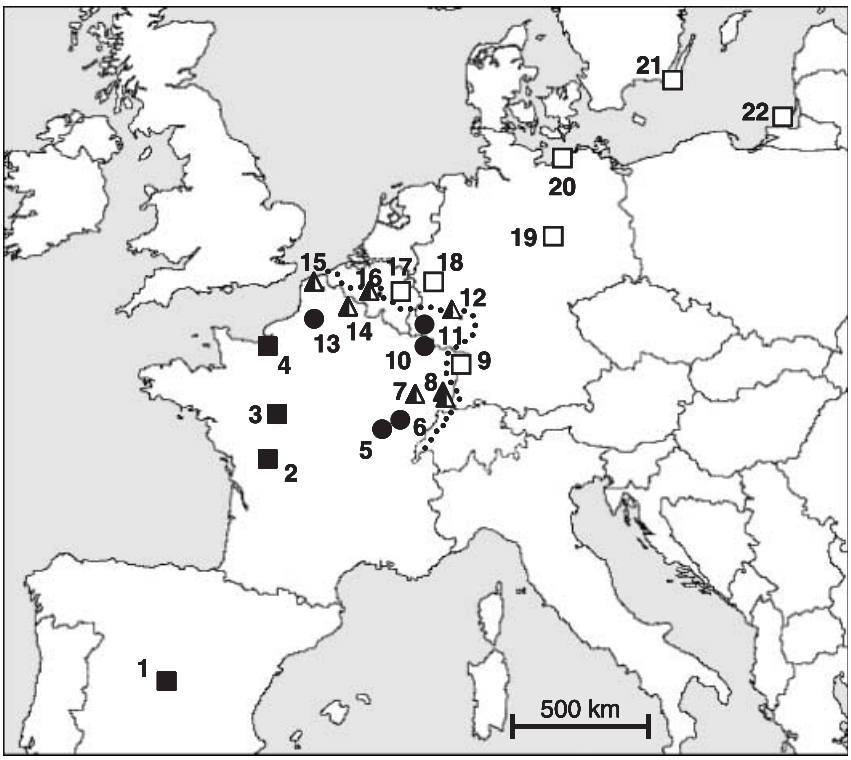

Fig. 1 Location of the study sites (see population names in Table 1). Populations of icterine warblers are represented with open symbols, and populations of melodious warblers with filled symbols. The position of each population relative to the contact zone is represented by squares (allopatry), circles (recent allopatry), or triangles (sympatry). The dashed line shows the northern limit of the current range of the melodious warbler.

Table 1 Number of birds screened for parasites, number of birds infected and harbouring mixed infections, and parasite counts in each population. Bird species names (MW, melodious warbler; IW, icterine warbler) are in bold in populations with cross-species infections (which involved the parasite lineages HIPOL1 and HIICT1). The lineages SGS1 and GRW2 belong to the genus *Plasmodium*, and the others to *Haemoproteus*. The location of each population with respect to the contact zone is indicated (A, allopatry; RA, recent allopatry; S, sympatry). Geographic locations and phylogenetic relationships and identity of parasite lineages are shown in Fig. 2

			Prevalence (number of birds infected)										
Population	Location	Species	*n*	Infected (*n*)	Multiple (*n*)	HIPOL 1	HIICT 1	ACDUM 2	HIICT 3	HIICT 4	HIPOL 2	SGS 1	GRW 2
1. Madrid	A	MW	19	11	1	9	0	2	0	0	0	0	1
2. Chizé	A	MW	12	12	2	12	0	2	0	0	0	0	0
3. Tours	A	MW	10	10	0	10	0	0	0	0	0	0	0
4. Caen	A	MW	10	10	0	10	0	0	0	0	0	0	0
5. C'teaux	A	MW	12	12	1	9	0	3	0	0	0	1	0
6. Auxonne	RA	MW	11	10	1	8	0	0	0	0	1	2	0
7. Conflans	S	**IW**	11	11	1	1	10	0	1	0	0	0	0
		MW	12	11	2	11	0	2	0	0	0	0	0
8. Mulhouse	S	IW	5	5	0	0	5	0	0	0	0	0	0
		MW	15	13	1	13	0	0	0	0	0	0	1
9. Strasbourg	A	IW	11	10	1	0	10	0	1	0	0	0	0
10. Saarlouis	RA	MW	11	11	0	11	0	0	0	0	0	0	0
11. Trier	RA	MW	10	10	0	10	0	0	0	0	0	0	0
12. Bingen	S	**IW**	4	3	0	1	2	0	0	0	0	0	0
		MW	23	17	1	15	1	2	0	0	0	0	0
13. Amiens	RA	MW	4	4	0	4	0	0	0	0	0	0	0
14. Le Quesnoy	S	**IW**	11	11	0	1	9	0	1	0	0	0	0
		MW	21	21	0	21	0	0	0	0	0	0	0
15. Hazebrouck	S	IW	3	3	0	0	3	0	0	0	0	0	0
		MW	8	7	0	7	0	0	0	0	0	0	0
16. Gembloux	S	IW	6	6	0	0	6	0	0	0	0	0	0
		MW	1	1	0	1	0	0	0	0	0	0	0
17. Liège	A	**IW**	10	10	0	1	9	0	0	0	0	0	0
18. Köln	A	IW	2	2	0	0	2	0	0	0	0	0	0
19. Magdeburg	A	IW	10	10	1	0	10	0	0	1	0	0	0
20. Rostock	A	IW	11	10	1	0	9	0	2	0	0	0	0
21. Öland	A	IW	12	11	0	0	11	0	0	0	0	0	0
22. Rybachy	A	IW	10	3	0	0	3	0	0	0	0	0	0

gene of *Plasmodium* and *Haemoproteus* parasites (Hellgren *et al*. 2004). In both steps, PCRs were set up in 25-μL total volumes including 50 ng of template DNA (1 μL of preamplified PCR product in the second reaction), 1 × PCR buffer (PerkinElmer), 0.125 mM of each nucleotide, 0.4 μM of each primer, 1.5 mM $MgCl_2$, and 0.5 U of Ampli*Taq* DNA polymerase (PerkinElmer). Reactions started with 3 min at 94 °C, followed by 20–35 cycles of 30 s at 94 °C, 30 s at 50 °C and 45 s at 72 °C, and they were terminated by a 10-min extension at 72 °C. We evaluated 2.5 μL of each final reaction on 2% agarose gels stained with ethidium bromide and using 0.5 × TBE buffer.

Positive infections were revealed in the gels by the presence of a PCR product of the expected length, and all negative results were confirmed by a second test. We included negative controls in all reactions (water instead of genomic DNA), and never observed false positives. The quality of all samples for PCR was guaranteed by successful amplification of sexing and AFLP markers. Samples with positive PCRs were precipitated by adding 11 μL of 8 M NH_4Ac and 33 μL ethanol, and resuspended in 10–15 μL water. We used 2 μL for direct sequencing using a dye-terminator AmpliCycle® sequencing kit and an ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems), following manufacturer's recommendations. All sequences were visually aligned and edited using BIOEDIT (Hall 1999). Sequences with too much background or weak signal were discarded, and the corresponding samples were amplified again. A few sequences (13 cases) showed double nucleotide peaks revealing multiple infections, which were resolved using TA-cloning (Pérez-Tris & Bensch 2005b). Parasite lineages were distinguished by one or more nucleotide differences, and classified as *Plasmodium* spp. or *Haemoproteus* spp. according to sequence similarity with known parasites.

Phylogenetic analyses

We used PAUP* 4.0 (Swofford 1998) to build an unrooted neighbour-joining phylogenetic tree based on parasite mitochondrial DNA sequences. We used a general time reversible (GTR) model of nucleotide substitution under a gamma distribution with $\alpha = 0.243$. The GTR + G model was selected as the best of 56 models, according to the Akaike information criterion implemented in MODELTEST 3.6 (Posada & Crandall 1998). We built the tree using the human parasite *Plasmodium falciparum* as an outgroup (Perkins & Schall 2002). 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.

Statistical analyses

We analysed overall differences in prevalence between species using Fisher exact tests. Then, we tested for variation in prevalence within species in relation to sex, age and population of origin of birds, using log-linear analyses. This method analyses multifactor cross-tabulations by partitioning the variation in frequency data into the different factors, testing for interactions in a way similar to the analysis of variance (ANOVA). The log-linear model is defined by the minimum number of interactions necessary to account for the distribution of frequencies. To obtain the best models in each case, we first proceeded hierarchically by fitting all interactions of order k to the corresponding null hypotheses that all of them are simultaneously zero. As soon as the reduction in k caused a lack of fit, we selected the terms of that order or lower which significantly contributed to explain the distribution of frequencies, thus generating the final model that best fitted to the data (StatSoft 2002).

We compared the average phylogenetic relatedness of parasites infecting each warbler species using ANOVA. In this analysis, the sample units were the pairwise genetic distances between parasites found in each species (Kimura 2-parameter distances computed by MEGA2; Kumar *et al*. 2001). Given that these measures are statistically not independent (because the same parasite sequence contributes to several pairwise distances), significance values were not derived from standard statistical tables, but they were estimated from an empirical distribution of F values obtained through the repetition of the ANOVA on 10 000 simulations of random distribution of parasite lineages between host species. In each simulation, a random subset of the whole parasite community (eight lineages, see Results) was assigned to each host species, keeping the same parasite lineage richness as observed in nature in each species. The critical value for significance was conventionally set as the 95% percentile of the distribution of simulated F values.

Results

Parasite prevalence

The vast majority of birds were infected by blood parasites, with 12 out of 22 populations reaching 100% prevalence (Table 1). Average local prevalence (with SE) was 92.6 ± 3.2% in melodious warblers and 90.7 ± 5.4% in icterine warblers. We found eight parasite lineages (Fig. 2). Parasite sequences were compared with the sequences of known parasites, either published in GenBank or found in our laboratory (in a database involving almost 100 passerine species, mostly European and sympatric to *Hippolais* warblers, and over 2000 infections of more than 165 parasite lineages). Two parasite lineages were identified as common *Plasmodium* parasites, which have already been retrieved from other birds [*Plasmodium* sp. strain SGS1, GenBank Accession no. AF495571, and strain GRW2 tentatively identified as *Plasmodium nucleophilum* (AF254962)]. The remaining six parasite lineages qualified as *Haemoproteus* spp. One of them had the same sequence as the lineage ACDUM2 (DQ000320) found in Blyth's reed warblers, *Acrocephalus dumetorum*, but the other five were mostly exclusive to *Hippolais* warblers [lineages HIPOL1 (DQ000324), HIPOL2 (DQ000325), HIICT1 (DQ000321), HIICT3 (DQ000322) and HIICT4 (DQ000323)]. The only exception was HIICT1, which had been found in whinchats, *Saxicola rubetra*, a species that shares breeding range with icterine warblers. We did not find the lineage HIICT2 (AF495556) previously found in icterine warblers in Nigeria by Waldenström *et al*. (2002). We found 13 birds harbouring mixed infections, of which 10 were infected by two *Haemoproteus* lineages, and three harboured parasites of both genera.

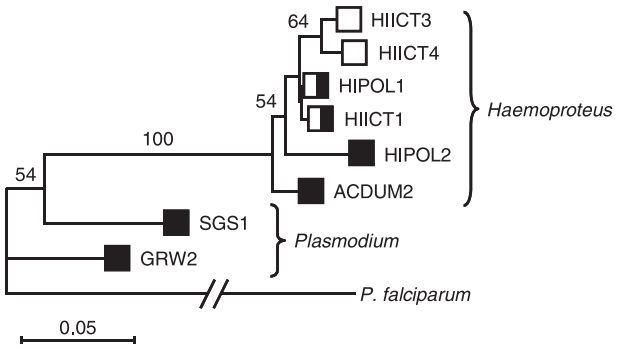

Fig. 2 A phylogeny of blood parasites found in icterine and melodious warblers, based on cytochrome *b* sequences. The occurrence of each parasite lineage in each host species is indicated by white squares (icterine warbler) or black squares (melodious warbler). Numbers on internal branches indicate bootstrap support if higher than 50% (based on 1000 replicates).

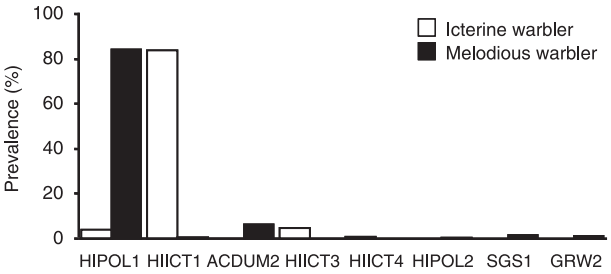

Fig. 3 Prevalence of the eight parasite lineages found in icterine and melodious warblers.

The prevalence of *Plasmodium* parasites was very low. We did not find this genus in icterine warblers, and only 2.5 ± 1.3% of melodious warblers were infected by parasites of this genus in each locality (*Plasmodium* parasites only occurred in 4 out of 15 melodious warbler populations, Table 1). Although *Haemoproteus* was the most prevalent genus, only two lineages (HIPOL1 and HIICT1) clearly dominated the parasite community (Fig. 3). These two parasites were very closely related (Fig. 2), differing by a single nucleotide substitution (0.21% sequence divergence). Interestingly, these two parasites were the only ones shared by both warblers (Fig. 3, Table 1), although they showed a highly biased distribution between host species, with 97.4% of HIPOL1 cases being detected in melodious warblers, and 98.9% of HIICT1 cases being found in icterine warblers (Fig. 3; Fisher exact $P < 0.0001$). Other parasite lineages had prevalence below 7%. Overall, both warbler species showed a similar prevalence of *Haemoproteus* parasites (Fisher exact $P = 0.85$; Fig. 3), even if this analysis was restricted to the two most prevalent parasite lineages (HIPOL1 and HIICT1: $P = 0.60$; Fig. 3). The only hybrid bird that we identified in our sample was not infected.

In melodious warblers, a log-linear analysis of parasite prevalence according to sex, age and population location (allopatry, recent allopatry or sympatry) produced a model with two-way interactions (fit to the absence of three-way interactions: $\chi^2_7 = 1.70$, $P = 0.97$; absence of two-way interactions: $\chi^2_9 = 72.2$, $P < 0.0001$; goodness of fit of the model: $\chi^2_{16} = 9.41$, $P = 0.90$). The model included a significant association between age of hosts and infection status (partial association: $\chi^2_1 = 44.82$, $P < 0.0001$), as only 7.7% of juvenile birds ($n = 13$) were infected (only one juvenile was infected, by the *Plasmodium* lineage SGS1), while 96.3% of adults ($n = 166$) were infected. Infection status was independent of the sex of birds ($\chi^2_1 = 1.34$, $P = 0.25$) and population location ($\chi^2_1 = 3.60$, $P = 0.16$).

In icterine warblers, a log-linear analysis of parasite prevalence according to sex, age and population location (allopatric or sympatric) also produced a model with two-way interactions (fit to the absence of three-way interactions: $\chi^2_7 = 2.99$, $P = 0.89$; absence of two-way interactions: $\chi^2_9 = 37.01$, $P < 0.0001$, goodness of fit of the model: $\chi^2_{16} = 16.69$, $P = 0.41$). In this species, parasite prevalence was also lower in juveniles (36.4%, $n = 11$) than in adults (95.7%), such an effect being statistically significant ($\chi^2_1 = 22.31$, $P < 0.0001$). Infection status in icterine warblers was also independent of the sex of birds ($\chi^2_1 = 0.19$, $P = 0.65$) and population location ($\chi^2_1 = 0.11$, $P = 0.74$). All these results remained the same when we restricted our analyses to *Haemoproteus* lineages, or to the most prevalent single parasite lineage in each species (results not shown).

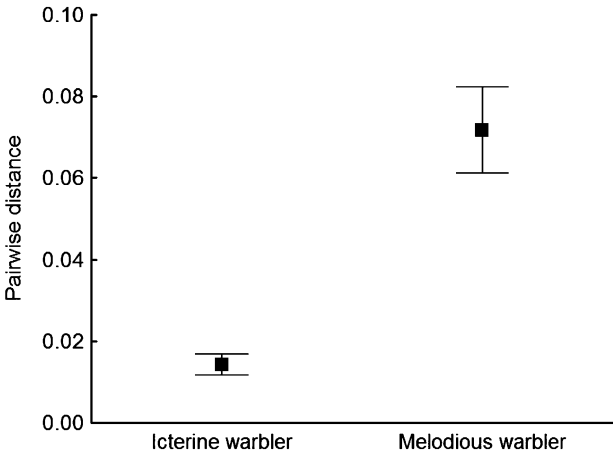

Fig. 4 Differences in phylogenetic diversity of the parasites infecting icterine and melodious warblers (mean and SE), measured by Kimura 2-parameter genetic distances between pairs of parasite DNA sequences.

Parasite phylogenetic diversity

Melodious warblers had a higher richness of parasite species (six lineages) than icterine warblers (four lineages). More importantly, the parasites of melodious warblers showed a higher phylogenetic diversity than the parasites of icterine warblers (Fig. 4). All parasites of icterine warblers clustered together into a monophyletic clade, while the parasites of melodious warblers were of more diverse ancestry (Fig. 2). Thus, an ANOVA revealed a higher average pairwise genetic distance between parasites of melodious warblers than between parasites of icterine warblers ($F_{1,19} = 11.39$), which was significant both when tested against standard significance tables ($P = 0.0031$) and when tested against an empirical distribution of 10 000 F values obtained from random simulations of parasite distribution between host species ($P = 0.027$).

Geographic range of parasites and cross-species infection

The geographic distribution of the two most prevalent parasite lineages matched the distribution of their respective main host species. Both parasite lineages co-occurred in sympatric populations, but even in these areas they

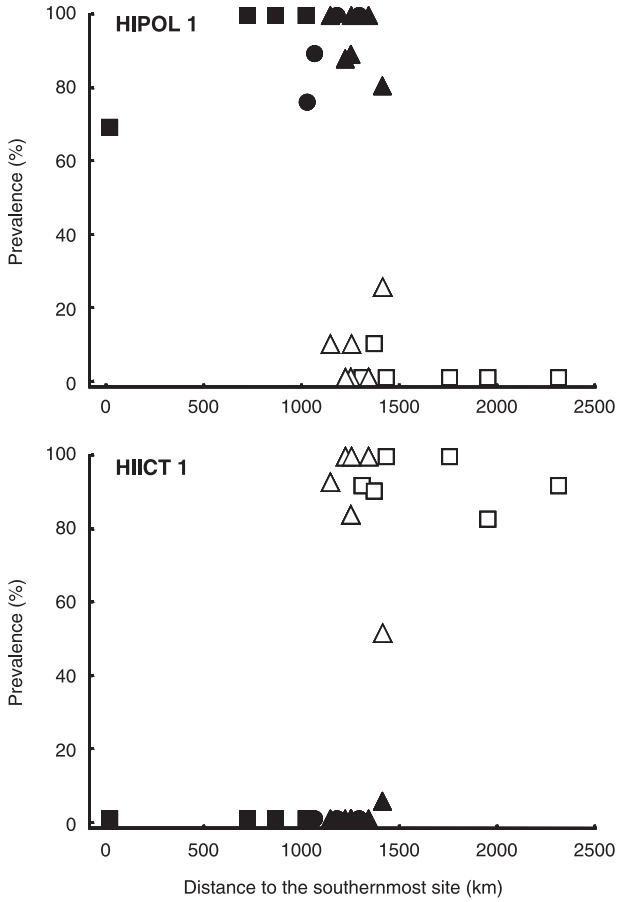

Fig. 5 Geographic distribution of adult prevalence of the two most common parasite lineages (HIPOL1 and HIICT1) in each host species (open symbols, icterine warblers; filled symbols, melodious warblers). Populations have been arranged in a geographic gradient from the southernmost locality. The position of each population relative to the contact zone is represented by squares (allopatry), circles (recent allopatry), or triangles (sympatry).

remained mostly confined to one single host species (Table 1; Fig. 5). However, we observed five instances of cross-species infection: one melodious warbler was infected by HIICT1 and four icterine warblers were infected by HIPOL1. All these cases were found in sympatric areas (four cases) or in other areas within the dispersal range of birds from the contact zone (one case in Liège, less than 50 km from the nearest sympatric site; Fig. 1, Table 1). Cross-species parasite interchange in sympatry was six times more frequent from melodious to icterine warblers (3 out of 39 infected icterine warblers from sympatric areas harboured the parasite lineage HIPOL1 = 7.7%) than from icterine to melodious warblers (1 out of 70 infected melodious warblers harboured HIICT1 = 1.4%), although such a trend was not statistically significant (Fisher exact $P = 0.14$; Fig. 5).

Discussion

Both melodious and icterine warblers showed a high prevalence of blood parasites across their geographic ranges. The prevalence of *Plasmodium* parasites was very low in melodious warblers (around 2%), and these parasites were not found in icterine warblers. However, the prevalence of *Haemoproteus* in these two warblers (higher than 90% in adults) was among the highest recorded so far in any bird species (Scheuerlein & Ricklefs 2004; Valkiūnas 2005). Such a result might be attributed to the high efficiency of parasite detection of the molecular method we used (Hellgren *et al*. 2004), which somewhat limits direct comparisons with other studies. Nevertheless, the high parasite prevalence recorded in these two warbler species suggests a high rate of parasite transmission in this host–parasite system, which should mean more opportunities for parasite interchange between species. This supports the idea that host–parasite interactions could influence the dynamics of the moving contact zone between these two warbler species.

History of host–parasite relationships

The distribution of parasite lineages in this host–parasite system can be interpreted as a result of the historical vicariance patterns of the two warbler species. Melodious and icterine warblers probably diverged from a common ancestor during periods of population isolation, probably more than 3 million years ago (the cytochrome *b* sequence divergence between these species is 6.5%; Helbig & Seibold 1999). After their divergence, the breeding areas of these warblers were repeatedly glaciated during the Quaternary, the most recent glacial maximum having occurred in the late Pleistocene 18 000 years ago (Hewitt 2000). As a consequence, these species are likely to have repeatedly retreated to glacial refugia and recolonized Europe during Quaternary periods, leading to the formation of different contact zones of which today we observe the most recent one (Blondel & Mourer-Chauviré 1998; Hewitt 2000).

Alternating periods of isolation and contact of host populations could have shaped the parasite community of these species. Host isolation periods were probably associated with parasite population bottlenecks, and probably with the loss of different parasite lineages in each refugium. Although both warbler species shared some parasites (HIPOL1 and HIICT1), other closely related parasites (HIICT3 and HIICT4) occurred in icterine but not in melodious warblers (Fig. 2). In melodious warblers, such parasites were substituted by a diverse parasite community, including lineages that are shared with other species (ACDUM2 and *Plasmodium* species). These parasites were probably acquired by melodious warblers through host switching from other species (Ricklefs *et al*. 2004).

The parasite community infecting these two warbler species was clearly dominated by two closely related *Haemoproteus* lineages (HIPOL1 and HIICT1), which accounted for more than 90% of infections. Although parasite release and acquisition in historical times are likely to explain the general distribution of parasites between both host species, these two parasite lineages were probably originated by other processes. HIPOL1 and HIICT1 are closely related parasites that show high prevalence in sibling melodious and icterine warblers, also demonstrating a high degree of specificity to these host species (we never found HIPOL1 in other species, and HIICT1 has only been found in whinchats among almost 100 passerine species screened). Moreover, each parasite is highly specialized to either sibling host species. Close relationships and host specialization suggest that these parasites could have diverged by cospeciation with their hosts (Coyne & Orr 2004; Ricklefs *et al*. 2004). However, these parasites are too similar at the cytochrome *b* gene (0.2% sequence divergence) to support the idea that they diverged in parallel to their hosts (which show 6.5% sequence divergence). Although these parasites may have a somewhat reduced rate of molecular evolution (Ricklefs & Fallon 2002), such a scenario would assume a rate of nucleotide substitution around 30-fold higher in the birds than in their parasites, which is very unlikely because these parasites have much shorter generation times than their hosts. An alternative possibility is that these two parasites diverged from each other far more recently, well after the divergence of their hosts. For example, the divergence of HIPOL1 and HIICT1 could have occurred during the last glaciation, which is enough time for the accumulation of a single nucleotide substitution.

Host–parasite interactions at the contact zone

Our study is the first to reveal a situation in which two closely related bird species are bringing together two closely related malarial parasite lineages (HIPOL1 and HIICT1). Given that both the hosts and the parasites are sister species in this system, cross-species transmission of parasites was expected to occur, and it was observed in five cases, involving both parasite lineages. However, the distribution of the two shared parasite lineages fitted almost perfectly to the distribution of their main host species, which indicates that parasite distribution has tracked the displacement of the zone, despite melodious warblers having expanded their range by about 300 km in 40 years (Jouard 1935; Yeatman-Berthelot & Jarry 1994; Faivre *et al*. 1999; Secondi *et al*. 2003). Cross-species infections were always found in sympatric populations, or within the dispersal range of individuals from the contact zone, but these populations were located hundreds of kilometres from each other, showing that parasite interchange is a pervasive process that is not restricted to particular local ecological conditions.

Winter transmission might contribute to increase host specificity of parasites, due to the fact that the wintering ranges of the two warblers show little overlap (Cramp 1992). However, such a process alone cannot account for the observed pattern, as 36% of juvenile icterine warblers were infected by HIICT1, demonstrating that at least this parasite is regularly transmitted in Europe. Indeed, we probably underestimated the frequency of juvenile infections, because we sampled most warbler populations early in the season (May to July, short after fledging of young warblers), while most infected juveniles were observed in two populations sampled in August (when newly acquired infections had had a longer time to become patent in the blood of birds; Valkiūnas 2005). If cross-species infections reveal the regular interchange of HIPOL1 and HIICT1 in sympatric areas, host specificity of these parasites could still be achieved if cross-infected birds incurred higher mortality. In fact, host switching commonly increases virulence in malaria parasites (Atkinson *et al*. 2000). In turn, different virulence in each host could impair the spread of parasites beyond the contact zone, thereby maintaining the observed fit in distribution between parasites and hosts.

A role for parasites in the movement of the contact zone?

A host species that is regularly exposed to a diverse parasite community is expected to have a more versatile immune system than a host species that always faces closely related parasites. Host susceptibility to parasites is directly linked to the host's genotype at resistance genes, such as the major histocompatibility complex. In fact, resistance to infection by particular genetic lineages of malaria parasites is associated with particular birds' *Mhc* alleles (remarkably, these results have been obtained with two *Plasmodium* lineages found in our study: GRW2 in great reed warblers, *Acrocephalus arundinaceus*, and SGS1 in house sparrows, *Passer domesticus*; Westerdahl *et al*. 2005; Bonneaud *et al*. in press). The phylogenetic relationships between parasites infecting two sibling host species may therefore reveal the relative versatility of hosts' immune systems (Møller & Rozsa 2005). It is important to note that melodious warblers harboured a more diverse community of blood parasites than icterine warblers as parasite phylogenetic diversity was significantly higher and more parasite lineages were identified in the former species (Figs 2 and 4). Importantly, such a difference was demonstrated at the contact zone, as all parasite lineages found in melodious warblers occurred in sympatric populations or in neighbouring recent allopatric areas (Table 1). Therefore, although increasing the sampling area towards eastern and central Europe might increase parasite diversity of icterine warblers, differences at the contact zone (which are relevant for sympatric interactions) would not be affected by this potential bias. Differences in parasite diversity might reflect

a better capacity of melodious warblers to face exposure to new parasites in the contact zone (Møller & Rozsa 2005). If the historical exposure to phylogenetically diverse parasites favoured the evolution of versatile resistance mechanisms against this type of parasites (Møller & Rozsa 2005), melodious warblers could be at an advantage when confronted with parasites acquired from the other species.

Furthermore, the occurrence of parasites from the sister species was six times higher in the receding icterine warbler than in the expanding melodious warbler, which may be biologically relevant although such a trend was not significant statistically ($P = 0.14$). Although the paucity of cross-species infections in this host–parasite system forces us to interpret these effects with extreme caution, at the least our results suggest an asymmetrical parasite spreading in the contact zone, from melodious to icterine warblers. Our study hints at an effect of parasitism on the contact-zone dynamics, but the impact of parasite interchange relative to other processes cannot be assessed with our data. A previous study suggested that icterine warblers incurred a higher rate of hybridization than melodious warblers (Faivre *et al*. 1999), although this pattern might have been a consequence of the rarefaction of conspecific sexual partners rather than a cause of the retreat of icterine warblers (Faivre *et al*. 1999; J. Secondi *et al*., unpublished). Differential nest predation has also been observed in the same population, revealing an indirect effect of coexistence between the two warblers (Faivre 1993). Parasite interchange might also affect host coexistence, but the actual effect of parasitism at the population level will be difficult to assess as long as the fitness costs of parasitism remain unknown. The fact that most *Hippolais* warblers were infected suggests that harbouring parasites does not entail important fitness costs. In these circumstances, the fitness consequences of parasitism are more likely dependent on the amount of parasites infecting the host than on parasite presence alone (Sol *et al*. 2003). However, our screening method cannot distinguish parasite intensities, scoring birds with extremely low, subclinical infection levels as identical to birds with highly pathogenic infection levels (Hellgren *et al*. 2004). Future studies should aim at investigating variation in intensity of infection of different parasite lineages in melodious and icterine warblers, particularly in cross-species infections. If malaria parasites play a role in the movement of the contact zone between *Hippolais* warblers, then we expect a higher parasitemia in the receding icterine warbler, particularly in individuals that are infected by HIPOL1 parasites acquired from the expanding melodious warbler.

In summary, our study reports the case of a narrow contact zone between closely related parasites matching both the distribution and the movement of their hosts' contact zone. Our results support the idea that if the contact zone between melodious and icterine warblers can be viewed as a barrier to the expansion of two closely related parasites (which keep nearly restricted to their main host species), it certainly also acts as a channel for parasite expansion into new hosts as shown by the limited but probably persistent cross-species parasite transfer. Finally, our data suggested both a better ability of the expanding species to deal with a diverse parasite fauna, and an asymmetric cross-species infection, with higher frequency of acquisition of parasites by the receding host. From the host perspective, these circumstances may promote parasite-mediated competitive asymmetries between sympatric warblers. From the parasite perspective, they illustrate a scenario in which some parasites might expand their geographic range at their host's contact zone, not by switching host, but by assisting their own host to outcompete its rival species by decreasing the rival's fitness (Price *et al*. 1988; Hudson & Greenman 1998). Interestingly, prevalence data contrast here with the continued existence of a mixed parasite community in the wake of the hosts' moving contact zone, which has been observed in less specialized host–parasite interactions (Hafner *et al*. 1998). Altogether, such results highlight the idea that parasitism plays an important, yet often complex role, in the population dynamics of sympatric species.

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