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Host races in *Ixodes ricinus*, the European vector of Lyme borreliosis

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ABSTRACT

Ixodes ricinus is a European tick that transmits numerous pathogenic agents, including the bacteria that cause Lyme disease (some genospecies of *Borrelia burgdorferi* sensu lato complex). This tick has been considered as a classic example of an extreme generalist vector. However, host-associations in such vector species are difficult to determine from field observations alone and recent work suggests that host specificity may be more frequent in ticks than previously thought. The presence of host-associated vector groups can significantly alter the circulation and evolutionary pathway of associated pathogens. In this paper, we explicitly test for host-associated genetic structure in *I. ricinus*. We analyzed genetic variability at 11 microsatellite markers in a large sample of ticks collected directly from trapped wild animals (birds, rodents, lizards, wild boar and roe deer) at five sites in Western and Central Europe. We found significant levels of genetic structure both among host individuals and among host types within local populations, suggesting that host use is not random in *I. ricinus*. These results help explain previous patterns of structure found in off-host tick samples, along with epidemiological observations of Lyme disease.

1. Introduction

Ticks are parasites of great interest in both applied and fundamental sciences. They present a wide array of life cycles, exploit a large range of host species and are second only to mosquitoes in their importance as vectors of human and livestock disease agents (Parola and Raoult, 2001). Ticks of the *Ixodes ricinus* complex are of notable significance; they are present across the Northern temperate zone and transmit a great variety of pathogens of medical and veterinary importance (e.g., Cotté et al., 2010), including the bacteria responsible for Lyme borreliosis (LB), some genospecies of *Borrelia burgdorferi* sensu lato species complex (Gern and Humair, 2002). The population dynamics, ecology, and basic biology of *I. ricinus* species complex, along with its interaction with *B. burgdorferi* s.l. are well documented, particularly for the European species *I. ricinus* (e.g., Gern and Humair, 2002; Kurtenbach et al., 2006 and references therein). Many studies have described the catholic feeding habits of *I. ricinus*; it can be observed on a wide array of vertebrate hosts including small, medium and large mammals, birds and lizards (Hoogstraal and Aeschlimann, 1982). For this reason, *I. ricinus* is traditionally considered as an extreme generalist vector. Nonetheless, we still have only limited knowledge about how these ticks interact locally with their different potential host species and the consequences of these interactions for disease transmission. One way to address such questions is by examining population genetic structure in association with local host exploitation (McCoy et al., 1999).

As in many other tick systems, genetic analyses of *I. ricinus* have focused on large scale patterns using questing ticks collected from the vegetation (e.g., Estrada-Peña et al., 1996; Delaye et al., 1997; De Meeûs et al., 2002, 2004a,b; Paulauskas et al., 2006; Kempf et al., 2010; Nourredine et al., 2011). Several of these studies have revealed unexpectedly high levels of heterozygote deficiencies within populations, deviations present even after correcting for potential technical biases (De Meeûs et al., 2002, 2004a). One cause for differences between expected and observed heterozygosities can be the erroneous sampling of individuals from different isolated sub-groups (i.e., a Wahlund effect). Indeed, recent work has demonstrated the existence of cryptic sub-groups within *I. ricinus* populations (Kempf et al., 2010), along with patterns of assortative

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pairing within certain populations (Kempf et al., 2009b). One explanation for these results is the existence of local host-specialized groups, a phenomenon that may be more common in ticks than previously thought (McCoy et al., 2001, 2005; Magalhães et al., 2007; De Meeûs et al., 2010). Evidence of host specialization in *I. ricinus* would shed new light on the basic biology of this species and on the epidemiology of the pathogens it transmits (Gooding, 1996; De Meeûs et al., 2007).

In the present paper, we test directly for host-associated specialization in this vector species by investigating the distribution of genetic variation at a series of microsatellite markers at the within-population level using *I. ricinus* ticks collected directly from multiple local host types (i.e., deer, wild boar, rodents, birds and lizards). In the analyses, we explicitly account for additional factors that may affect within-population genetic structure, including host individual and temporal variation (i.e., year, tick life stage). Finally, we discuss how our findings of host-associated structure may substantially alter our vision of Lyme disease epidemiology, particularly given the sensitivity of these bacteria to local host diversity (e.g., Ostfeld and Keesing, 2000).

2. Materials and methods

2.1. Sampling and study sites

We sampled a total of 598 ticks between 2002 and 2007 (Table 1) directly from their hosts in five sites in Western and Central Europe (Fig. 1). The host species included five main types: bird (11 species), lizard (1 species), rodent (4 species), roe deer and wild boar (see Supplementary Table S1 for species details). The sampled ticks included all life stages (larva, nymph, and adult) and were stored in 70–90% ethanol until DNA extraction.

The use of different host type categories was done for several reasons. First, for the bird and rodent groups, we did not expect strong selection at the species level given relative infestation intensities on these hosts and their phylogenetic and ecological similarities (e.g., Talleklint and Jaenson, 1997; Estrada-Peña et al., 2005). If each host species was considered independently, sample sizes would not have been sufficient for analyses. Moreover, whenever the sample sizes were sufficient for comparison, local among-species differentiation was either non-significant or negligible compared to within-species differentiation among distant sites (not shown). For large mammals, we did not group the different species (roe deer and wild boar), but rather considered them as independent host types. In this case, there is no clear consensus emerging from the literature suggesting a grouping for these hosts (i.e., patterns of relative infestation are poorly known) and these host types can support large tick populations that include all life stages (in contrast to bird and small mammal groups).

2.2. Genotyping

Conserved ticks were washed three times in distilled water to eliminate ethanol and were cut in half. One half was ground with a mixer mill 301 (Retsch GmbH, Haan, Germany) and DNA was extracted using a Dneasy Tissue Kit (Qiagen, Valencia, USA). Ticks were genotyped at 11 microsatellite loci, including IRN15, IRN37 (Røed et al., 2006), IR25, IR27, IR39, IR32 (Delaye et al., 1998) amplified following the PCR protocols proposed by the authors; marker IRN15 was found to be duplicated within the genome of *I. ricinus*, and was split into two distinct and non-overlapping markers, IRN15a and IRN15b. Four additional markers were specifically developed for this study: Iric04, Iric05, Iric08, Iric11 (GenBank accession numbers: JF724082–JF724085). For these four markers, we used a M13 protocol where each forward primer was 5′-tagged with the M13 sequence (5′-CACGACGTTGTAAAACGAC-3′) and a 5'-dye labeled M13 added to the reaction mix. PCR amplifications were performed in a 10 μL mixture containing 20–50 ng of genomic DNA, 25 μM of each dNTP, 0.15 μM of each primer, 0.15 μM of labeled M13, 1 μL of 10× PCR buffer and 0.25 U of Taq DNA polymerase (Roche Diagnostics GmbH, Mannheim, Germany). Amplifications were performed using a "touch down" PCR procedure consisting of an initial 2 min denaturation step at 94 °C, followed by 16 cycles with 45 s at 94 °C, 45 s at 60 °C with

Table 1
Sampling sites, host types, years, tick life stage, number of ticks (Ticks), and number of hosts (Hosts) in our sample. For each subsample category, we report the values of Nei's (1987) unbiased index of genetic diversity H_s and Weir and Cockerham's (1984) estimator of F_{IS}.

Site	Host type	Year	Stage	Ticks	Hosts	H_s	F_{IS}
Lier (Belgium)	Rodent	2006	Larva	35	21	0.782	0.637
Chizé (France)	Bird	2007	Larva	18	5	0.792	0.480
			Nymph	12	3	0.724	0.519
	Roe deer	2007	Nymph	12	5	0.824	0.589
			Adult	44	25	0.794	0.473
	Wild boar	2007	Adult	28	13	0.765	0.533
Gardouch (France)	Bird	2007	Larva	69	25	0.841	0.596
			Nymph	15	9	0.750	0.597
	Roe deer	2007	Nymph	13	10	0.667	0.44
			Adult	22	12	0.778	0.508
Brzotín/Plešivec (Slovakia)	Bird	2002	Larva	12	6	0.772	0.488
			Nymph	21	7	0.664	0.433
	Bird	2003	Larva	33	3	0.731	0.466
			Nymph	23	2	0.828	0.491
	Lizard	2004	Larva	15	3	0.737	0.324
			Nymph	25	3	0.770	0.414
Drienovec (Slovakia)	Bird	2002	Larva	20	5	0.879	0.482
			Nymph	7	3	0.911	0.339
	Bird	2006	Larva	31	11	0.768	0.588
			Nymph	23	8	0.760	0.634
	Bird	2007	Larva	24	3	0.780	0.488
			Nymph	11	3	0.843	0.581
	Lizard	2007	Larva	26	3	0.810	0.491
			Nymph	24	3	0.830	0.388
	Rodent	2006	Larva	35	10	0.826	0.507