MOLECULAR ECOLOGY

Molecular Ecology (2009) **18**, 4450–4462 doi: 10.1111/j.1365-294X.2009.04356.x

Recent evolution of host-associated divergence in the seabird tick *Ixodes uriae*

FLORENT KEMPF,* THIERRY BOULINIER,† THIERRY DE MEEÛS,‡ CÉLINE ARNATHAU* and KAREN D. MCCOY*

**Génétique et Evolution des Maladies Infectieuses), UMR CNRS-IRD 2724, IRD, 911 avenue Agropolis, BP 64501, 34394 Montpellier Cedex 5, France, †Centre d'Ecologie Fonctionnelle et Evolutive, CNRS UMR 5175, 1919 Route de Mende 34293 Montpellier, France, ‡Laboratoire de recherches et de coordination sur les Trypanosomoses, UMR177 IRD/CIRAD, TA A-17/G, Campus International de Baillarguet, 34398 Montpellier Cedex 5, France*

Abstract

Ecological interactions are an important source of rapid evolutionary change and thus may generate a significant portion of novel biodiversity. Such changes may be particularly prevalent in parasites, where hosts can induce strong selection for adaptation. To understand the relative frequency at which host-associated divergences occur, it is essential to examine the evolutionary history of the divergence process, particularly when it is occurring over large geographical scales where both geographical and host-associated isolation may playa part. In this study, we use population genetics and phylogeography to study the evolutionary history of host-associated divergence in the seabird tick *Ixodes uriae* (Acari, Ixodidae). We compare results from microsatellite markers that reflect more ecological timescales with a conserved mitochondrial gene (COIII) that reflects more ancient divergence events. Population structure based on microsatellites showed clear evidence of host-associated divergence in all colonies examined. However, isolated populations of the same host type did not always group together in overall analyses and the genetic differentiation among sympatric host races was highly variable. In contrast, little host or geographical structure was found for the mitochondrial gene fragment. These results suggest that host race formation in *I. uriae* is a recent phenomenon, that it may have occurred several times and that local interactions are at different points in the divergence process. Rapid divergence in *I. uriae* implies a strong interaction with its local host species, an interaction that will alter the ecological dynamics of the system and modify the epidemiological landscape of circulating micropathogens.

Keywords: co-evolution, colonial seabirds, cytochrome oxidase III, host–parasite interactions, microsatellites

Received 7 April 2009; revision received 10 July 2009; accepted 14 July 2009

Introduction

Historical debates on the evolution of genetic divergence have particularly addressed the role played by the geographical arrangement of populations (i.e. allopatry, sympatry, peripatry and parapatry). Efforts have been made to draw a general picture of the interplaying processes (Kirkpatrick & Ravigné 2002), and particularly to assess the possibility of divergence in the case of ongoing ecological interactions (Via 2001). In addition to its spatial context, a growing amount of work now focuses on its pace and frequency (Thompson 1998; Yoshida *et al.* 2003; Schwarz *et al.* 2005; Carroll *et al.* 2007). Thus, some authors make the distinction between 'ecological' (i.e. rapid) and 'evolutionary' timescales of divergence (Gingerich 2001). However, beyond a simple question of scale, this distinction highlights the importance of the underlying mechanisms and their long-term consequences. For

Correspondence: Florent Kempf, Fax: (33) 4 67 41 62 99; E-mail: florent.kempf@mpl.ird.fr

example, ecological interactions are probably an important cause of rapid evolutionary changes (Orr & Smith 1998), but these changes can in turn influence the dynamics of ecological interactions (Fussmann *et al.* 2007).

Parasites are ideal model organisms to address the mechanisms and consequences of population divergence in the case of ecological interactions (e.g. Criscione *et al.* 2005). Indeed, the host is the main component of the parasite's environment, including both its food resource and its breeding site, and this habitat is constantly changing over time. This means that parasites may be particularly prone to ecological divergence (De Meeûs *et al.* 1998; Gandon & Van Zandt 1998; Whiteman *et al.* 2007). Well-known case studies on phytophagous insects have revealed the importance of host-associated divergence for the evolution of biodiversity (Drès & Mallet 2002; Feder *et al.* 2005; Stireman *et al.* 2006), but ecological speciation is predicted to be a general tendency among parasitic species (De Meeûs *et al.* 1998) and will depend on their life history characteristics (McCoy 2003; Barrett *et al.* 2008).

Facing entangled recent and ancient events acting at various spatial scales, the choice of suitable markers and methods of data analysis are of primary interest to understand the divergence process. Microsatellite markers are inherited biparentally and have relatively high mutation rates (Jarne & Lagoda 1996). They are thus expected to reflect contemporary processes; size homoplasy is expected to suppress the signature of ancient events (e.g. Howes *et al.* 2006). In contrast, detection of recent events using more conserved genetic markers can be limited by incomplete lineage sorting (i.e. lineages may not yet reflect current population divergence), but can be used to investigate historical processes (Avise 1998; Ballard & Whitlock 2004). In this study, we use population genetics and phylogeography to investigate the evolutionary history of host-associated divergence in the seabird tick *Ixodes uriae* (Acari, Ixodidae).

Ixodes uriae is a common haematophagous ectoparasite that exploits colonial seabirds in the polar regions (Northern and Southern hemispheres). Although it was initially characterized as a seabird generalist because of its extensive host range (Guiguen 1988), microsatellite-based studies have shown evidence of host-associated divergence in this tick for several host species under sympatric conditions (McCoy *et al.* 2001, 2005a). These observations call into question the habitat-centred hypothesis of tick evolution, whereby the abiotic environment, and not the host, plays a primary role in tick adaptation (Klompen *et al.* 1996), and suggest that the ecological interaction with the host could be of key importance in such species (Magalhaes *et al.* 2007). However, to understand the relative ease at which such divergences occur, it is essential to understand the evolutionary history of the divergence process, particularly when it is occurring over large spatial scales where both geographical and host-associated isolation may play a role. Understanding the nature of host-associated adaptation is particularly important for organisms like ticks, as they are vectors for numerous microparasites (Parola & Raoult 2001), including the bacteria *Borrelia burgdorferi sensu lato* causing human Lyme disease (Gern & Humair 2002). Rapid divergence in ticks can greatly modify the epidemiological landscape of such important micropathogens (McCoy 2008).

Previous studies examined host-associated divergence of *I. uriae* populations in seven colonies where different seabird species were breeding sympatrically (McCoy *et al.* 2001, 2005a). These results suggested that the divergence of local tick populations was spatially dynamic in nature. However, current gene flow between formerly isolated colonies and homoplasy may alter the signature of past events. In this study, we first examined host-associated population structure at eight microsatellite markers in four new colonies where ticks were collected from two to four sympatric seabird species, and we compared this structure with that of two previously analysed colonies. We then studied how the phylogeographical relationships among ticks from these colonies are related to their host associations by using a mitochondrial marker (Cytochrome oxidase III). In particular, our aim was to determine if host-associated tick races result from a few historical specialization events or whether specialization may have occurred repeatedly and independently at a local scale.

Materials and methods

Sampling design

In 1998, 2001 and 2003, we collected ticks directly from their hosts in six different seabird colonies in the North Atlantic (Fig. 1). Ticks were collected from four host species, the black-legged kittiwake *Rissa tridactyla*, the Atlantic puffin *Fratercula arctica*, the common guillemot (also known as common murre) *Uria aalge* and the razorbill *Alca torda*. These host species were chosen for several reasons. All four species are widespread, highly abundant and frequently infested by *Ixodes uriae*. Therefore, they represent stable and reliable hosts for ticks. Although these seabirds share many general life history features (long lived, pelagic and exhibit strong natal and breeding philopatry), they may still represent very different environments for their parasites. For example, these different species tend to use different substrates within the breeding colonies; kittiwakes build

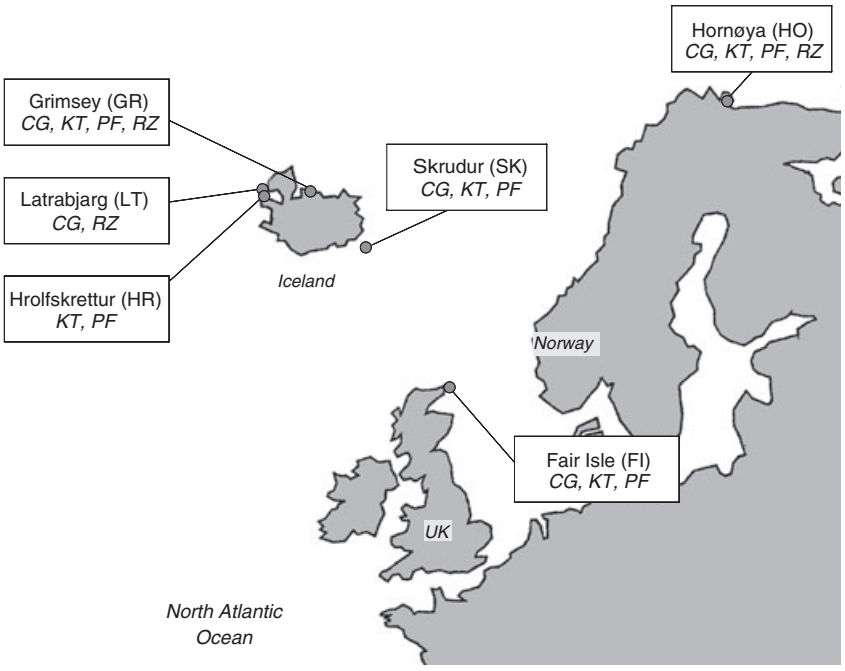

Fig. 1 Sampling sites of the ticks used for the mitochondrial DNA and microsatellite analyses. See Table 1 for exact locations and host sampled.

individual nests on vertical sea cliffs, puffins dig burrows on grassy slopes or use rock cavities in boulder piles, common guillemots breed on cliff ledges in very dense numbers with no true nest and razorbills tend to be interspersed among these species in relatively open, pre-existing cavities. Breeding phenology can also vary among these species depending on the locality, such that optimum periods for exploitation by ticks may differ (McCoy *et al.* 2001). Finally, prospecting habits of immature individuals and failed breeders differ among these seabird species, and probably translate into different dispersal opportunities for their ticks (McCoy *et al.* 2003). All of these features may favour the host-related divergence observed in previous studies for three of these four species (McCoy *et al.* 2001, 2005a).

We collected *I. uriae* directly from these four seabird host species in six different seabird colonies in the North Atlantic (Fig. 1). Ticks were collected from as many individual birds as possible to maximize the representativeness of the sample. In most colonies, each tick came from a different bird. In colonies where this was not possible, no more than three ticks per bird were included in the analyses. Sampled ticks were stored in 70–90% ethanol after collection.

DNA extraction

Adult female and nymphal ticks were used for genetic analyses (see McCoy *et al.* 2001, for more details). Preserved ticks were washed three times in distilled water to remove any traces of ethanol and were cut in half. One half was kept for future analyses, and the other was placed in a 1.5-mL tube with a steel bead, frozen using liquid nitrogen and ground with a mixer mill 301 (Retsch, Germany). DNA extractions of the ground product were then performed using the procedure outlined in the Dneasy Tissue Kit (QIAGEN).

Microsatellite markers: amplification, genotyping and data analysis

We analysed the distribution of genetic variation at eight microsatellite markers (McCoy & Tirard 2000) from ticks collected in 17 populations (Table 1). For the Icelandic ticks, PCR amplifications were performed as detailed by McCoy & Tirard (2000) and were visualized using an automated sequencer (ABI Prism 310 Genetic Analyser; Applied Biosystem). These new genotypic data were combined with the previously published data from tick populations collected on Fair Isle, UK, and Hornøya, Norway (McCoy *et al.* 2005a).

Independence of the different microsatellite markers was tested using the software Genepop v. 3.4 (Raymond & Rousset 1995). Significance levels were corrected using a sequential Bonferroni correction for multiple tests (Rice 1989). Genetic variability was assessed using Nei's unbiased estimator of heterozygosity H_s (Nei 1987) and differences in these observed values among populations of each host type were tested using a permutation test (10 000 permutations; FSTAT v. 2.9.3.2; Goudet 2002). All populations were tested for departure from Hardy–Weinberg proportions in genotypic frequencies

Table 1 Sample details and genetic variation of the studied *Ixodes uriae* populations

Colony	Code	Latitude/longitude	Host	No. ticks (COIII)	π (COIII)	No. ticks (microsat.)	Gene diversity
Grimsey, Iceland (2003)	GR	66°33′N/18°00′W	CG	22	0.00627	30	0.7331 (0.0410)
			KT	20	0.00539	30	0.7310 (0.0421)
			PF	19	0.00306	38	0.5479 (0.1282)
			RZ	16	0.0054	15	0.6335 (0.0712)
Hornøya, Norway (1998)	HN	70°22′N/31°10′E	CG	18	0.00566	24	0.6082 (0.0730)
			KT	14	0.00463	29	0.5873 (0.0906)
			PF	20	0.00494	31	0.5895 (0.1155)
			RZ	2	0.00648	—	—
Hrolfskrettur, Iceland (2003)	HR	65°29′N/24°32′W	KT	19	0.00361	20	0.5922 (0.1015)
			PF	23	0.00406	30	0.5406 (0.1343)
Fair Isle, UK (2001)	FI	53°33′N/1°36′W	CG	19	0.00531	27	0.5173 (0.1195)
			KT	19	0.00421	42	0.5428 (0.0974)
			PF	18	0.00383	38	0.5213 (0.1360)
Latrabjarg, Iceland (2003)	LT	65°28′N/24°28′W	CG	16	0.00596	11	0.5417 (0.1021)
			RZ	12	0.00452	14	0.5947 (0.1084)
Skrudur, Iceland (2003)	SD	64°54′N/13°37′W	CG	—	—	29	0.6435 (0.0796)
			KT	—	—	27	0.6884 (0.0623)
			PF	—	—	30	0.5236 (0.1254)

Host species are coded as: CG – common guillemot (*Uria aalge*), KT – black-legged kittiwake (*Rissa tridactyla*), PF – Atlantic Puffin (*Fratercula arctica*), RZ – Razorbill (*Alca torda*). Nucleotide diversity (π) and gene diversity (±standard errors) are based on Nei's (1987) estimators.

(Genepop v. 3.4; Raymond & Rousset 1995). The overall test compared Weir & Cockerham's (1984) unbiased estimator of Wright's F_{IS} (Wright 1965) with its chance distribution resulting from the randomization of alleles among individuals within samples (15 000 permutations). These computations were performed using FSTAT v. 2.9.3.2 (Goudet 2002).

Next, we assessed whether ticks were locally structured among host species within each colony. Pairwise differentiation among local host-related groups was calculated using Wright's F_{ST} (Wright 1965) by following Weir & Cockerham's (1984) approach. The significance of the estimator was assessed using the randomization *G*-based test of Goudet *et al.* (1996; 15 000 permutations of individuals among populations). For these computations, we used the software FSTAT version 2.9.3.2. The same approach was employed to examine among-site structure within a given tick host race.

The between-group analysis method implemented in the ade4TkGUI package (Thioulouse & Dray 2007) was used to examine the role of host and geography in the distribution of allelic frequencies. The method includes an initial principal component analysis, followed by a second analysis where population is included as a qualitative explanatory variable. The linear combinations of the variables that maximize the interpopulational variance are then used to plot individuals and the barycentre of each population. Next, a permutation test is performed to test departure from a random distribution of individuals among populations (between-class ratio test, 10 000 permutations, Chessel *et al.* 2004; Dolédec & Chessel 1987). These computations were performed using R software (R-Development Core Team, 2006).

To assess the robustness of groups, we carried out two different analyses. First, we determined the likelihood of correctly assigning the ticks to their original host-related group given their multilocus genotype, without explicit consideration of the geographical location. We used the assignment criterion proposed by Cornuet *et al.* (1999) and the associated test implemented in the software GENECLASS2 (Piry *et al.* 2004). Assignment tests were first run with only microsatellite data and then were repeated including the COIII haplotype (see below). Next, we employed the software STRUCTURE 2.3.1 (Pritchard *et al.* 2000), to estimate the number of groups and their composition within each multi-host colony. All runs were performed on the basis of the admixture model with correlated allelic frequencies, which may best correspond to the hypothesis of a recent evolution of host-related groups. Runs included 100 000 iterations after an initial burn-in of 100 000 iterations. This was sufficient to observe the convergence of the summary statistics describing the partition of the data (e.g. the likelihood of *K* clusters, the differentiation among the clusters, etc.). For each colony, *K* was set from 1 to 10 and six independent runs were performed.

mtDNA: amplification, sequencing and data analyses

Ticks from HR, LT, GR, FI and HN (Fig. 1) were selected for mtDNA sequencing (Table 1). We amplified a fragment of the mitochondrial gene COIII (cytochrome oxidase, subunit III) using primers IuCO3F (5′-CGT GAA GCC TCT TTT CAA GG-3′) and IuCO3R (5′-TCA TGC TGC AGC TTC AAA TC 3′) designed using the complete mitochondrial genome sequence of *I. uriae* (Shao *et al.* 2005; NC 006078). The 50-μL PCR reaction mix contained 10× buffer (Tris-HCl, pH 9.0, KCl, Triton_X-100), 100 nmol $MgCl_2$, 10 nmol dNTP, 10 pmol of each primer, 1 U Taq polymerase (Promega), 20 ng DNA and distilled water. The PCR amplification procedure began with an initial denaturation step (95 °C, 5 min), followed by 30 cycles of denaturation (94 °C, 40 s), annealing (48 °C, 30 s) and extension (72 °C, 90 s), and ended with a final extension step (72 °C, 5 min). The PCR products were visualized on 2% agarose–ethidium bromide gels; positive amplifications were sent for direct sequencing (Cogenics, Meylan, France). The chromatographs of all sequences were manually verified and sequence alignment was performed using the software Clustal_X (Thompson *et al.* 1997).

Phylogeographical interpretations can be deeply influenced by non-neutral evolution at the locus of interest (Ballard & Whitlock 2004). We therefore assessed the neutrality of the COIII fragment using the tests of Tajima (1989) and McDonald & Kreitman (1991). To calculate the latter, we used the COIII sequence of *Ixodes pacificus* as an outgroup (Kain *et al.* 1999; AF082986). Both tests were performed using DnaSP v.4.50.3 (Rozas *et al.* 2003).

For each population, we computed the haplotype and nucleotide diversities (Nei 1987) using DnaSP (Rozas *et al.* 2003). We tested for differences in these values among host types using Kruskal–Wallis tests (Table 1).

To better understand the evolutionary history of *I. uriae* populations, we then performed a nested clade analysis (Templeton 1998). Gene genealogies were inferred using the software TCS v. 1.21 (Clement *et al.* 2000). Ambiguities in the resulting cladograms (i.e. loops) were resolved by applying the criteria described by Pfenninger & Posada (2002). Nesting categories were identified by following the rules proposed by Templeton (1998). The distribution of haplotypes was analysed by considering either the host or the geographical location as a categorical variable. We tested for an association between geography, host use, haplotypes/clade within each nesting clade, using a permutational contingency test implemented in GeoDis 2.5 (Hudson *et al.* 1992; Posada *et al.* 2006). As the validity of inferences made using Templeton's inference key is strongly criticized (Petit *et al.* 1998; Petit & Grivet 2002; Knowles 2008), we limited our analysis to the contingency test.

Comparison between mtDNA and microsatellite data

Finally, we compared population differentiation computed using microsatellite and mitochondrial data. First, we used Weir & Cockerham's (1984) analysis of variance framework to compute the pairwise differentiation among all pairs of populations characterized using the two marker types (Table 1). This was carried out using FSTAT 2.9.3.2. for microsatellite data (see above) and Arlequin 3.01 (Excoffier *et al.* 2005) for mitochondrial data. We then tested for a correlation in the differentiation measured by two marker types using the 'Mantelize it' procedure of the program FSTAT v. 2.9.3.2 (15 000 permutations). Second, we examined whether pairwise patterns of differentiation for each marker type was better explained by the effects of host or geographical distance by using a generalized linear model (S-PLUS 2000 Professional release 2; MathSoft, Inc., Seattle, WA, USA). The model included the direct geographical distance between populations, the host used (coded 0 if the populations were sampled on the same host and 1 if not) and the interaction between these two variables. Third, we performed a similar analysis by recoding geographical distance in a qualitative fashion, where each pairwise distance was coded as a categorical variable (e.g. 1914 km was recoded as 'A', 1248 km as 'B', etc.); the corresponding model included the categorical geographical distance and the host used.

Results

Microsatellite markers

All possible pairs of loci were in linkage equilibrium. Gene diversity varied among populations from 0.52 to 0.73 (Table 1), but did not differ significantly among host groups ($P = 0.1877$). The overall F_{IS} across populations was statistically significant ($F_{IS} = 0.052$, $P < 0.0001$). However, only one population showed a significant overall deviation from Hardy–Weinberg proportions (CG ticks in FI; $\chi^2 = 35.0$, d.f. = 14, $P = 0.0015$), a pattern which was previously associated with local substructuring (see McCoy *et al.* 2005a). Other deviations were associated with certain locus–population combinations concerning primarily three loci (T47, T38 and T39); tests of population structure were run with and without these loci and the overall structure was not significantly altered (results not shown).

At the within-colony level, there was evidence for significant differentiation among ticks sampled from

different sympatric host species, except for ticks from razorbills, which tended to be similar to the other local populations (Table 2). Local divergence was always strongest between ticks from guillemots and puffins, and was variable between the kittiwake race and the other local races. Patterns of by-host divergence were confirmed in the between-group analysis (Fig. 2); tick populations were grouped principally by host species, except for razorbill ticks which clustered with other host groups. Interestingly, ticks sampled from guillemots formed two isolated groups based on this analysis, one that included the western Icelandic population (LT) and the more southerly Scottish population of Fair Isle (FI), and another that included the northern and eastern Icelandic populations (GR, SD) and the colony in northern Norway (HN). This distribution was strongly supported by the between-class inertia ratio test (P-value = 0.00001). The host dependency of tick population structure was also reflected in patterns of among-site differentiation within each host race (PF: F_{ST} = 0.009, P = 0.0856; KT: F_{ST} = 0.030, P = 0.00007; CG: F_{ST} = 0.093, P = 0.00007; RZ: F_{ST} = 0.111, P = 0.00007). Finally, assignment analyses of individual ticks echoed these results. Razorbill ticks had a low probability of being correctly assigned to their host group. Kittiwake ticks showed an intermediate probability of correct assignment, whereas both puffin and guillemot tick assignment probabilities were high (Table 3). The addition of haplotype data to this analysis greatly improved assignment results and suggested that host-associated

Table 2 Pairwise differentiation between host-associated tick groups, based on microsatellite data, as estimated by Weir & Cockerham's (1984) estimator of F_{ST}

Geographical location	Host races	F_{ST}	P-values
Grimsey (GR)	CG–KT	**0.037**	0.00007
	CG–PF	**0.164**	0.00007
	CG–RZ	**0.066**	0.00233
	KT–PF	**0.102**	0.00007
	KT–RZ	0.021	0.03600
	PF–RZ	**0.100**	0.00040
Hornøya (HN)	CG–KT	**0.163**	0.00007
	CG–PF	**0.224**	0.00007
	KT–PF	**0.060**	0.00007
Hrolfskrettur (HR)	KT–PF	**0.030**	0.00007
Fair Isle (FI)	CG–KT	**0.059**	0.00173
	CG–PF	**0.163**	0.00007
	KT–PF	**0.073**	0.00007
Latrabjarg (LT)	CG–RZ	−0.0002	0.72216
Skrudur (SD)	CG–KT	**0.130**	0.00007
	CG–PF	**0.293**	0.00007
	KT–PF	**0.080**	0.00007

Significant F_{ST} (after Bonferroni correction) appears in bold. Host abbreviations as outlined in Table 1.

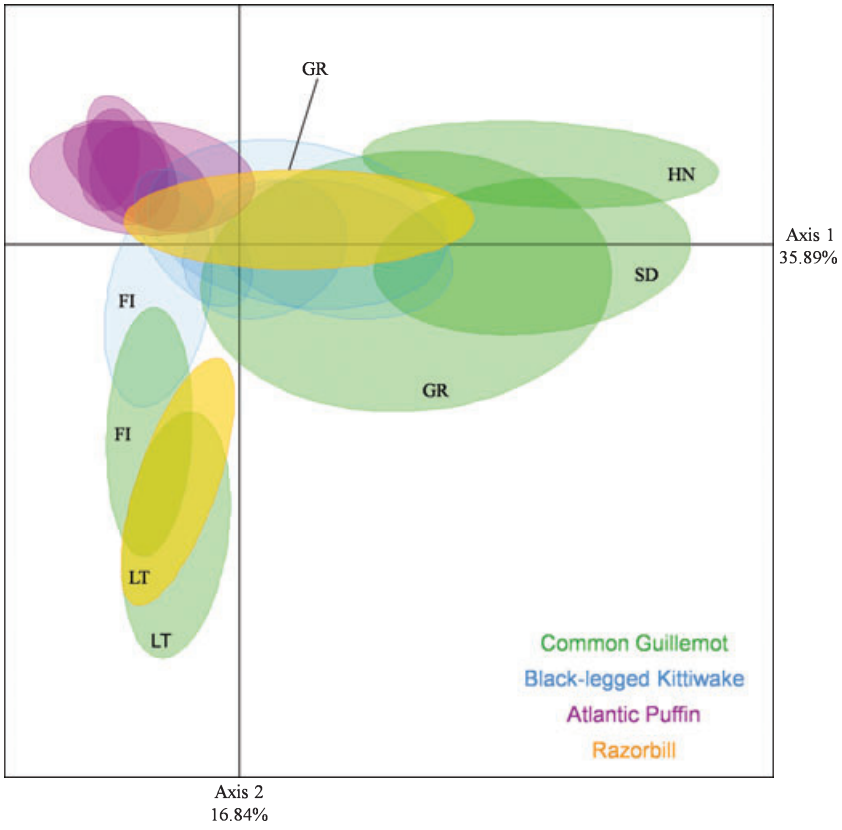

Fig. 2 Between-group analysis of *Ixodes uriae* populations revealed two significant axes that explained 35.89% (Axis 1) and 16.84% (Axis 2) of the total inertia of the microsatellite data. Different colours refer to the host species of origin. For ease of interpretation, we have only labelled certain colonies (GR = Grimsey; HR = Hrolfskrettur; LT = Latrabjarg; SD = Skrudur; FI = Fair Isle; HN = Hornøya). Ellipses represent the distribution of 67% individuals around the population barycentre.

Table 3 Average percentage of tick assignments (±standard error) to host race using only microsatellite markers and both microsatellite and the COIII gene fragment

Genetic markers	Tick race	Assigned to			
		CG	KT	PF	RZ
μsats only	CG	**77.7 (12.2)**	4.0 (1.9)	2.2 (0.9)	16.1 (10.8)
	KT	37.5 (9.1)	**35.9 (5.5)**	11.3 (3.9)	15.3 (2.8)
	PF	4.3 (1.2)	4.3 (1.9)	**86.1 (1.5)**	5.3 (0.9)
	RZ	59.1 (12.4)	6.7 (6.7)	13.4 (13.4)	**21.0 (7.7)**
μsats + COIII	CG	**96.2 (1.9)**	2.2 (2.7)	3.4 (1.7)	0
	KT	19.4 (7.6)	**72.2 (5.7)**	7.9 (3.2)	2.9 (2.2)
	PF	4.2 (2.8)	0	**93.5 (1.9)**	1.8 (1.3)
	RZ	25.5 (7.2)	11.5 (8.2)	0	**63.0 (1.0)**

Sample sizes were reduced in μsats + COIII so as to include only ticks that were typed at both microsatellite and mitochondrial markers. Host species abbreviations as outlined in Table 1. Assignments to the sampled host species are in bold.

structure may be starting to appear at this marker (see below). Clustering analyses corresponded well with assignment tests and identified the major host-associated groups in most colonies. As results were consistent among the six runs per colony, we only present the highest likelihood run in this study. In two colonies (HN and SD), three groups were identified and principally contained ticks from the respective host races. The analysis of the site GR gave two equally likely partitions, including either two or four clusters. However, the different groups also tended to contain only Puffin and Guillemot ticks, whereas Kittiwake and Razorbills ticks clustered with these two host races. In FI, only two groups were found, one that contained both puffin and kittiwake ticks and another that contained both guillemot and kittiwake ticks. Only a single group could be distinguished for colonies HR and LT.

mtDNA: sequence diversity and haplotype distribution

We observed 39 variable sites in the 463-bp-length fragment of the mitochondrial cytochrome oxidase III subunit. A total of 49 haplotypes were found among the 257 ticks sequenced (GenBank numbers: EU849503–EU849551). The hypothesis of neutral evolution could not be rejected for the COIII gene fragment according to a McDonald–Kreitman *G*-test with Yates's correction ($P = 0.60$). Tajima's test revealed an excess of rare haplotypes ($D = -2.26$, $P < 0.01$), which can reflect recent population history (e.g. population bottleneck followed by a radiation event; Tajima 1989). The degree of genetic variation did not differ strongly among the different host-associated groups (Table 1). There was no significant difference among host groups in the number of haplotypes (Kruskal–Wallis test: $\chi^2 = 5.7652$, d.f. = 3, $P = 0.1236$). However, we did observe a significant difference in nucleotide diversity (Kruskal–Wallis test: $\chi^2 = 8.2667$, d.f. = 3, $P = 0.0408$); guillemot ticks showed the highest diversity and puffin ticks the lowest.

The cladogram of all haplotypes (Figs 3 and 4) was supported by a maximum parsimony probability greater than 95% (see Templeton *et al.* 1992 for details). All cladogram ambiguities were resolved according to Pfenninger & Posada's (2002) criteria (disregarded relationships are represented as dotted lines in Figs 3 and 4). First, the connections between several haplotype pairs (H33–H1, H36–H38, H31–H49 and H3–H21) had little support from the different criteria used (i.e. geographical, topological and frequency criteria). The relationship between H2 and H11 was more strongly supported than the alternative between H10 and H11 (i.e. topological and frequency criteria favoured the H2–H11 link, whereas the geographical criterion favoured the H10–H11 link). The most abundant haplotypes were H1 and H2 (representing respectively 37.7% and 22.9% of all individuals). Most of the other haplotypes radiated from H1 and H2 via a single step connection or by haplotypes with an intermediate abundance.

In general, the different haplotypes were homogenously distributed among the host-associated tick groups (Fig. 3) and a marginal nonsignificant association between host of origin and haplotype clade was found (*P*-value = 0.0764; Table 4). Although only 11 of the 49 haplotypes were shared among groups, these haplotypes represented the majority of the collected ticks (215 of the 257 sampled). Among all one-step and two-step nested clades, four were composed of ticks from a single host race (1–2, 1–4, 1–8 and 1–11). We tested for an association between host type and haplotypes/clades in the 12 other clades; a significant χ^2 test was observed in only three of them (Table 4). These significant tests reflect that most haplotypes in the clade, other than the interior haplotype, are associated with a single host race (Fig. 3).

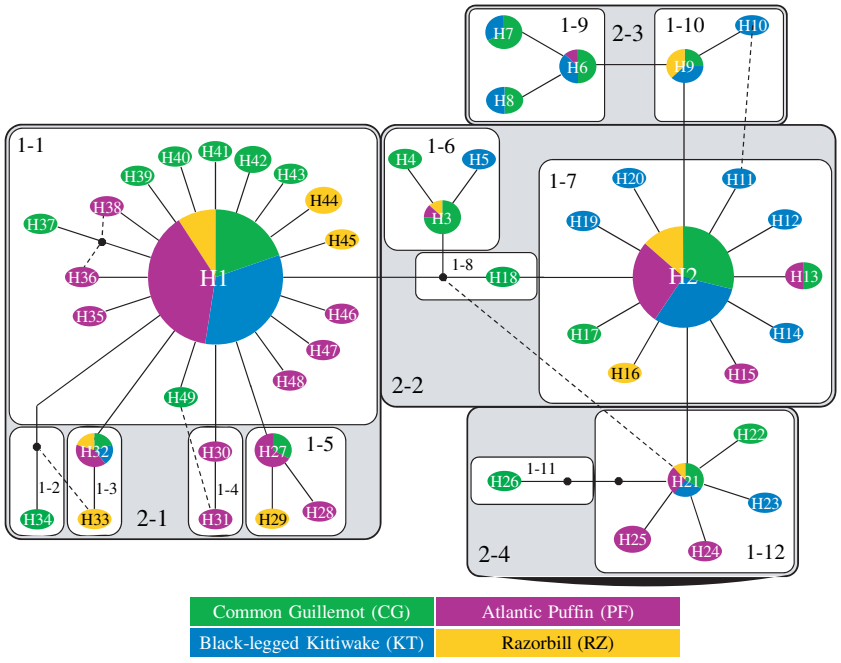

Fig. 3 Nested cladogram of COIII haplotypes of *Ixodes uriae* for examining host-associated structure. Circle size is representative of overall haplotype frequency. The proportion of ticks sampled from each host species is represented with different colours. Dotted lines correspond to the genetic relationships disregarded according to Pfenninger and Posada's criteria (Pfenninger & Posada 2002).

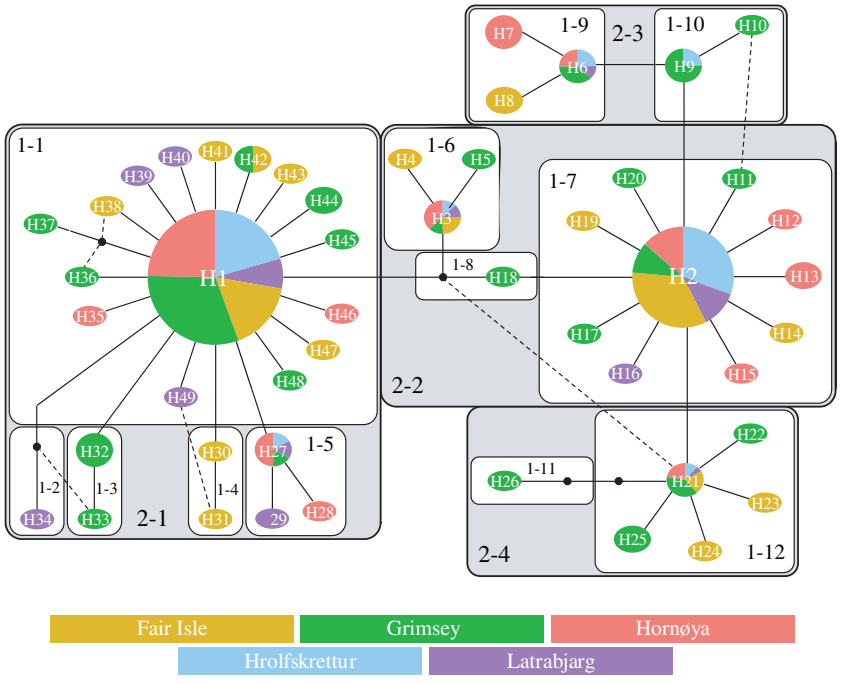

Fig. 4 Nested cladogram of COIII haplotypes of *Ixodes uriae* for examining geographical structure. Circle size is representative of overall haplotype frequency. The proportion of ticks sampled in the five different geographical locations is represented with different colours. Dotted lines correspond to the genetic relationships disregarded according to Pfenninger and Posada's criteria (Pfenninger & Posada 2002).

The cladogram showed stronger support for an association between the genetic variation of *Ixodes uriae* and its geographical location (Table 4). Four of the 11 χ^2 tests for a geographical association and overall test across the entire cladogram were significant (Fig. 4, Table 4). However, the interpretation of this structure is not obvious from the cladogram.

Comparison between mtDNA and microsatellite data

Differentiation estimated using microsatellite and mitochondrial data was not significantly correlated ($r = 0.098$, $P = 0.355$). The GLM suggested that neither host nor geographical distance (or their interaction) could significantly explain pairwise differentiation

Table 4 Chi-square tests for association between haplotypes and geographical location/host of origin within each clade

	Geographical location		Host used	
Clades	χ^2-statistic	*P*-value	χ^2-statistic	*P*-value
1–1	65.95	0.254	62.86	**0.019**
1–3			2.40	1.000
1–5	6.67	0.462	8.53	0.247
1–6	6.87	0.671	10.36	0.534
1–7	52.29	**0.009**	24.08	0.773
1–9	18.85	**0.008**	0.84	1.000
1–10	0.32	1.000	1.41	1.000
1–12	10.18	0.804	46.91	**<0.001**
2–1	37.46	**0.001**	12.78	0.376
2–2	7.74	0.481	11.64	**0.045**
2–3	9.18	**0.028**	6.37	0.099
2–4	1.23	1.000	2.09	1.000
Cladogram	21.70	**0.037**	15.43	0.076

Clades and the distribution of host types/geographical locations are found in Figs 3 and 4. Significant *P*-values appear in bold.

among populations for the mitochondrial gene (all $P < 0.75$). However, there was a significant effect of host (same or different) on pairwise genetic differentiation for the microsatellite loci ($F_{distance} = 0.457$, $P = 0.500$; $F_{host} = 5.818$, $P = 0.0179$, $F_{interaction} = 0.0171$, $P = 0.896$). The effect of host group was similar when nonsignificant variables were removed from the model in a stepwise fashion (final model: $F = 5.208$, $P = 0.0249$), supporting the primary influence of host species on tick population structure.

In contrast, when geographical distance was considered as a qualitative variable, it explained a significant part of mitochondrial pairwise differentiation ($F_{distance\ categories} = 6.681$, $P < 0.001$), and was the only remaining effect of the final model after stepwise removal of nonsignificant factors. In this analysis, pairwise differentiation at microsatellite markers depended on both the host used and the geographical distance category ($F_{distance\ categories} = 8.397$, $P < 0.001$; $F_{host} = 31.265$, $P < 0.001$). These results suggest that geographical structure exists among colonies, but cannot be explained by distance alone. Other factors such as colony dynamics probably explain tick dispersal among sites (see the following).

Discussion

In this study, we examined population and phylogeographical structure of a common seabird ectoparasite, the tick *Ixodes uriae*, to better understand the history of host-associated divergence in this system. For this, we compared two types of genetic markers, microsatellite markers that reflect more ecological timescales and a conserved mitochondrial gene that should reflect more ancient divergence events. As previously inferred from other population genetic studies (McCoy *et al.* 2001, 2005a), we found significant divergence among tick populations sampled from most sympatric seabird species, indicating that the evolution of host-associated races is a general pattern in this ectoparasite. However, similar patterns were not evident in the mitochondrial genome; analyses of the COIII gene fragment supported the hypothesis that host races have evolved relatively recently.

Population structure of Ixodes uriae

Except for ticks sampled from razorbills (see below), significant differentiation at nuclear markers was found between all tick populations sampled from the different local host species. Indeed, the exploited host was associated with a large part of the genetic variation in the tick populations as indicated by the relatively high probabilities to assign ticks to the host of origin, and the significant effect of host in explaining pairwise genetic differentiation between populations with the microsatellite markers. The fact that many seabird species breed in dense and temporally predictable patches probably favours the evolution of host specialization in this system.

Razorbill ticks did not show evidence of host-associated divergence in the two colonies examined in this study. This result may not be completely surprising given the reproductive behaviour of this species; compared with the other seabird species sampled, razorbills are relatively isolated breeders with nests spread throughout the colony and often intermixed among other, more densely, breeding birds. This behaviour may select against the evolution of host specificity for this particular host. Nevertheless, a more extensive sample of RZ colonies will be necessary to understand fully the relationship of their ticks with those of other local hosts, in particular KT and CG, and also for consideration of the potential role of non-neutral loci in host specialization. However, these results do support the general notion that constraints imposed by host life-history traits may be an essential component driving parasite population structure (McCoy *et al.* 2003; Barrett *et al.* 2008; Bruyndonckx *et al.* 2009) and the evolution of specialization (e.g. Tripet & Richner 1997).

Assignment probabilities were lower for kittiwake ticks than for the other two races and, particularly so when only microsatellite markers were employed (Table 3). Similarly, the degree of sympatric divergence was highest between ticks collected from guillemots and puffins, and lower and more variable when comparing kittiwake ticks with the those of other races

(Table 2). In the clustering analyses, kittiwake ticks grouped with either PF or CG ticks in three of the colonies considered (i.e. GR, FI and SD). These patterns are difficult to explain readily, as kittiwakes are phylogenetically distant from both puffins and guillemots and breed in dense numbers. Therefore, these patterns may be because of the relative age and/or origin of this race (i.e. kittiwake races have evolved recently within colonies from other local races). Alternatively, this pattern could also be a result of reduced specificity at the host–tick interface (i.e. ticks adapted to other species can still feed on kittiwakes). As the mitochondrial gene provided no signal at this level, other genetic markers or experimental tests will be required to distinguish between these hypotheses.

Within each tick host race, patterns of geographical population structure were variable (Fig. 2). For example, puffin ticks from different colonies were grouped tightly together in the between-group analysis and showed no significant differentiation among colonies (F_{ST} = 0.009, *P*-value = 0.085), suggesting substantial gene flow among these populations. Population structure was intermediate in kittiwake ticks. In guillemot ticks, two population groups could be identified; one that included tick populations from northern Norway (Hornøya) and from two Icelandic populations (Grimsey and Skrudur) and the other that grouped the western Icelandic population (Latrabjarg) with the UK population (Fair Isle). These groups were more closely related to other local races than to each other. Previous results showed that the population of guillemot ticks from Hornøya was genetically distant from other southern European populations (McCoy *et al.* 2005a). As *I. uriae* can only disperse with its host during the breeding season (McCoy *et al.* 2005b), the observation of two well-differentiated tick groups within Iceland suggests the presence of at least two groups of guillemots in this country that function independently despite geographical proximity; birds from the north and east of Iceland may interact more strongly with birds from the Barents Sea, whereas those from the west coast of Iceland may interact with populations to the south. Studies of population genetic structure in guillemots have indicated little to no differentiation among populations within the North Atlantic, even among morphologically distinguishable subspecies (Riffaut *et al.* 2005; Morris-Pocock *et al.* 2008). Therefore, other types of data, such as those from capture-mark-recapture studies, will now be required to test this hypothesis.

Host specialization and phylogeography

Despite strong patterns of geographical and host-associated structure in the microsatellites of *I. uriae*, the distribution of COIII haplotypes was found to be relatively homogenous among host types and colonies. Most haplotypes, including the most common, were shared among host groups and Tajima's test suggested a recent radiation in this gene with an excess of rare haplotypes. As stated above, we expected that host association and geographical isolation would interact to produce patterns of population structure in *I. uriae*. However, the interaction of these two variables could not explain patterns of pairwise population differentiation in either mitochondrial or microsatellite markers. The χ^2 tests for associations between host exploitation and haplotype distribution within each nested clade yielded a nonsignificant pattern overall (*P* = 0.0764). The historical relationships among the host races could therefore not be clearly deduced from the COIII cladogram. In line with these observations, pairwise differentiation could not be explained by a host effect. However, the fact that the association was only marginally nonsignificant could suggest that the signature of host-associated divergence is starting to appear at this marker. This is supported by the fact that results of assignment tests improved substantially with the addition of the mitochondrial data (Table 3). If this is indeed the case, we would predict that host-associated structure should be more obvious at faster evolving markers in the mitochondrial genome.

Although significant geographical structure was deduced from the COIII-haplotype network (*P* = 0.037, Table 4), the nature of this structure was also not obvious at a first glance and there was no correlation between pairwise differentiation in the two marker types. Nevertheless, geographical distance explained a significant part of mitochondrial pairwise differentiation when taken as a qualitative variable. This confirms the results obtained with the nested clade analysis, indicating that mitochondrial genetic diversity is more readily explained by geography than by host. The fact that distance taken as a categorical variable better explained the data than distance as a continuous variable is likely because of the highly vagile nature of the birds (i.e. distance is not a barrier if they decide to move among colonies) and the fact that decisions to prospect or disperse to new colonies are conditioned by local colony dynamics rather than simply by intercolony distance (see McCoy *et al.* 2005b for a more detailed discussion). Significant, albeit limited, geographical structure, combined with the variable patterns of host-associated groups within colonies, suggests that geographical isolation has evolved before host-related divergence at the COIII gene and supports the hypothesis that races have evolved independently in isolated geographical areas. If this is the case, the signature of host-related divergence may not yet have had time to appear at the slower evolving mitochondrial gene,

and/or may never appear at such genes because of the long-term stability of tick populations (i.e. host-associated groups frequently evolve and become extinct). Other mitochondrial (cytB) and nuclear (ITS2) genes were screened in a preliminary study and showed little geographical or host-associated variation, further supporting the recent nature of host-associated divergence (results not shown). For now, the alternative hypothesis of a single divergence event followed by secondary contact cannot be ruled out. However, given the results presented in this study and the recent establishment of many of the more northern seabird colonies, this hypothesis seems less parsimonious. Future work should now focus on obtaining reliable estimates of within- and between-colony divergences.

Based on results to date, it therefore appears that the evolution of host races is recent and may evolve recurrently across the distribution of *I. uriae*. An experimental study has demonstrated the existence of local adaptation in *I. uriae* at the within-colony scale in kittiwakes (McCoy *et al.* 2002). In particular, kittiwake chicks were transplanted among isolated breeding cliffs such that each experimental nest contained one resident and one nonresident chick. Local ticks were then followed during infestation and were found to perform better on local host birds, suggesting that despite a lack of neutral genetic structure at this spatial scale, ticks had adapted to the local host population. Rapid adaptive divergence, even at a fine spatial scale, may therefore be a general aspect in the evolution of the *I. uriae*–host interaction, whether it is associated with different host species or distinct subgroups of a particular host species. In line with theoretical work (Carroll *et al.* 2007; Garant *et al.* 2007), this supports the notion that local ecological interactions, and particularly antagonistic interactions, may change quickly and can differ strongly in relation to local conditions and the extent of among-population gene flow.

As ticks can have a significant impact on the reproductive success of their hosts (e.g. Boulinier & Danchin 1996), the evolution of host-associated tick races and the relative stability of these races will have important consequences for the population dynamics of seabirds. For example, the presence of specialized ticks could alter the outcome of interspecific competition for nesting space within local colonies (Oro 2008). These races may also have significant implications for the epidemiology of pathogens vectored by *I. uriae*, including *Borrelia burgdorferi sensu lato*, the bacterial complex responsible for human Lyme disease (Duneau *et al.* 2008). Indeed, both vector diversity and rapid genetic change can strongly influence the dynamics of the host/vector/pathogen interaction (Fussmann *et al.* 2007; Power & Flecker 2008). Therefore, the seemingly rapid host-associated divergence found in *I. uriae* highlights the need to consider such patterns of divergence in epidemiological modelling and calls for more explicit tests of the host-associated structure in other tick species, particularly in systems of significant medical and economic importance.

Acknowledgements

We thank A. Pedersen, R.T. Barrett, T. Tveraa, J. Gray and the staff of the Fair Isle Bird Observatory for assistance with sampling. We also thank M. Dietrich, N. Galtier, B. Godelle, E. Gomez-Diaz, J. Goudet, J. Michaux, O. Plantard and members of the 'Ticks and tick-borne diseases' working group of the Réseau Ecologie des Interactions Durables (REID) for useful discussions. This study was financed by grants from the Bureau des Ressources Génétiques, the Agence National de la Recherche (ANR-06-JCJC-0095-01) and the Institut Polaire Français – Paul Emile Victor (Programme no. 333). Florent Kempf is supported by the French Ministry for National Education and Research.

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This work was part of F. Kempf's dissertation on the evolution of host specialization in two vectors of Lyme disease bacteria, *Ixodes uriae* and *Ixodes ricinus*. T. De Meeûs' major interests focus on theoretical and empirical population genetic studies in parasitic systems. C. Arnathau works on the population genetics of vectors and associated microparasites. The results presented in this study are part of a long-term study conducted by K.D. McCoy and T. Boulinier on the evolutionary ecology of the interaction among seabirds, *I. uriae* and Lyme disease bacteria.
