Host specificity of a generalist parasite: genetic evidence of sympatric host races in the seabird tick *Ixodes uriae*

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Abstract

Due to the close association between parasites and their hosts, many 'generalist' parasites have a high potential to become specialized on different host species. We investigated this hypothesis for a common ectoparasite of seabirds, the tick *Ixodes uriae* that is often found in mixed host sites. We examined patterns of neutral genetic variation between ticks collected from Black-legged kittiwakes (*Rissa tridactyla*) and Atlantic puffins (*Fratercula arctica*) in sympatry. To control for a potential distance effect, values were compared to differences among ticks from the same host in nearby monospecific sites. As predicted, there was higher genetic differentiation between ticks from different sympatric host species than between ticks from nearby allopatric populations of the same host species. Patterns suggesting isolation by distance were found among tick populations of each host group, but no such patterns existed between tick populations of different hosts. Overall, results suggest that host-related selection pressures have led to the specialization of *I. uriae* and that host race formation may be an important diversifying mechanism in parasites.

Introduction

When new host preference alleles arise in a population, and if mating is linked to host choice, they can initiate a host shift that may lead to the establishment of genetically distinct host races of parasites (Maynard Smith, 1966; Jaenike, 1981; Rice, 1987). Provided that selection is strong relative to gene flow, over time these races may evolve into distinct species (Rice, 1987; Bush, 1994). There now exists experimental evidence for the evolution of reproductive isolation among groups as a result of strong selection, pleiotropy and/or hitchhiking, with or without physical isolation (see Rice & Hostert, 1993; Feder *et al.*, 1997) and divergence after a host shift is thought to account for the majority of speciation events in phytophagous insects and other such host/habitat specialists (Bush, 1994; de Meeûs *et al.*, 1998).

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Most documented examples of host/habitat specific races come from groups of phytophagous insects (e.g. Emelianov *et al.*, 1995; Via, 1999; Groman & Pellmyr, 2000; and see Tauber & Tauber, 1989). For more 'traditional' parasites, there are numerous examples of taxa found to consist of cryptic species and where speciation events are thought to be related to the specialization of the parasite on different host species or to different niches within individual hosts (e.g. Renaud & Gabrion, 1988; Giraud *et al.*, 1999). Fewer examples exist of parasites in the process of speciation, i.e. specialized races (but see de Meeûs *et al.*, 1992; Théron & Combes, 1995; Norton & Carpenter, 1998; Tompkins & Clayton, 1999; Bucheli *et al.*, 2000), even though this is likely to be an important diversifying force in the evolution of host–parasite associations (Price, 1980; Thompson, 1994; de Meeûs *et al.*, 1998; Timms & Read, 1999). Knowledge of the degree of host specialization of parasites is fundamental if we are to understand the ecological and evolutionary patterns we see in studies of host–parasite interactions (Tripet & Richner, 1997).

The evolution of host specificity in parasites is thought to be related to the relative availability and predictability

of their hosts. In this sense, host specialization is favoured by host-dependent fitness trade-offs (Fry, 1990), the advantage of using a single host species more profitably compared to interacting less profitably with several infrequently encountered hosts (Jaenike, 1990). When hosts are found in high abundance and predictable, parasites should specialize, and when hosts are in low abundance and ephemeral, parasites should generalize (Jaenike, 1990; Combes, 1997). In addition to their distribution and abundance, host availability will also depend on parasite dispersal abilities and the success rate of dispersing parasites to find and establish on new host individuals (Ward *et al*., 1998).

If some degree of host specialization has occurred in parasites, and if it is to have the potential to lead to reproductive isolation, there should be genetic differentiation between sympatric host groups (Jaenike, 1981). Direct genetic evidence can inform us about whether different host groups exchange genes at all or to what extent. Here, we address the question of host specificity by examining patterns of genetic variation in a host–parasite system where the parasite is considered to be a generalist but where there seems to be a high potential for specialization to occur.

The tick *Ixodes uriae* is considered to be catholic in terms of the seabird species it parasitizes; it has been found on over 50 different species (Rothschild & Clay, 1957; Guiguen, 1988). Some studies have suggested that host preferences exist, but these studies have largely been based on uncontrolled laboratory or field observations involving a limited number of host individuals (e.g. Eveleigh & Threlfall, 1974; Mehl & Traavik, 1983). *Ixodes uriae* appears to have a high potential for specialization; most of its seabird host species are colonial nesters found in dense, temporally predictable colonies; its hosts encompass a taxonomically diverse group and, thus, are likely to impose different selection pressures in relation to differences in their timing of reproduction, habitat use and immune response; and, finally, this ectoparasite probably has limited active dispersal thereby restricting its ability to access new hosts (Falco & Fish, 1991). Due to *I. uriae*'s large geographical range and high number of possible host species, Guiguen (1988) suggested that it was composed of a species complex; this was never tested. More recently, observations on prevalence and abundance of this species have indicated a potential segregation of populations among different host species in multispecific colonies (Barton *et al*., 1996; McCoy *et al*., 1999).

Here, we examine genetic variability and population differentiation between ticks collected from two host species, the Black-legged kittiwake (*Rissa tridactyla*) and the Atlantic puffin (*Fratercula arctica*), to determine whether *I. uriae* populations exploiting different sympatric hosts exhibit any degree of host specialization. If host races exist, there should be significant differentiation between tick populations of different sympatric host species. This differentiation should be larger than that between geographically close tick populations exploiting the same host species. In order to test this, and to eliminate the potentially confounding influence of microgeographical distance between different host groups, we also estimate population subdivision between allopatric tick populations of each host species independently (Rousset, 1999). We examine patterns of isolation by distance within and between tick populations of different hosts by comparing differentiation between groups at two different spatial scales. If there is high reproductive isolation between ticks of different host species, we predict that we should find patterns consistent with isolation by distance between tick populations of a single host, but not between ticks of different host species.

Materials and methods

Study species

Ixodes uriae is a common ectoparasite of seabirds found in the circumpolar regions of both hemispheres. It typically has a 4-year life cycle consisting of three stages, larvae, nymph and adult. At each stage (usually corresponding to one stage per year), the parasite will take a single blood meal from its host. The length of this blood meal changes depending on the stage of the parasite, but in general corresponds to 5–7 days for larvae and nymphs and 5–10 days for adults (e.g. Eveleigh & Threlfall, 1974). Except for this short period on the host, *I. uriae* is found in the substrate surrounding the host breeding site.

Independent dispersal abilities in this tick genus are generally considered low. For example, a study on the dispersal abilities of the deer tick, *Ixodes dammini*, found that adult ticks moved an average of 1.8 m in 6 days (Falco & Fish, 1991). Thus, most dispersal in ticks likely takes place in association with host movements. However, host-mediated dispersal may be limited for *I. uriae*, as most of its host species are pelagic (i.e. only on land during the breeding season and at sea the rest of the year) and, during the reproductive season, most breeding adults take trips between the nest and the feeding areas only and thus do not provide many opportunities for tick dispersal to occur.

In this study we restrict our focus to two host species of *Ixodes uriae*, the Black-legged kittiwake (*Rissa tridactyla*) and the Atlantic puffin (*Fratercula arctica*). These two species are both colonial nesting seabirds that are found in large numbers in the North Atlantic (Cramp & Simmons, 1983; Cramp, 1985). Despite their similar distributions, there are many features of their life histories that might distinguish them for ectoparasites. First, they are phylogenetically distant from each other (kittiwakes; family Laridae; puffins; family Alcidae) meaning that physiological conditions and immunological responses to ticks are potentially quite different. They can also differ in their timing of reproduction when they breed at the same location. For instance, on Hornøya,

Norway, kittiwake chicks hatch and fledge approximately 2–3 weeks before puffins (R. Barrett, unpublished data). This means that the availability of hosts at the nest does not completely overlap for the two species. Ticks that do not time their meal correctly could end up at sea when chicks start to fledge and adults leave the colony for the winter. Another potentially important difference between these two host species is that they use slightly different nesting substrates during reproduction; kittiwakes build grass nests on the vertical parts of cliffs, while puffins typically dig burrows on grassy slopes. In this sense, during the off-host period, ticks are exposed to different environmental conditions depending on which host they parasitize. There has been considerable debate about the relative importance of host vs. habitat in the evolution of ticks (Klompen *et al.*, 1996) and, thus, these two aspects can have a confounding influence on results of studies examining the influence of one or the other.

Study sites and sampling

Ticks were collected from two main sampling locations: Baccalieu Island, Newfoundland, Canada (48°08′N, 52°48′W) in July 1997 and Hornøya, an island in northern Norway (70°22′N, 31°10′W) in July 1998 (Fig. 1). On each island, we sampled ticks at the same time from both kittiwakes and puffins on a single, mixed breeding cliff. On Baccalieu, the puffin slope was above the vertical nesting area of kittiwakes with a mixed area of both species at the cliff edge. On Hornøya, puffin burrows were intermixed among areas with nesting kittiwakes. Ticks were also sampled from nearby breeding cliffs/colonies that contained only a single host species. On the two islands considered, there were other cliffs with only breeding kittiwakes: B.G. on Baccalieu Island, approximately 1 km from the mixed host colony (N.W.) and Cliff G on Hornøya, 0.5 km from the mixed site (Main). Ticks from puffin hosts were collected at the island of Hernyken, Røst, Norway (67°26′N, 11°52′W) in late June/early July 1999 and at Gull Island, Newfoundland (47°15′N,52°46′W) in July 1997 (see Fig. 1 for relative locations).

For both host species, ticks were sampled from nestlings at the nest site. Only ticks that were found feeding on the birds or found inside a puffin burrow at the actual nest site were considered for the analysis. Each nestling was searched for ticks using visual inspection and skin palpation. After ticks were removed, nestlings were weighed, ringed and returned to the nest site. All ticks found were stored in 70% alcohol for later DNA extraction. For a given sampling location and host species, ticks were searched for at as many nest sites as possible (typically >30). Ticks were selected for genotyping from a maximum number of nests possible within a breeding cliff.

The abundance of different tick stages varies among different hosts and populations (McCoy *et al.*, 1999). For the populations examined here, we tended to find only nymphal ticks on puffins whereas we found a mix of adult and nymphal ticks on kittiwakes. In order to have

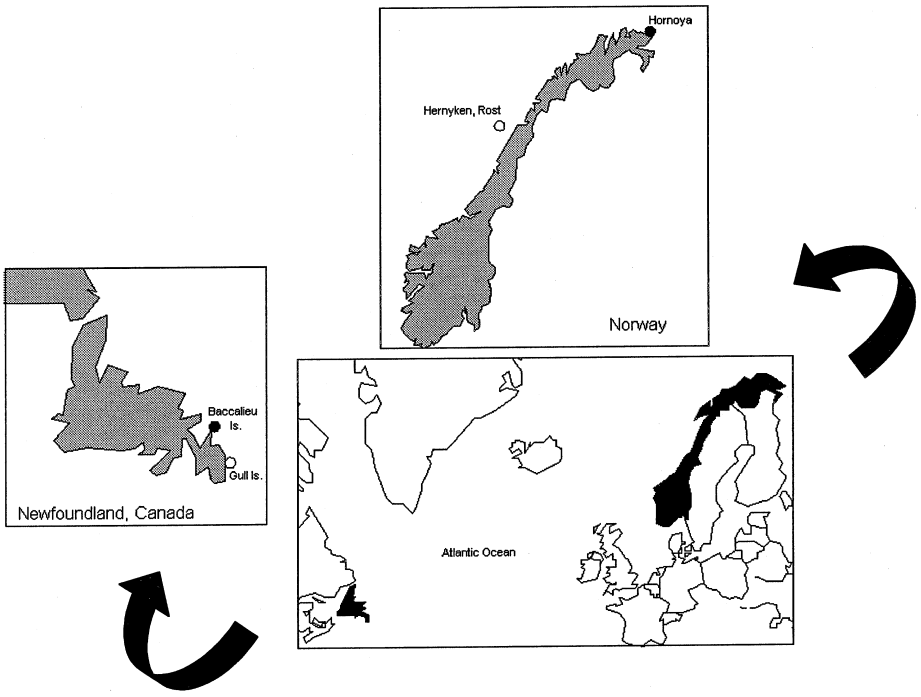

Fig. 1 Tick sampling locations in Newfoundland, Canada, and Norway. Black dot: islands with mixed breeding areas and monospecific kittiwake cliffs; white dot: islands with monospecific puffin breeding areas.

a comparable number of individuals for different host species, we did not control for tick stage in our analysis. To test the assumption that we could compare different tick stages between host species, we examined genotypic differentiation between adult and nymphal ticks collected from two kittiwake host populations (Main, Hornøya and N.W., Baccalieu Island) from which we sampled enough individuals of each stage to enable a test to be performed.

Genotyping

Ticks were genotyped for nine different microsatellite loci designed using ticks from Atlantic puffins (McCoy & Tirard, 2000). For each population, DNA extractions were carried out on a minimum of 24 ticks. Extraction and PCR procedures followed those outlined by McCoy & Tirard (2000) and resulting products were run on 6% acrylamide gels using size controls. To avoid technical problems that could influence results, extractions, PCR amplifications and electrophoreses were performed at several different occasions for a given population. Two observers scored each gel.

There was strong variation in amplification success across different tick populations and loci; the number of ticks successfully genotyped from the eight populations and among the nine loci ranged from 7 to 33 (Table 1). This difference in performance could be related to DNA quality or to the presence of null alleles. Null alleles, the absence of PCR products due to mutations in primer sequences, were suspected when DNA from an individual did not amplify for certain loci, but were successfully amplified for at least two others, indicating that DNA quality was probably not the cause of the non-amplification. However, as we cannot say for certain that non-amplifications were indeed homozygotes for null alleles, we will refer to suspected cases as 'blanks'.

Statistical methods

All populations and loci were examined for conformity to Hardy–Weinberg equilibrium using exact probability tests employing a Markov chain method to estimate exact *P*-values (Raymond & Rousset, 1995). To ensure independence among loci, data were tested for linkage disequilibrium using the exact probability test. Allelic and gene diversities (Nei, 1987) were calculated for each tick population and comparisons between different host species were performed using Wilcoxon two-sample tests (Zar, 1996).

Data were divided into three groups for analysis: among tick populations of kittiwakes, among tick populations of puffins and between tick populations of different host species. Genetic structure among populations was examined in two ways. The first tested population differentiation using *G*-based exact tests for examining the distribution of genotypes among populations. Test results for each locus were combined using Fisher's method (Raymond & Rousset, 1995). The second examined the degree of structure using Wright's *F*-statistics estimated according to Weir & Cockerham (1984). It is well recognized that F_{ST} will tend to underestimate levels of genetic differentiation when applied to microsatellite data (Slatkin, 1995; Rousset, 1996; Hedrick, 1999; Balloux *et al*., 2000). While other measures have been proposed to take into account some of the characteristics of microsatellites, in particular, an analogue to F_{ST}, R_{ST} (Slatkin, 1995), its reliability has been questioned in different circumstances (e.g. Estoup *et al*., 1995; Rousset, 1996; Balloux *et al*., 2000). Thus, we use only F_{ST} estimates to examine the differentiation between tick populations; we consider these measures to provide conservative estimations of the divergence between populations. The maximum possible value of F_{ST} corresponds to the value given by the average expected within-sample homozygosity (Hedrick, 1999); we report these values to provide a reference for interpreting population differentiation. We first present global *F*-statistics for all populations and then for each of the three analytical groups. The standard errors of estimates were calculated by bootstrapping over loci and their significance was determined using permutation tests based on resampling alleles or genotypes either among individuals or subpopulations using 1000 randomizations (Goudet, 1995).

The frequency of blanks for each population was calculated by summing the number of non-amplifications across loci for individual ticks with amplified products for at least two other loci. Owing to the presence of these potential null alleles, we performed population differentiation statistics both with and without a correction. The correction added the same null allele to all populations with blanks by computing maximum likelihood estimates of gene frequencies in the presence of a null allele using the EM algorithm of Dempster *et al*. (1977) implemented in GENEPOP v3.1d (Raymond & Rousset, 1995). Analyses are first presented without the correction, and changes caused by it are discussed at the end of the Results section. As blanks also provide relevant information on genetic differentiation, i.e. potential divergence in primer sequences, host species-related and population differences in the frequency of blanks were tested using Chi-square analyses of contingency tables (Zar, 1996).

Patterns associated with isolation by distance were examined in each group by comparing average pairwise genetic distances of populations that were 'nearby' (<1000 km) and far apart (>4000 km). Considering each locus as an independent replicate, Wilcoxon paired-sample tests (one-tailed) were performed on the two distance classes to determine whether populations far apart had significantly greater genetic distances than 'nearby' populations (Zar, 1996).

Calculations of allele frequencies, gene diversities, estimations and significance tests were carried out using

Table 1 Summary of variability parameters for each tick population and locus. Average values (± standard error) are given across tick populations for each host species.

		T1			T3			T5			T22		
	N_{all}	*n*	n_a (SR)	*h*	*n*	n_a (SR)	*h*	*n*	n_a (SR)	*h*	*n*	n_a (SR)	*h*
Kittiwake													
NW Baccalieu Is.	25	20	5 (159–163)	0.77	24	2 (114–116)	0.16	17	7 (118–138)	0.67	22	8 (157–187)	0.84
B.G. Baccalieu Is.	21	14	4 (159–162)	0.62	20	2 (114–116)	0.18	9	4 (118–138)	0.79	13	6 (169–183)	0.71
Main. Hornoya	32	31	5 (158–162)	0.64	29	3 (112–116)	0.60	26	5 (118–182)	0.65	26	8 (165–183)	0.75
Cliff G. Hornoya	23	23	5 (158–162)	0.70	20	3 (112–116)	0.48	19	6 (118–184)	0.74	18	9 (169–185)	0.79
Avg Kittiwake		22.0 ± 3.54	4.75 ± 0.25	0.68 ± 0.03	23.3 ± 2.14	2.5 ± 0.29	0.36 ± 0.11	17.8 ± 3.5	5.5 ± 0.65	0.71 ± 0.03	19.8 ± 2.78	7.75 ± 0.63	0.77 ± 0.03
Puffin													
NW Baccalieu Is.	32	32	6 (158–164)	0.69	33	1 (114)	0	32	9 (122–138)	0.84	33	10 (157–187)	0.85
East. Gull Is.	26	26	6 (159–164)	0.75	26	1 (114)	0	26	8 (122–140)	0.84	25	8 (169–187)	0.82
Main. Hornoya	31	31	5 (159–163)	0.72	31	2 (112–114)	0.15	31	9 (118–182)	0.85	31	8 (157–181)	0.79
Hernyken. Rost	22	21	4 (159–162)	0.76	22	2 (112–114)	0.05	11	5 (128–136)	0.81	16	9 (157–181)	0.87
Avg Puffin	27.5	5.25 ± 2.53	0.73 ± 0.48	28 ± 0.02	1.5 ± 2.48	0.05 ± 0.29	25 ± 0.04	7.75 ± 4.85	0.84 ± 0.95	26.3 ± 0.01	8.75 ± 3.82	0.83 ± 0.48	± 0.02

n = number of individuals genotyped, n_a = number of alleles, SR = size range of alleles, *h* = gene diversity (Nei, 1987).

Table 1 Continued.

T35			T38			T39			T44			T47		
n	n_a (SR)	*h*	*n*	n_a (SR)	*h*	*n*	n_a (SR)	*h*	*n*	n_a (SR)	*h*	*n*	n_a (SR)	*h*
Kittiwakes														
20	7 144–158	0.72	21	2 (161–163)	0.42	15	9 (160–225)	0.87	20	1 (169)	0	18	2 (152–158)	0.06
18	5 (144–158)	0.63	9	4 (159–169)	0.60	7	7 (151–217)	0.93	10	2 (169–173)	0.10	10	1 (158)	0
31	7 (144–162)	0.84	23	4 (159–169)	0.60	23	12 (160–215)	0.82	22	4 (153–173)	0.13	20	3 (152–158)	0.27
22	8 (144–162)	0.81	19	6 (159–169)	0.73	18	13 (157–217)	0.83	16	4 (151–169)	0.29	14	3 (152–158)	0.26
22.8 ± 2.87	6.75 ± 0.63	0.75 ± 0.05	18 ± 3.11	4 ± 0.82	0.59 ± 0.06	15.8 ± 3.35	10.25 ± 1.38	0.86 ± 0.03	17 ± 2.65	2.75 ± 0.75	0.13 ± 0.06	15.5 ± 2.12	2.25 ± 0.48	0.15 ± 0.07
Puffins														
33	10 (142–162)	0.72	33	3 (159–163)	0.44	32	18 (183–227)	0.93	32	4 (155–169)	0.48	33	3 (152–158)	0.52
26	8 (144–162)	0.60	25	5 (155–165)	0.38	26	20 (177–223)	0.95	26	2 (163–169)	0.40	26	2 (152–158)	0.42
27	8 (144–162)	0.82	31	6 (155–167)	0.64	31	21 (160–225)	0.95	31	4 (153–185)	0.10	30	3 (152–158)	0.43
18	7 (144–162)	0.80	14	5 (155–167)	0.63	13	9 (185–217)	0.90	16	2 (163–169)	0.06	15	3 (150–158)	0.35
26 ± 3.08	8.25 ± 0.63	0.74 ± 0.05	25.8 ± 4.27	4.75 ± 0.63	0.52 ± 0.07	25.5 ± 4.37	17 ± 2.74	0.93 ± 0.01	26.3 ± 3.66	3 ± 0.58	0.26 ± 0.11	26 ± 3.94	2.75 ± 0.25	0.43 ± 0.07

GENEPOP (v.3.1d, Raymond & Rousset, 1995), FSTAT (v.1.2, Goudet, 1995) and SAS (SAS Institute, 1990). When required, significance levels were adjusted for multiple tests (Rice, 1989).

Results

Initial examination for agreement with Hardy–Weinberg equilibrium showed that three loci, T1, T5 and T39, had significant heterozygote deficiencies after correcting for multiple tests. To reduce their influence on estimates, all presented analyses have been performed excluding these three loci. It should be noted, however, that their inclusion did not change the general results or their significance. With their elimination, Hardy–Weinberg expectations were obtained globally for all populations (Table 2). No linkage disequilibrium was detected between any of the nine loci either within each population or across all populations.

G-like exact tests performed between nymphal and adult ticks collected from kittiwakes showed no significant genotypic differences among stages for either of the two breeding cliffs (Combined test, Main, $\chi^2_{18} = 20.08$, $P = 0.33$; NW, $\chi^2_{16} = 15.85$, $P = 0.46$). Thus, we were able to make comparisons between tick populations of different host species even though they were represented by unequal proportions of different tick stages.

Within host species differentiation

Variation in allele and gene diversities was high among populations and loci (Table 1). Among the four tick populations from kittiwake hosts, the average number of alleles across loci varied from 3.33 (±0.80) to 5.50 (±1.06) and average gene diversities between 0.37 (±0.13) and 0.56 (±0.10) (Table 2). The frequency of blanks among kittiwake tick populations differed significantly among populations (Chi-square, $\chi^2_3 = 16.19$, $P < 0.005$; Fig. 2). Likewise, there was significant overall differentiation between populations indicated by both genotypic tests (Combined test, $\chi^2_{12} =$ infinity, $P < 0.0001$; note that *P*-values from independent tests that are close to zero result in a Chi-square value of infinity for the combined test) and by the estimated value of F_{ST} (Table 3). However, there were no differences between tick populations from nearby kittiwake cliffs after correcting for

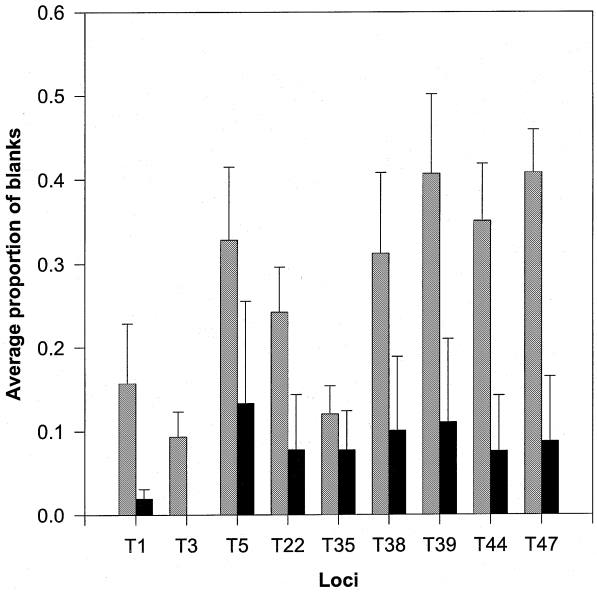

Fig. 2 Average frequency of 'blanks' (±standard error) in populations of *I. uriae* for nine microsatellite loci. Shaded bar: tick populations from kittiwake hosts; black bar: tick populations from puffin hosts.

Table 2 Average estimates of variability parameters (± standard error) for tick populations. Values are calculated using only the six loci used in analyses. H_{HW} refers to estimates of F_{IS} for each population and the corresponding *P*-values to tests for Hardy–Weinberg equilibrium. No *P*-values were significant after sequential correction for multiple tests (Rice, 1989).

	n_{avg}	n_a (se)	h (se)	H_o (se)	H_{HW}	P
Kittiwake						
NW, Baccalieu Is.	20.83 (±0.83)	3.67 (±1.23)	0.37 (±0.14)	0.30 (±0.15)	0.18	0.01
BG, Baccalieu Is.	13.33 (±1.89)	3.33 (±0.80)	0.37 (±0.13)	0.33 (±0.28)	0.10	0.47
Main, Hornøya	25.17 (±1.74)	4.83 (±0.87)	0.53 (±0.11)	0.52 (±0.12)	0.03	0.87
Cliff G, Hornøya	18.17 (±1.17)	5.50 (±1.06)	0.56 (±0.10)	0.50 (±0.10)	0.11	0.32
Avg Kittiwake	19.38 (±2.48)	4.33 (±0.50)	0.46 (±0.05)	0.41 (±0.06)	0.09	0.06
Puffin						
NW, Baccalieu Is.	32.83 (±0.17)	5.17 (±1.58)	0.50 (±0.12)	0.50 (±0.14)	–0.001	0.48
East, Gull Is.	25.67 (±0.21)	4.33 (±1.28)	0.44 (±0.11)	0.37 (±0.11)	0.16	0.26
Main, Hornøya	30.17 (±0.65)	5.17 (±1.05)	0.49 (±0.13)	0.43 (±0.14)	0.12	0.12
Hernyken, Røst	16.83 (±1.17)	4.67 (±1.17)	0.46 (±0.15)	0.34 (±0.11)	0.25	0.16
Avg Puffin	26.38 (±3.51)	4.84 (±0.21)	0.47 (±0.01)	0.41 (±0.04)	0.11	0.05

n = number of individuals genotyped, n_a = number of alleles, h = gene diversity (Nei, 1987), H_o = observed heterozygosity.

Table 3 Distribution of microsatellite variation among tick samples of each host species as measured by *F*-statistics (Weir & Cockerham, 1984). 95% confidence intervals, shown in parentheses, were calculated by bootstrapping over loci. Max F_{ST} is equivalent to the average homozygosity within samples.

Host	F_{IS}	F_{IT}	F_{ST}	Max F_{ST}
Kittiwake	0.080	0.149	0.075	0.54
	(0.022, 0.174)	(0.090, 0.249)	(0.054, 0.096)	
Puffin	0.107	0.147	0.045	0.53
	(0.039, 0.221)	(0.091, 0.251)	(0.020, 0.081)	
All	0.096	0.159	0.069	0.54
	(0.045, 0.193)	(0.111, 0.272)	(0.051, 0.111)	

multiple tests; the pairwise value between populations in Newfoundland (1 km apart) was close to significance (Table 4). Estimates of pairwise F_{ST} were significantly different between 'nearby' and far populations, suggesting isolation by distance among these four tick populations (Wilcoxon paired-sample test, $n = 6$, $P = 0.03$; Fig. 3).

Similar ranges of average allele and gene diversities were found between tick populations from puffin hosts, ranging between 4.33 (±1.28) and 5.17 (±1.05) for allele diversity and between 0.44 (±0.11) and 0.50 (±0.12) for gene diversity (Table 2). The frequency of blanks among puffin tick populations was very low, except for one colony, Hernyken (Røst), for which a large proportion of blanks was found (Chi-square, $\chi^2_3 = 93.64$, $P < 0.001$; Fig. 2). Again, there was significant overall genotypic differentiation between tick populations from puffin hosts (Combined test, $\chi^2_{12} =$ infinity, $P < 0.0001$), and a significant overall value of F_{ST} (Table 3). Isolation by distance among populations was suggested by the difference between the average pairwise genetic distance of 'nearby' and far populations (Wilcoxon paired-sample test, $n = 6$, $P = 0.03$; Fig. 3).

From the results presented here, patterns of differentiation among tick populations seem to depend on host species. Tick populations of both hosts show patterns consistent with isolation by distance, but estimates of F_{ST} tended to be greater for tick populations from kittiwakes than for those from puffins over both spatial scales (Tables 3 and 4; Fig. 3).

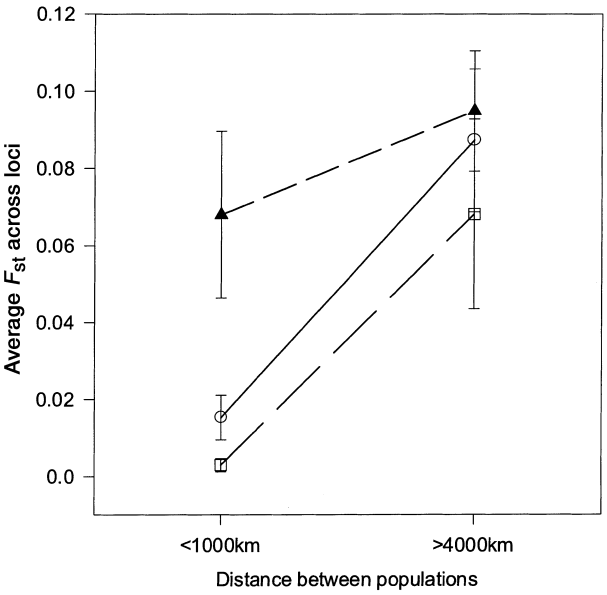

Fig. 3 Relationships between the average pairwise genetic distance ($F_{ST}/1 - F_{ST}$) of tick populations and the geographical distance of host colonies ('nearby' or far). Circles: average values (±SE) across loci of populations parasitizing kittiwake hosts; squares: average values (±SE) across loci of populations parasitizing puffin hosts; solid triangles: average values (±SE) across loci of populations parasitizing different host species.

Between host species differentiation

There were no significant differences between the allele and gene diversities of ticks from the two host species. The average allele diversities were 4.33 (±0.50) and 4.84 (±0.21), and average gene diversities were 0.46 (±0.05) and 0.47 (±0.01) for kittiwake and puffin hosts, respectively (Wilcoxon two-sample tests, allele diversity, $n_{1,2} = 4$, $P = 0.66$; gene diversity, $n_{1,2} = 4$, $P = 1.0$; Table 2).

There was a significantly higher proportion of blanks found for ticks sampled from kittiwakes compared to those sampled from puffins (Chi-square, $\chi^2_1 = 91.59$, $P < 0.001$) (Table 1, Fig. 2). As microsatellites for *I. uriae* were developed using ticks collected from puffins (McCoy & Tirard, 2000), this difference in amplification

Table 4 Pairwise estimates of F_{ST} (Weir & Cockerham, 1984) between nearby allopatric tick populations from the same host species and between sympatric tick populations from different host species. *P*-values for F_{ST} were calculated by permutating genotypes among samples and is based on 1000 randomizations. Max F_{ST} is equivalent to the average homozygosity within samples.

Host	Populations	km	Max F_{ST}	F_{ST}	*P*
Kittiwake	NW vs. BG, Baccalieu Is.	1.0	0.63	0.019	0.012
	Main vs. Cliff G, Hornøya	0.5	0.46	0.010	0.265
Puffin	NW, Baccalieu Is. vs. Gull Is.	50	0.53	–0.003	0.802
	Main, Hornøya vs. Hernyken, Røst	700	0.53	–0.005	0.692
Multi-host	NW, Baccalieu Is.	0	0.57	0.072	<0.001
	Main, Hornøya	0	0.49	0.037	<0.001

performance suggests population level differences among ticks from the two host species. Indeed, pairwise exact tests across loci indicated strong genotypic differences between sympatric host populations of ticks (Combined test, both NW and Main, $\chi^2_{12} =$ infinity, $P < 0.0001$). Furthermore, estimates of F_{ST} showed significant structure between ticks from the two host species in both mixed host locations (Table 4). The estimated values of F_{ST} between ticks from different hosts were 0.037 and 0.073, for NW and Main, respectively, and were more than three times greater than those estimated between 'nearby' allopatric tick populations for the same host species (Table 4). These values corresponded to theoretical maximum possible values of F_{ST} of less than 0.57 and 0.49 (Table 4).

Unlike for each host species, there was no evidence suggesting isolation by distance of tick populations of different host species; no significant difference was found between the average pairwise genetic distance estimates of 'nearby' and far populations (Wilcoxon paired-sample test, $n = 6$, $P > 0.10$). Furthermore, differentiation between sympatric tick populations of different hosts was greater than between 'nearby' populations and equivalent to values for very far (>4000 km) populations of the same host. Nonetheless, these estimates were still somewhat low relative to the maximum possible (Fig. 3). Overall, results are consistent with the existence of host-associated races of *Ixodes uriae*.

Due to the possible presence of null alleles in our data, analyses of population structure among tick populations were also performed using corrected gene frequencies. Here, we present only the non-corrected results for several reasons. First, the mutations causing non-amplification affect primers and not the microsatellites themselves. Thus, unlike other genetic markers, e.g. allozymes, in which a null allele provides relevant information on the kind of genetic variability scored, null alleles for microsatellites provide information on a different kind of genetic variability. There is no a priori reason that an individual homozygous for mutations in the primers should also be homozygous for the microsatellite allele. Second, not correcting would be conservative with respect to our major question, i.e. differentiation between tick populations exploiting different hosts, if there was an association between the frequency of non-amplification and the frequency of certain microsatellite alleles. In our study, blanks could be statistically associated with, and therefore mask, 'kittiwake' tick microsatellite alleles leading to an underestimation of the degree of differentiation. Indeed, as discussed earlier, most blanks occurred in ticks sampled on kittiwakes. Finally, and most importantly, the correction for a possible null allele does not qualitatively alter our main results. The correction decreased estimated structure between tick populations of kittiwakes and increased structure among tick populations of puffins. This change is logical in light of the pattern of blanks in our data; the correction added the same new allele to all kittiwake tick populations, even to those very far apart (>4000 km), and to only one puffin tick population among the four (Hernyken, Røst). Nonetheless, there was still greater structure (higher pairwise estimates) between tick populations of different sympatric hosts species than between 'nearby' allopatric tick populations of the same host. Thus, as our conclusions are qualitatively unchanged whether we correct or not, we present only results without a correction because this procedure is conservative with respect to the questions addressed.

Discussion

In many instances, reports of host species ranges for parasites are of little value because they do not distinguish between typical and accidental hosts and do not provide information about potential host specialization (Tripet & Richner, 1997). In agreement with this, *Ixodes uriae* has typically been considered to be a host generalist because of its long list of host species (Rothschild & Clay, 1957). Our results suggest that, in fact, this parasite may be specialized among its different host species; stronger genetic differentiation was found between tick populations of different sympatric host species than between isolated tick populations in 'nearby' colonies (< 1000 km) of the same host species.

Clear differences in the amplification performance of loci between ticks of the different host species were evident and could indicate high levels of isolation between host groups. Similarly, the lack of evidence for isolation by distance between tick populations of different host species also suggests low gene flow between races. Nonetheless, estimates of differentiation found between populations were not extremely high, even at large spatial scales (maximum pairwise F_{ST} of 0.17) and after considering maximum possible F_{ST} values of between 0.46 and 0.63. These low values could suggest that some limited gene flow may still be occurring between host groups. The populations we consider here only cover areas that are either relatively close or very distant. More detailed information at intermediate distances and field studies examining reproductive isolation will be required to better examine current gene flow between host groups.

Genetic differences between host groups match earlier observational studies in this parasite. Eveleigh & Threlfall (1974) examined host preferences in *I. uriae* by introducing ticks to a variety of different host species. It was not stated from which host species ticks were collected, but extreme differences in tick feeding success among host species were found. Only 25% of adult ticks fed successfully on kittiwakes compared to a 75% success rate on puffins. All ticks successfully fed on razorbills

(*Alca torda*) and common guillemots (*Uria aalge*). Razorbills and guillemots belong to the same family as puffins (Alcidae), thus if the ticks used in these experiments originated from guillemot or razorbill hosts, these results could make sense in light of our finding of tick host races. It should be stated, however, that very few host individuals were used in this study (one or two of each species), and therefore individual differences in host susceptibility to tick parasitism alone could account for the observed patterns (Boulinier *et al*., 1997). More recent observations noted host-related differences in the prevalence and abundance of different tick stages (McCoy *et al*., 1999). In particular, at the period that observations took place, significantly more nymphal ticks were found on species of Alcidae compared to kittiwakes, which hosted mainly adult ticks. Considering the assumed limits of active tick movement, the authors suggested that this could be the result of separate populations functioning asynchronously, rather than stage-related host preferences. A similar observation of isolation of tick stages among host species has been made between kittiwake and common guillemot hosts in Scotland (Barton *et al*., 1996).

To understand how tick host races might have formed, it is important to investigate the possible mechanisms driving divergence. Typically, the formation of host races is thought to be linked to disruptive selection exerted by different hosts and host-dependent trade-offs (Maynard Smith, 1966; Rice & Hostert, 1993; Bush, 1994; but see Kawecki, 1998). Differences in host phenology are considered to be one such disruptive force. In phytophagous insects, adaptation to host/resource phenology appears to be key for sympatric race formation and speciation (e.g. Tauber & Tauber, 1989; Filchak *et al*., 1999; Groman & Pellmyr, 2000). This mechanism has also been implicated in the isolation of a trematode parasite between different definitive host species (Théron & Combes, 1995). Here, differences in the host breeding period might explain the formation of tick races; bird species differ in their timing of reproduction such that ticks feeding at the wrong time could end up at sea when the birds leave for the winter.

Other potential selection pressures could stem from differences in host immune responses to infestation or to differences in the physical host environment. Likewise, we cannot eliminate differences in microhabitat usage between different host species as a potential selective pressure. As kittiwakes and puffins use different types of nesting substrates, ticks will be exposed to different conditions during the off-host period. Thus, we cannot say whether the genetic differences seen here are due to selection pressures exerted from the host species or from the off-host environment; both factors are likely to play a role. Controlled preference and cross-infection experiments, and further genetic comparisons between tick populations of other sympatrically occurring host species may provide some elements to help distinguish among these different possible forces.

Finally, host-associated dispersal could have played an important role in the isolation of tick groups, particularly if isolation occurred in allopatry. This dispersal mechanism is essential for gene flow to occur between isolated subpopulations, but it is unclear what effect it has in mixed host sites where the distance between different host species is within the range of the independent dispersal abilities of the parasite. In our case, for both mixed sites considered, both host species were easily found within this range. Thus, not all tick dispersal was likely associated with host movements between nest sites. Furthermore, we found no significant deviations from Hardy–Weinberg equilibrium within each group, suggesting that we did not have substructuring at this scale. Nonetheless, detailed information on the genetic distance and the distance between different host species could allow one to look at the effect of non-random host movements on tick gene flow at the scale of the breeding cliff.

While much attention has been given to the role of sympatric host races as a diversifying force in phytophagous insects (e.g. Bush, 1994), relatively little work has been done on this mode of evolution in more 'traditional' parasites, despite their potential to speciate in this fashion. Morphological, behavioural and genetic differences have been demonstrated between sympatric host populations of *Schistosoma mansoni* (Théron & Combes, 1995). After its recent introduction to Guadeloupe, this trematode underwent a host shift, from humans to rats. In this case, asynchrony in the emergence time of cercariae from the common gastropod intermediate host was considered to be responsible for the sympatric differences between host groups. Evidence for host switching events has also been found to explain the diversity of mistletoe parasites (Norton & Carpenter, 1998). Recently, host-related genetic differences were shown for the anther-smut fungus *Microbotryum violaceum* (Bucheli *et al*., 2000); investigators found that there was almost no gene flow between different host plants and that, at least within the *Silene* genus, co-speciation with the host plant was not responsible for the genetic differences found.

Here, we find evidence of sympatric tick races infesting different seabird host species. As the two host species we examined are phylogenically distant, tick races did not likely form through co-speciation events. However, we cannot say with certainty whether these races formed in sympatry or represent secondary contact after forming in allopatry. Nor can we say whether tick races arose through a single host shift or through several such events. Nonetheless, our findings reinforce the idea that host race formation, or specialization after a host shift, could be an important mechanism for explaining the rich diversity of parasites and that it merits further examination in other host–parasite systems.

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