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ORIGINAL ARTICLE

Inter-oceanic variation in patterns of host-associated divergence in a seabird ectoparasite

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ABSTRACT

Aim Parasites with global distributions and wide host spectra provide excellent models for exploring the factors that drive parasite diversification. Here, we tested the relative force of host and geography in shaping population structure of a widely distributed and common ectoparasite of colonial seabirds, the tick *Ixodes uriae*.

Location Two natural geographic replicates of the system: numerous seabird colonies of the North Pacific and North Atlantic Ocean basins.

Methods Using eight microsatellite markers and tick samples from a suite of multi-specific seabird colonies, we examined tick population structure in the North Pacific and compare patterns of diversity and structure to those in the Atlantic basin. Analyses included population genetic estimations of diversity and population differentiation, exploratory multivariate analyses, and Bayesian clustering approaches. These different analyses explicitly took into account both the geographic distance among colonies and host use by the tick.

Results Overall, little geographic structure was observed among Pacific tick populations. However, host-related genetic differentiation was evident, but was variable among host types and lower than in the North Atlantic.

Main conclusions Tick population structure is concordant with the genetic structure observed in seabird host species within each ocean basin, where seabird populations tend to be less structured in the North Pacific than in the North Atlantic. Reduced tick genetic structure in the North Pacific suggests that host movement among colonies, and thus tick dispersal, is higher in this region. In addition to information on parasite diversity and gene flow, our findings raise interesting questions about the subtle ways that host behaviour, distribution and phylogeographic history shape the genetics of associated parasites across geographic landscapes.

Keywords
Co-evolution, host race, host–parasite interactions, *Ixodes uriae*, microsatellite, North Atlantic, North Pacific, tick.

INTRODUCTION

The geographic mosaic theory of coevolution predicts that coevolution between interacting species can vary across geographic landscapes (Thompson, 2005). In host–parasite systems, spatial heterogeneity in the interaction is expected to be intimately linked to host population structure, to relative host and parasite dispersal abilities, as well as to parasite specificity in relation to local host community composition and history (e.g. Dybdahl & Lively, 1996; Johnson *et al.*, 2002; McCoy *et al.*, 2003; Stefka *et al.*, 2009). Coevolutionary interactions can be strong in some locations, but weak in others, leading to differential levels of parasite adaptation and specialization over space (e.g. Thompson & Cunningham,

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2002). In this way, parasites with global distributions and wide host spectra provide excellent models for exploring the factors driving parasite diversification.

The tick *Ixodes uriae* White, 1852 is a common ectoparasite of seabirds, widely distributed across the polar regions of both hemispheres. In addition to its direct effects on seabird populations (e.g. Boulinier & Danchin, 1996), this tick can also vector a wide array of pathogens, some of which cause human disease, e.g. the Lyme disease bacterium, *Borrelia burgdorferi sensu lato* (Dietrich *et al.*, 2011). Hosts include a great diversity of seabird species (more than 60), which may impose different selection pressures on ticks in relation to their timing of reproduction, habitat use, behaviours (e.g. grooming) and immune response. These hosts generally breed in large aggregations (i.e. colonies) that are spatially and temporally predictable (Coulson, 2001). Moreover, natal and breeding philopatry is common in seabirds and may significantly restrict tick dispersal and gene flow (McCoy *et al.*, 2003). Indeed, tick dispersal is thought to be limited to the within-breeding season movements of seabirds among colonies (McCoy *et al.*, 2003). The combination of high host diversity, a wide geographic distribution and the spatial isolation of seabird colonies creates multiple opportunities for geographical structuring and host specialization in the tick. Indeed, previous studies in the North Atlantic and Southern Indian Ocean have shown that geography and seabird host species are both key factors in determining the population genetic structure of this tick. Sympatric tick host races occur recurrently in *I. uriae* among seabird species that are phylogenetically and ecologically distinct (McCoy *et al.*, 2001, 2005a) and within-race geographic structure is largely host-dependent (McCoy *et al.*, 2003). Furthermore, recent mitochondrial DNA analyses suggest that the divergence of tick races in the North Atlantic is a dynamic process that has occurred relatively recently (Kempf *et al.*, 2009).

The world-wide distribution of *I. uriae* means that the diversity of its seabird hosts and local environmental conditions can vary greatly among geographically isolated areas, possibly leading to different patterns of adaptation among regions. In the Northern Hemisphere, two such areas can be distinguished: the North Atlantic and North Pacific Oceans. The community assemblage of seabird species are largely distinct in each region (del Hoyo *et al.*, 1996). Among the shared species, significant genetic differentiation has been demonstrated, suggesting little to no contemporary gene flow between the North Atlantic and North Pacific (e.g. Brünnich's guillemot, *Uria lomvia* – Birt-Friesen *et al.*, 1992; black-legged kittiwake, *Rissa tridactyla* – Patirana, 2000; common guillemot, *Uria aalge* – Morris-Pocock *et al.*, 2008). Moreover, the extent of host population structure within each region is variable; while common guillemot and black-legged kittiwake populations are differentiated within the Atlantic over very large distances, this is not the case in the Pacific (Patirana, 2000; Riffaut *et al.*, 2005; Morris-Pocock *et al.*, 2008). Such differences in seabird populations can be expected to have major effects on the genetic structure of their ectoparasites, and potentially on the evolution of host specificity. In this way, the Atlantic and Pacific basins constitute interesting natural replicates with which to test mechanisms of tick population differentiation.

In this study we test whether patterns of population structure in seabirds are reflected in their parasites. In particular, we examine the relative force of host and geography in shaping tick population structure in the North Pacific Ocean basin and compare patterns of diversity and structure to those in the Atlantic basin. Given the lower genetic structure of seabird hosts in the Pacific, we expected to find reduced genetic structure of tick populations in the Pacific compared to the Atlantic. If this lower structure is due to patterns of high contemporary gene flow, but selection for host specialization is occurring, we expected patterns of host-associated divergence with little geographic structure within each host-associated group.

MATERIALS AND METHODS

Study sites, hosts and genotyping

Field sampling took place in 2008 and 2009 across the North Pacific in nine heterospecific colonies where different seabirds breed within mixed colonies (Fig. 1a and Table 1). Seabird communities in the North Pacific are relatively rich and colonies can be very large (Byrd *et al.*, 2005). Hosts included eight species found in large number in the North Pacific, including two sister species of kittiwake [the black-legged kittiwake (BKT), *Rissa tridactyla*, and the red-legged kittiwake (RKT), *Rissa brevirostris*], two sister species of guillemot [the common guillemot (CG), *Uria aalge*, and the Brünnich's guillemot (BG), *Uria lomvia*], one puffin species [the tufted puffin (TPF), *Lunda cirrhata*], two species of auklet [the Cassin's auklet (CAA), *Ptychoramphus aleuticus,* and the rhinoceros auklet (RHA), *Cerorhinca monocerata*] and one species of cormorant [the red-faced cormorant (RC), *Phalacrocorax urile*]. Phylogenetically, the rhinoceros auklet is more closely related to puffins (tribe Fraterculini) than to other auklets, but nomenclature has not yet been modified to reflect this relationship. For the majority of colonies, ticks were collected directly from their hosts and from as many distinct individual birds as possible to maximize the representativeness of the sample. In Bogoslof and Aiktak colonies (Fig. 1), TPF ticks were also collected off-host (in the burrows). We attempted to account for possible micro-allopatric conditions among the nest sites of different host species by sampling different intermingled patches of a given host species within the larger mixed colony. After collection, sampled ticks were stored in 70% ethanol. Based on geographic location and host species of origin, we considered initially that 26 distinct tick populations were collected (Table 1).

Ticks were genotyped using eight previously described microsatellite loci (McCoy & Tirard, 2000). Full details on the preparation and procedure for genotyping samples can be found in Kempf *et al.* (2009). Sample genotypes were visualized using an ABI PRISM 3130xl Genetic Analyser and allele sizes were assigned using GeneMapper v.4 (Applied Biosystems, Foster City, CA, USA).

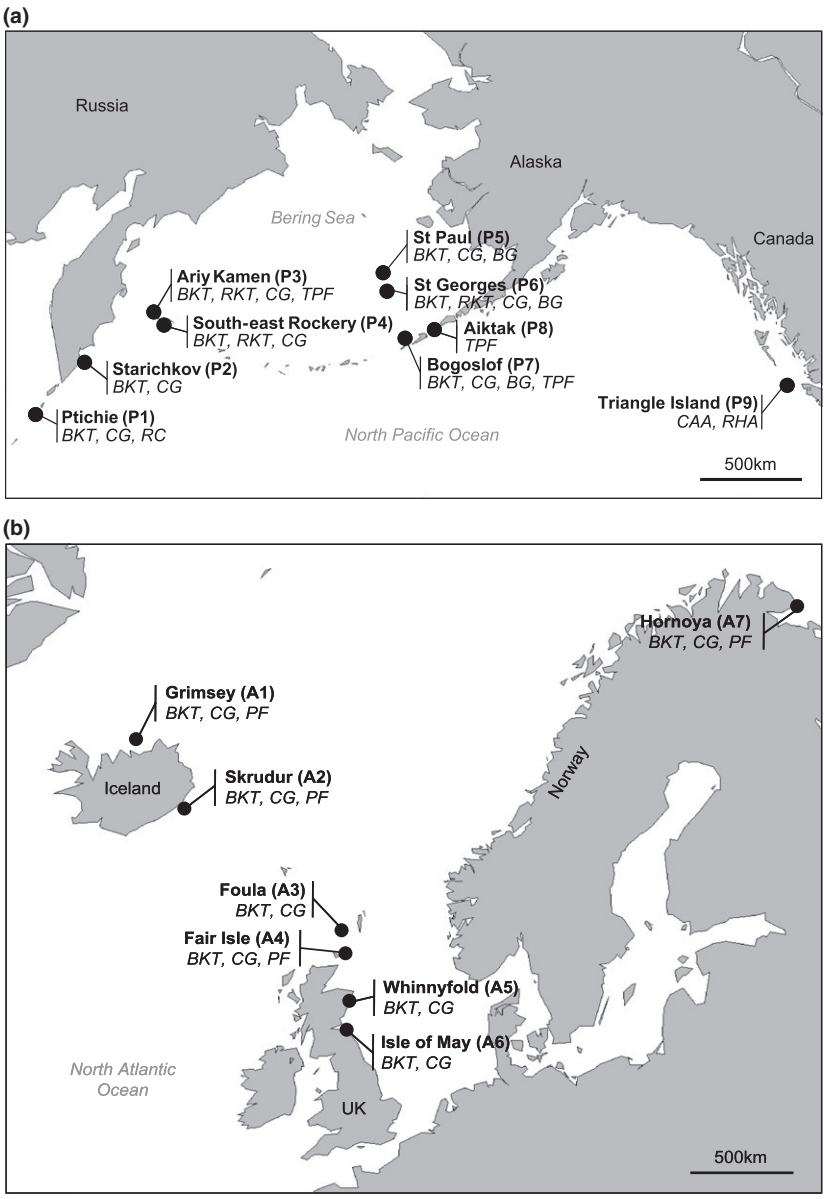

Figure 1 Sampling sites in seabird colonies of the (a) North Pacific and (b) North Atlantic. See Table 1 for exact locations and details of the species sampled.

To compare patterns of structure and diversity with those in the Atlantic Ocean basin, we used a dataset of 529 ticks collected from seven mixed colonies within the North Atlantic (see Fig. 1b and Table 1). These sites were selected in order to have both a similar list of host species and a comparable range of geographic distances among colonies within each ocean basin. These data were previously obtained by McCoy *et al.* (2005a) and Kempf *et al.* (2009).

Data analysis

Population structure in the North Pacific

To ensure the independence of the markers employed, the loci were tested for linkage disequilibrium. In all populations, departures from Hardy–Weinberg (HW) proportions in genotypic frequencies were investigated by estimating the inbreeding coefficient (F_{IS}). The significance of this estimator was assessed by randomizing alleles among individuals within samples (5000 permutations). Gene diversity (H_S) was also assessed and differences among host species (BKT, RKT, CG, BG and TPF) were tested using a permutation test of populations among host species (5000 randomizations). These computations were performed with the software FSTAT v.2.9.3.2 (Goudet, 2002). To compare gene diversity among colonies of each host species, we used a Kruskal–Wallis test.

To estimate tick genetic structure among host species, we first carried out a between-group analysis (BGA), implemented in the 'ade4TkGUI' package (Thioulouse & Dray, 2007). The purpose of this analysis is to ordinate groups of samples (i.e. here, group is the host species) rather than individuals. The method includes an initial principal components analysis,

Table 1 Sample details and genetic variation of *Ixodes uriae* in North Pacific and North Atlantic populations. For each population, we report the number of ticks, the number of hosts on which ticks were collected, F_{IS} (where F_{IS}* is this estimator without locus T44; see Materials and Methods) and gene diversity (H_S). Significant deviations from Hardy–Weinberg proportions are indicated in bold. Host species are coded as: BKT, black-legged kittiwake (*Rissa tridactyla*); RKT, red-legged kittiwake (*Rissa brevirostris*); TPF, tufted puffin (*Lunda cirrhata*); PF, Atlantic puffin (*Fratercula arctica*); CG, common guillemot (*Uria aalge*); BG, Brünnich's guillemot (*Uria lomvia*); RC, red-faced cormorant (*Phalacrocorax urile*); CAA, Cassin's auklet (*Ptychoramphus aleuticus*); RHA, rhinoceros auklet (*Cerorhinca monocerata*).

Colony	Code	Latitude Longitude	Host species	No. of ticks	No. of hosts	F_{IS}	F_{IS}*	H_S
North Pacific								
Ptichie, Kuril Island, Kamtchatka	P1	N 50°30′ E 156°12′	BKT	26	20	0.145	0.063	0.66
			CG	26	25	0.013	−0.028	0.65
			RC	30	16	**0.146**	0.076	0.65
Starichkov Island, Kamtchatka	P2	N 52°43′ E 158°40′	BKT	25	13	**0.251**	0.113	0.63
			CG	30	16	0.089	0.051	0.64
Ariy Kamen Islet, Bering Island, Kamtchatka	P3	N 55°12′ E 165°55′	BKT	27	15	0.111	0.056	0.68
			RKT	14	3	0.092	0.023	0.64
			CG	30	26	0.031	0.002	0.62
			TPF	30	23	0.066	0.035	0.64
South-East Rockery, Bering Island, Kamtchatka	P4	N 54°48′ E 166°26′	BKT	32	18	0.101	0.025	0.59
			RKT	30	17	0.103	0.048	0.58
			CG	23	16	0.087	0.072	0.57
St Paul Island, Alaska	P5	N 57°11′ W 170°16′	BKT	30	29	0.095	−0.003	0.69
			CG	13	6	0.098	0.000	0.65
			BG	30	27	0.046	0.026	0.64
St George Island, Alaska	P6	N 56°33′ W 169°31′	BKT	21	17	0.164	0.089	0.58
			RKT	31	17	**0.260**	0.193	0.64
			CG	31	20	0.064	0.047	0.64
			BG	20	20	0.113	0.039	0.63
Bogoslof, Alaska	P7	N 53°55′ W 168°1′	BKT	27	11	**0.169**	0.118	0.66
			CG	27	14	0.061	0.008	0.65
			BG	28	13	**0.137**	0.075	0.63
			TPF	16	> 1†	0.015	−0.010	0.67
Aiktak, Alaska	P8	N 54°10′ W 164°53′	TPF	16	> 1†	**0.227**	0.170	0.70
Triangle Island, British Columbia	P9	N 50°51′ W 129°5′	CAA	29	18	0.015	0.015	0.43
			RHA	28	28	0.060	0.063	0.46
North Atlantic								
Grimsey, Iceland	A1	N 66°33′ W 18°00′	BKT	30	24	0.041	0.060	0.73
			CG	30	22	0.024	0.021	0.73
			PF	38	27	0.028	0.015	0.56
Skrudur, Iceland	A2	N 64°54′ W13°37′	BKT	28	17	0.084	0.057	0.69
			CG	29	24	0.059	0.048	0.64
			PF	31	19	0.037	0.031	0.55
Foula, UK	A3	N 60°08′ W 02°04′	BKT	17	6	0.078	0.079	0.52
			CG	14	6	0.033	0.013	0.56
Fair Isle, UK	A4	N 53°33′ W 01°36′	BKT	43	15	0.055	0.027	0.56
			CG	27	16	0.125	0.137	0.52
			PF	38	7	−0.053	−0.053	0.52
Whinnyfold, UK	A5	N 57°30′ W 01°48′	BKT	35	19	0.061	0.063	0.48
			CG	27	10	0.042	0.044	0.51
Isle of May, UK	A6	N 56°12′ W 02°42′	BKT	33	24	0.170	0.167	0.47
			CG	24	24	0.020	−0.009	0.52
Hornoya, Norway	A7	N 70°22′ E 31°10′	BKT	29	12	0.087	0.090	0.60
			CG	24	15	0.068	0.078	0.60
			PF	31	14	0.132	0.135	0.59

†For these populations, ticks were collected both on- and off-host (see Materials and Methods).

followed by a second analysis where sample group is included as a qualitative explanatory variable. The linear combination of variables that maximize the intergroup variance in allele frequencies are used to plot individuals and the barycentre of each group. We then performed a permutation test to evaluate departure from a random distribution of individuals among groups (10,000 permutations; Dolédec & Chessel, 1987; Chessel *et al.*, 2004). These computations were performed using the R statistical package (v.2.10.0).

We then tested whether tick populations clustered genetically according to host species, by using a Bayesian clustering approach with the STRUCTURE v.2.3.1 software (Pritchard *et al.*, 2000). We included all North Pacific tick populations and set the number of potential clusters (K) from 1 to 26 (maximum number of populations), with 10 independent runs performed. Computations were run under the admixture model with correlated allelic frequencies. We used the new LOCPRIOR model developed in Hubisz *et al.* (2009) to take into account the host species of each tick individual. This model is recommended in situations where the signal of genetic structure is weak. Simulations were carried out using a burn-in of 100,000 iterations, allowing for the stabilization of summary statistics, followed by a run length of 100,000 iterations. The *ad hoc* statistic ΔK was then used to detect the most likely number of clusters in each colony (Evanno *et al.*, 2005). However, this method is unable to make inferences if the actual number of populations is 1 or the maximum value of K tested ($K = 26$), and thus, we also computed the posterior probability $\Pr(K)$ to infer the number of populations (Pritchard *et al.*, 2000).

Pairwise differentiation (F_{ST}) among host-related tick groups in each colony was estimated with the FSTAT software. Although there has been debate about the use of F_{ST} for estimating gene flow rates (Bossart & Prowell, 1998; Whitlock & McCauley, 1999), this index remains a useful tool for inferring population structure (Neigel, 2002). The significance of the global differentiation between the same pair of host species was calculated by combining the independent pairwise tests. When the number of tests was low ($k < 4$), this was done using Stouffer's Z approach (Whitlock, 2005). When the number of tests was greater than 4, we used a generalized binomial procedure implemented in the software MULTITEST v.1.2 (De Meeûs *et al.*, 2009). To interpret the population differentiation observed, we computed the maximal theoretical value of genetic differentiation (F_{ST} max) as a reference, using data from both ocean basins (Hedrick, 2005).

Geographic structure across colonies was quantified for each identified host-associated tick race using F_{ST} estimates (FSTAT software). Only tick races for which more than two colonies were sampled were included in this analysis (i.e. kittiwake tick race: BKT and RKT ticks; guillemot tick race: CG and BG ticks; and puffin tick race: TPF). The significance of the estimator was assessed using 5000 permutations of genotypes among populations and standard errors were calculated by jackknifing over loci (Goudet *et al.*, 1996). We then performed a test of isolation by distance with IBDWS v3.14 (Jensen *et al.*, 2005) with 1000 permutations to assess the statistical significance of the correlation between genetic and geographic distances. Geographic distances were log-transformed for a two-dimensional organization of populations (Rousset, 1997). This latter analysis was only performed for kittiwake (BKT and RKT) and guillemot (CG and BG) tick races because several colonies were sampled for these species, making them the most robust for the analysis.

Comparison of Pacific and Atlantic tick populations

We used an AMOVA test to determine the significance of the a priori geographic isolation between Atlantic and Pacific tick populations using ARLEQUIN v.3.1 (Excoffier *et al.*, 2005). All tick populations ($n_{Pacific} = 26$, $n_{Atlantic} = 18$, see Table 1) were included in the analysis. The F_{CT} value was used to estimate genetic differentiation between ocean basins and the significance of the test was assessed by 10,000 permutations of populations between ocean basins. Next, we calculated the mean percentage of private alleles across loci in each ocean basin and tested for differences between regions using a Student's t-test. We then compared the average allelic richness (AR), unbiased gene diversity (H_S) and heterozygosity (H_O) of populations between the ocean basins using a permutation test implemented in FSTAT (1000 randomizations of populations among ocean basins).

Finally, we compared the level of geographic and host-related structure of tick populations between ocean basins. We focused on BKT and CG tick populations as both of these tick host races are present and have been sampled in the Atlantic and the Pacific. In each ocean basin, we first computed the mean F_{ST} of tick populations among colonies of the same host species (geographical structure) and tested for a significant difference between regions using a permutation test implemented in FSTAT (1000 randomizations of populations between ocean basins). Second, we compared the mean F_{ST} between sympatric BKT and CG ticks (i.e. local host-related structure) of each region using a Student's t-test.

RESULTS

Genetic structure in the North Pacific

In line with previous studies, all tests of pairwise genotypic combinations failed to reject the null hypothesis of linkage equilibrium among loci. Gene diversity (H_S) ranged from 0.43 to 0.70 (Table 1), but did not vary either among host species ($P = 0.907$) or among colonies of the same host species (BKT: $P = 0.997$, RKT: $P = 0.706$, CG: $P = 0.985$, BG: $P = 0.990$, TPF: $P = 0.949$). After a standard Bonferroni correction for multiple tests, significant deviations from Hardy–Weinberg proportions were observed in six populations (Table 1). A locus-by-locus analysis revealed that these population estimators were strongly affected by heterozygote deficits at a single locus (T44). The elimination of this locus led to a significant decrease in global F_{IS} values (F_{IS}*) (Student's t-test, $P = 0.002$,

Table 1) and to HW proportions in all populations. Given the deviations present at locus T44, we repeated all tests including and excluding this locus, but results were unchanged (data not shown). We therefore report values for all eight loci.

The between-group analysis resulted in two principal components which explained more than 60% of the total variance of the data and revealed four genetically different groups of ticks (Fig. 2, $P = 0.0001$). The first group is composed of RHA and CAA ticks, which are well-differentiated from all other populations and explain most of the variance along the first axis. The second and third groups include, respectively, ticks from the two sister species of kittiwakes (BKT and RKT) and of guillemots (CG and BG). Finally, ticks from tufted puffins (TPF) and cormorants (RC) group in a fourth cluster. When we eliminated ticks from Triangle Island (group 1: RHA and CAA) from the between-group analysis, the differentiation among the remaining tick host races became more pronounced (see Appendix S1 in Supporting Information).

Results from the Bayesian clustering analysis revealed that ticks grouped primarily by host species (Fig. 3). The most probable number of genetic clusters within the North Pacific basin was indeed four [$\Pr(K = 4) > 0.99$], which is concordant with results of the between-group analysis. Differentiation of TPF ticks (in red) in the P3, P7 and P8 colonies was supported by high, almost maximal, assignment probabilities compared to that of the other tick races. In the same way, RC ticks (in red) in the P1 colony were well-differentiated from those collected on other species, but assignment probabilities were lower. Divergence between kittiwake (in blue) and guillemot (in grey) ticks was evident across colonies, but low in the P1, P3 and P5 colonies (i.e. the assignment probability of kittiwake ticks to the kittiwake group were reduced). Overall, kittiwake ticks always showed lower assignment probabilities compared to guillemot ticks. Finally, ticks from Triangle Island (P9) were well differentiated from all other tick populations but there was no evidence of genetic differentiation between CAA and RHA ticks within the colony (Fig. 3).

The pattern of host-related divergence found in the between-group analysis and the Bayesian clustering analysis was supported by pairwise F_{ST} estimates between sympatric hosts. Significant local structure between sympatric kittiwake and guillemot ticks was found across colonies (Table 2). However, in the P1, P3 and P5 colonies, differentiation was low between these tick groups (data not shown). These results suggest that overall differentiation between kittiwake and guillemot ticks can be site-dependent. In contrast, strong divergence was always observed between TPF ticks and other local tick races (i.e. higher values of pairwise F_{ST}), particularly given the maximal theoretical value of genetic differentiation (F_{ST} max = 0.397). RC ticks were also significantly divergent from kittiwake and guillemot ticks, but the level of differentiation was lower. On Triangle Island, the degree of divergence between RHA and CAA ticks was relatively high compared to other colonies ($F_{ST} = 0.0264$) but was only marginally significant (Table 2). Finally, ticks from the sister species of

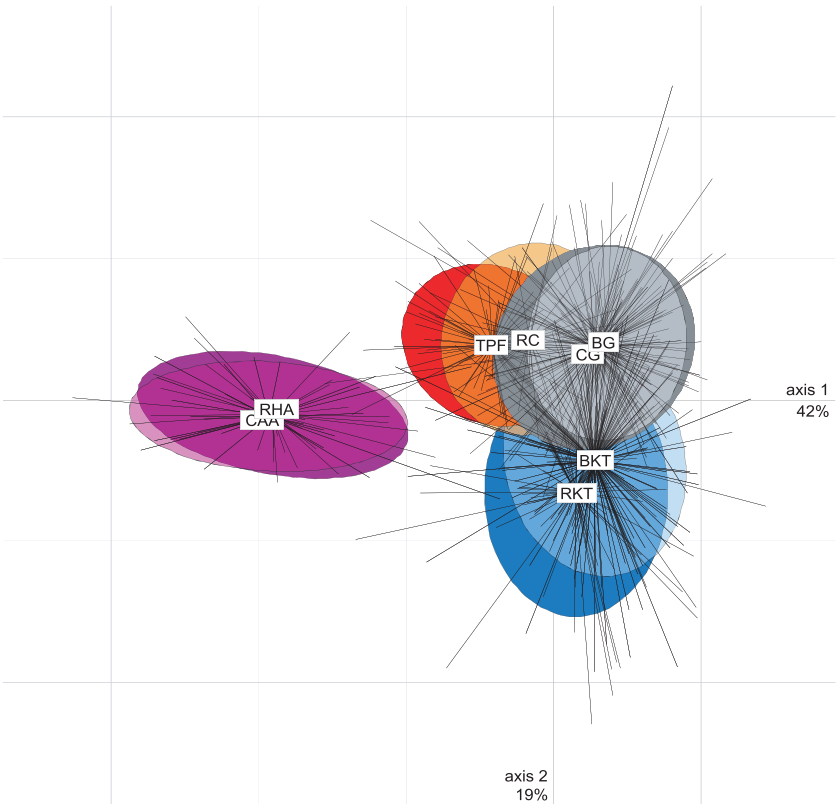

Figure 2 Between-group analysis of North Pacific *Ixodes uriae* populations. Ticks from all nine geographic locations were combined for each host type. Ellipses represent the distribution of 67% individuals around the population barycentre and each individual is connected to the barycentre of the group by a line. Acronyms for host species as outlined in Table 1.

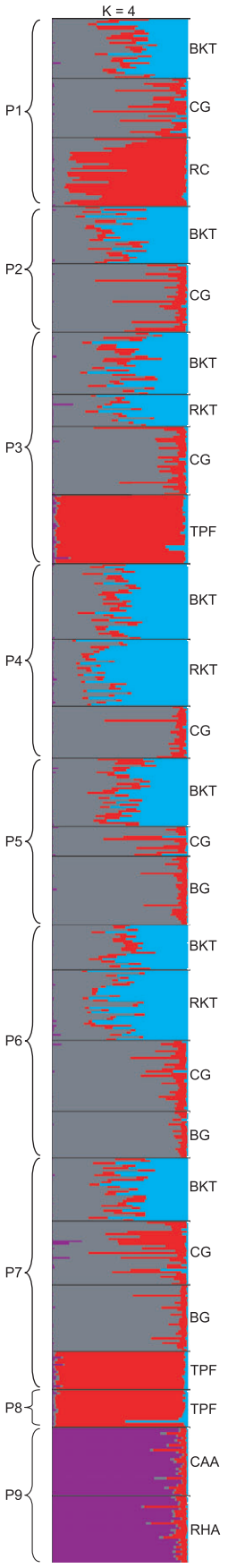

Figure 3 Tick population structure inferred by Bayesian clustering within the North Pacific basin. Each individual tick is shown as a thin vertical line partitioned into *K* coloured segments, the length of each colour being proportional to the estimated membership coefficient. Here, the most probable number of genetic clusters is $K = 4$ [$\Pr(K = 4) > 0.99$]. Black lines separate different population (geographic sites and host species are labelled on the left and right of the figure, respectively, with abbreviations corresponding to those in Table 1).

Table 2 Average pairwise population differentiation between sympatric host-related tick groups in the North Pacific. When necessary, multiple *P*-values were combined using a generalized binomial procedure (number of tests $k > 4$) or Stouffer's *Z* test ($k < 4$). Significant F_{ST} estimates appear in bold. See Table 1 for host species abbreviations.

Host race comparisons	Number of combined tests	Genetic differentiation	
		F_{ST}	*P*-value
BKT–CG	7	**0.0152**	0.001
BKT–BG	3	**0.0142**	0.001
RKT–CG	3	**0.0270**	< 0.001
RKT–BG	1	**0.0238**	0.050
TPF–BKT	2	**0.0418**	< 0.001
TPF–CG	2	**0.0377**	< 0.001
TPF–BG	1	**0.0522**	0.008
TPF–RKT	1	**0.0315**	0.008
RC–CG	1	**0.0116**	0.017
RC–BKT	1	**0.0152**	0.017
CAA–RHA	1	**0.0264**	0.050
BKT–RKT	3	0.0073	0.056
CG–BG	3	−0.0012	0.304

kittiwake (BKT and RKT) and guillemot (CG and BG) were undifferentiated, with low and non significant average F_{ST} estimates (Table 2).

In terms of geographic structure, low differentiation was found across colonies of each tick race (kittiwake ticks: $F_{ST} = 0.014$ ($\pm$ 0.004), $P = 0.0002$; guillemot ticks: $F_{ST} = 0.007$ ($\pm$ 0.003), $P = 0.0004$; puffin ticks: $F_{ST} = 0.008$ ($\pm$ 0.005), $P = 0.0036$). Moreover, for kittiwake and guillemot tick populations, there was no evidence for an association between genetic and geographic distances among colonies ($P = 0.531$ and $P = 0.546$, respectively).

Comparison of Pacific and Atlantic tick population structure

When colonies were grouped by ocean basin for the hierarchical AMOVA, the amount of variation explained by ocean basin was highly significant ($P < 0.0001$). The average estimate of genetic differentiation between Pacific and Atlantic tick populations was high ($F_{CT} = 0.237$) with respect to the maximum value possible given marker diversity (F_{ST} max = 0.397). There was also a high percentage of private alleles within each ocean basin (only 32% of alleles were

Table 3 Comparison of genetic diversity and structure between North Pacific and North Atlantic tick populations. Diversity indices and F_{ST} values were averaged over loci and populations in each ocean basin. Standard errors are shown in parentheses. *P*-values were computed by population permutations between ocean basins. The comparison of private alleles was tested using a Student's *t*-test (see text for details) and standard errors over loci are indicated in parentheses. Geographic structure within each ocean basin was only compared for black-legged kittiwake (*Rissa tridactyla*, BKT) and common guillemot (*Uria aalge*, CG) ticks as only these host species are present and were sampled in both the Pacific and the Atlantic.

	North Pacific	North Atlantic	*P*-value
Allelic richness (*AR*)	5.559 (± 1.000)	5.060 (± 0.879)	0.027
Heterozygosity (H_O)	0.555 (± 0.089)	0.549 (± 0.084)	0.523
Gene diversity (H_S)	0.623 (± 0.084)	0.575 (± 0.082)	0.056
Private alleles/locus (%)	39.8 (± 9.4)	43.1 (± 3.7)	0.747
F_{ST} BKT	0.011	0.090	0.313
F_{ST} CG	0.009	0.193	0.034

shared), but both ocean basin contained the same approximate percentage (Table 3). Allelic richness, heterozygosity and gene diversity were higher in the Pacific, but a significant difference was only found for allelic richness (Table 3).

For both BKT and CG ticks, geographic structure was more pronounced in Atlantic than in Pacific populations, but was only statistically different for CG ticks (Table 3). Local host-related divergence between sympatric BKT and CG ticks ranged from 0.037 to 0.155 in the Atlantic and from 0.010 to 0.025 in the Pacific. As shown in Fig. 4, the level of host-related structure between sympatric BKT and CG ticks in the Atlantic was significantly higher than in the Pacific (Student's *t*-test, $P = 0.006$).

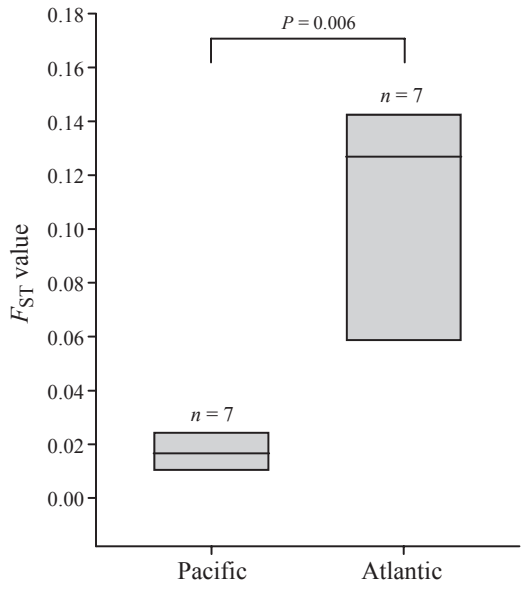

Figure 4 Genetic differentiation (F_{ST}) between sympatric black-legged kittiwake (*Rissa tridactyla*, BKT) and common guillemot (*Uria aalge*, CG) tick populations in seabird colonies within the North Pacific and within the North Atlantic. Median value, lower and upper quartiles are shown. The number of pairwise comparisons used is indicated above the box for each basin.

DISCUSSION

Here, we used microsatellite markers to examine the population structure of *Ixodes uriae* in the North Pacific and compared the patterns found with those of the North Atlantic. As predicted based on the lower genetic structure of seabirds in the Pacific, we found that tick populations of Pacific seabirds were less structured that those of the Atlantic. Here, we contrast patterns of structure and diversity within each ocean basin to make some inferences on the processes behind these patterns.

Host-associated population structure of *I. uriae* in the North Pacific

Genetic structure was found among *I. uriae* ticks exploiting different seabird species within colonies of the North Pacific and ticks grouped primarily by host species, regardless of the geographic distance between colonies. This is consistent with previous reports for the North Atlantic and the Southern Hemisphere and supports the recurrent evolution of host-associated races in this globally-distributed ectoparasite (McCoy *et al.*, 2005a; Kempf *et al.*, 2009). However, the degree of divergence in sympatry was host-dependent. A lack of specialized tick host races for the sister species of kittiwake (BKT and RKT) and guillemot (CG and BG) was observed in all colonies. This is not surprising given that these sister species are close phylogenetically and have similar breeding habitats, phenologies and physiologies (Gaston & Jones, 1998; Kildaw, 1999). Similar results have been found in ticks exploiting sister species of penguins in sub-Antarctic Islands (McCoy *et al.*, 2005a). In contrast, the other host-associated comparisons were consistently significant.

TPF ticks were highly differentiated from other co-occurring host races in all colonies where they were sampled (Fig. 3). This echoed previous results found in Atlantic colonies where local divergence was always strong for ticks from Atlantic puffins (McCoy *et al.*, 2005a; Kempf *et al.*, 2009). This pattern may be explained by a more ancient evolution of puffin ticks, and/or by selection for higher host specificity in this tick race.

Divergence of this tick race may also be favoured by differences in the breeding habitats of puffins and the other co-occurring species (e.g. dirt burrows versus cliff faces for kittiwakes and guillemots). Indeed, ticks can be highly sensitive to microhabitat conditions (i.e. variation of temperature and humidity; Klompen *et al.*, 1996) and these parameters may differ between burrows and rock faces thereby reducing genetic exchanges among different host-associated groups. However, these differences should not completely eliminate tick gene flow in most multi-specific seabird colonies as different breeding birds are typically close enough to enable a tick to use an alternative host and return to the appropriate microhabitat via homing behaviours (Benoit *et al.*, 2008).

Cormorant ticks were also divergent from co-occurring kittiwake and guillemot ticks but to a lesser extent than puffin ticks (Table 2 and Fig. 2). Auklet ticks from Triangle Island likewise showed no clear evidence of within-colony divergence, but here, we were limited to a single comparison. These ticks were quite distinct from the other tick races; however, we can not disentangle the relative role of host and geographic factors in this divergence, as the two species of auklet were sampled in a single geographic location. Oceanographically, Triangle Island is clearly separated from Alaskan colonies, and thus, geography may be an important factor driving the observed divergence. Clearly, a more extensive sampling of auklet and cormorant colonies across the North Pacific, as well as of related species in the North Atlantic, will be necessary to understand whether these tick groups represent host specific races.

Divergence between sympatric kittiwake and guillemot tick races was observed across colonies, but the degree of differentiation varied in intensity across sites. This could be due to independent divergence events, as suggested to have occurred in the North Atlantic (Kempf *et al.*, 2009), or to reduced barriers to gene flow in certain colonies. For example, different seabird species can group into monospecific patches of differing size within mixed colonies; the presence and organization of such patches may reinforce isolation among host-associated tick groups in some cases and favour exchange in other cases. In the colonies where kittiwake and guillemot tick races were well-differentiated (i.e. P2, P4, P6 and P7), kittiwake ticks always showed lower assignment probabilities compared to guillemot ticks. This pattern has been previously observed in the North Atlantic (Kempf *et al.*, 2009) and may be the result of (1) the relative age and/or origin of the kittiwake tick race (i.e. they have evolved more recently within colonies from other local races), and/or (2) reduced specificity at the host-tick interface (i.e. ticks adapted to other species can still feed on kittiwakes).

Geographic structure of *I. uriae* within the North Pacific

For the three tick races with at least three sampled colonies, i.e. kittiwake, guillemot and puffin tick races, only weak geographic structure was found among colonies, suggesting high levels of contemporary gene flow within tick races. This pattern corresponds to that found in the seabird host. For example, common guillemots in the North Pacific are suggested to encompass a single genetic population that is maintained by contemporary migration between colonies, favoured by the stepping-stone distribution of seabird colonies within the North Pacific (see Fig. 1a; Morris-Pocock *et al.*, 2008). Given that tick dispersal between colonies is largely limited to the movements of seabirds during the breeding season (Danchin, 1992; McCoy *et al.*, 2005b), we expected to detect signals of genetic isolation-by-distance (IBD) for kittiwake and guillemot tick races. This was not found. This may mean that among-colony tick dispersal is either high enough to eliminate any traces of IBD or that factors other than colony configuration favour high seabird movement among the colonies of this region, and thus tick dispersal.

Comparison of Pacific and Atlantic tick population structure

Our analyses revealed that tick populations of the North Pacific and North Atlantic oceanic basins are strongly isolated and can therefore be considered as natural replicates with which to test mechanisms of tick population differentiation. This again matches what we know about seabird population structure. Indeed, many seabird species that live both in the North Pacific and North Atlantic exhibit significant population differentiation between ocean basins (e.g. see Friesen *et al.*, 2007). Interestingly, we found lower levels of both geographic and host-related genetic structure in Pacific tick populations compared to North Atlantic tick populations. Such differences may be associated with historical population demographic events, where, for example, the number of glacial refugia differed between ocean basins. These differences may also be related to variation in contemporary gene flow due to basin-specific differences in seabird population functioning and inter-colony dispersal (Morris-Pocock *et al.*, 2008). Given that genetic diversity tended to be higher in North Pacific tick populations, and that we have no evidence to suggest substantial differences in the effective population sizes of ticks from the two regions, our results lend support to the gene flow hypothesis.

The factors, present and historical, that influence population structure throughout a species' range are often difficult to disentangle. Understanding the elements driving population structure and host-related differentiation in *I. uriae* is particularly challenging as many of its host species have undergone multiple cycles of range expansion and contraction during glacial episodes of the Pleistocene (see Friesen *et al.*, 2007). Given the wide geographic range of *I. uriae* and the contrasting results we obtained in the Pacific and Atlantic oceanic basins, a phylogeographical approach at a world-wide scale that investigates the history of colonization and host race formation in this globally distributed parasite could provide important elements for understanding the factors that have shaped the regional population structure of this tick.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Between-group analysis of North Pacific *Ixodes uriae* populations, excluding ticks from Triangle Island.

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BIOSKETCH

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Author contributions: M.D., T.B. and K.D.M. conceived the study. M.D. carried out the lab work and most of the analyses with the help of F.K. Tick collections were performed by E.G.D., A.S.K., J.M.H., T.B. and K.D.M. All authors contributed to writing the manuscript.

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