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Body size and shape evolution in host races of the tick *Ixodes uriae*

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The tick *Ixodes uriae* is a common ectoparasite of seabirds, and is widely distributed across the circumpolar regions of both hemispheres. Previous work demonstrated the existence of genetically distinct host races of this ectoparasite, occurring across its current range. The objective of the present study was to examine whether these host races have evolved measurable morphological differences. We measured a set of morphological variables on 255 non-engorged ticks (nymphs and adults) collected from three sympatrically occurring host species in the North Atlantic. Genotyping at eight microsatellite markers enabled us to analyse the relationship between patterns of morphological and neutral genetic variation. Multivariate analyses showed that most morphological variation was associated with size differences among tick individuals. Body size differed among races, but only in adult life stages. A linear discriminant analysis based on shape variation revealed three distinct morphological clusters corresponding to the three tick host races. These results, along with correlated patterns of host-related genetic variation, suggest that differences among host-related groups are not simply the result of phenotypic plasticity or drift, but rather reflect host-associated adaptations. Experimental work and observations across the range of *I. uriae* will now be required to test the genetic basis and adaptive nature of morphological differences. *Biological Journal of the Linnean Society*, 2012, ••, ••–••

ADDITIONAL KEYWORDS: adaptation – bird – morphometry – parasite – sympatric speciation.

INTRODUCTION

Molecular genetic studies have revealed an increasing number of cryptic parasite lineages that are genetically divergent, but considered to be morphologically indistinguishable (Bickford *et al*., 2007). A key mechanism driving the evolution of such cryptic diversity is the adaptation of parasites to different host habitats or resources (De Meeûs, Michalakis & Renaud, 1998). However, parasite adaptation often involves morphological changes (Poulin, 2007), thus begging the question of whether these lineages are indeed cryptic. Direct evidence for an association between morphological variation and the diversification process is provided by a number of studies on phytophagous insects (e.g. Schmidt, Walter & Moore, 2000; Pappers *et al*., 2002; Svensson, Althoff & Pellmyr, 2005), but remains scarce among other groups of parasites (e.g. Huyse & Volckaert, 2002; Klimov, Bochkov & OConnor, 2006; Malenke, Johnson & Clayton, 2009). Understanding the frequency and nature of the morphological changes that accompany host-associated divergence are essential if we want to better comprehend the factors that favour parasite diversification.

With the increasing popularity of molecular methods, morphological approaches have been overshadowed by population genetics studies, thereby

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overlooking potentially valuable information on the processes driving diversification. Indeed, changes in the morphology of closely related species can provide key information on the selection pressures that may act in divergence (e.g. Smith *et al*., 2011). Although advances in multivariate methods and geometric morphometrics have improved our ability to discriminate subtle morphological differences, few studies have tried to determine the extent to which host-related genetic differentiation is associated with morphometric variation (e.g. Edwards & Labhart, 2000; Diegisser *et al*., 2004; Svensson *et al*., 2005). It is thus of particular interest to assess morphological variation in conjunction with previously identified host-related genetic units (e.g. host races).

The tick *Ixodes uriae* (Acari: Ixodidae) is an ideal model species for examining the role of morphology in the evolution of host-associated divergence. This seabird ectoparasite is widely distributed across the circumpolar regions of both hemispheres (Dietrich, Gómez-Díaz & McCoy, 2011). Most of the seabird host species are colonial, and breed in dense and temporally predictable colonies, that can be either monospecific or heterospecific (several sympatric species) in composition (Coulson, 2001). These potential hosts include a great diversity of species (more than 60), which may impose different selection pressures in relation to differences in their timing of reproduction, habitat use, physiology, and immune response. Previous genetic studies have indeed shown that gene flow between ticks of different sympatric host species is strongly restricted, supporting the existence of distinct host races, and that these host-associated groups have evolved recurrently across the global distribution of the parasite (McCoy *et al*., 2001, 2005; Kempf *et al*., 2009; Dietrich *et al*., 2012).

However, even if host race formation is a general pattern across the range of *I. uriae*, it does not imply that morphological changes have accompanied genetic divergence. Indeed, *I. uriae* host races have to date been considered to be cryptic, as no obvious morphological differences have been reported. On the one hand, the extreme environmental conditions that *I. uriae* encounters in polar regions might impose stabilizing selection on morphology, reducing or eliminating the morphological changes that can accompany speciation (Bickford *et al*., 2007). Alternatively, host-race formation of *I. uriae* is relatively recent in North Atlantic colonies (Kempf *et al*., 2009), and changes in morphological traits post-isolation may not have occurred or be obvious yet.

Here we examine whether morphometric differences have evolved in the host races of the tick *I. uriae*. As the optimal morphology for a particular environment may vary throughout the course of the growth and development of an organism, we might expect stage-specific patterns of host-related morphological variation in *I. uriae* (i.e. during its life cycle, *I. uriae* goes through a larval and nymphal instar before reaching the adult stage). Changes in optimal size and shape over an individual's lifetime may also differ between males and females, given their distinct roles in mating and reproduction (Fairbairn, 1997), and the fact that males do not take a blood meal in the adult stage. In this way, gender-specific patterns of host-related morphological variation could also be expected. With these aspects in mind, our study focused on answering the following questions: (1) do *I. uriae* host races differ in subtle morphological traits; (2) does this morphological variation vary according to sex and life stage of the tick; and (3) can the morphological differences be associated with the degree of genetic isolation among host races? We analysed ticks collected from three sympatric seabird host species breeding in a heterospecific seabird colony in the North Atlantic. We measured a set of morphological variables and performed multivariate analyses based on size and shape data. Genotypic data at eight microsatellite markers were obtained from the same tick individuals to analyse the relationship between morphological and neutral genetic variation. Patterns resulting from host-related variation in morphological traits and genetic markers are discussed in relation to the insights they can provide on the factors driving population differentiation, and ultimately speciation, in *I. uriae*.

MATERIAL AND METHODS

Sampling design

The study was based on *I. uriae* tick samples (larvae and nymphs) collected in 2009 on Hornøya, an island in northern Norway (70°22′N, 31°10′E). We collected ticks from three sympatric seabird host species: the black-legged kittiwake (KT) *Rissa tridactyla*, the common guillemot (CG) *Uria aalge*, and the Atlantic puffin (PF) *Fratercula arctica*. To ensure that ticks sampled from CG and PF were at complete repletion, we collected them in the environment where the birds were nesting after they had dropped off of their host. It was difficult to collect KT ticks from the off-host environment because after repletion ticks return deep into cracks of the cliff face. For this reason, we used KT ticks collected directly from the host (nestlings) during the last stages of engorgement (Table 1). Collected ticks were kept alive in the laboratory at 4 °C and high relative humidity (80–90%). After moulting, 255 non-engorged ticks (female adult, male adult, and nymphs) were preserved in 70% ethanol and used for morphological measurements. The use of

Table 1. Sample sizes for *Ixodes uriae* ticks used in the study

	Females	Males	Nymphs	Total
KT	30	32	21	83
PF	20	32	30	82
CG	30	30	30	90
Total	80	94	81	255

Host species: black-legged kittiwake (KT; *Rissa tridactyla*), the common guillemot (CG; *Uria aalge*), and the Atlantic puffin (PF; *Fratercula arctica*).

non-engorged ticks for the measurements allowed us to rule out possible morphological distortion from the blood meal.

Morphological measurements

Ticks were photographed with a digital camera fitted to a Motic SMZ-140 stereomicroscope and digitized with the MOTIC IMAGE PLUS 2.0 software. Ticks were placed in the centre of the visual field to reduce the risk of optical distortion, and only one side of the body (the right side) was used to avoid variation from patterns of asymmetry. To reduce potential bias, all measurements were performed without reference to the tick's origin and were carried out by one individual (M. Dietrich). In total, 31 morphological variables were measured for males and females, and 29 morphological variables were measured for nymphs (Fig. S1; Table S1, for abbreviations), including the majority of those used in previous morphological studies on *Ixodes* ticks (e.g. Hutcheson & Oliver, 1998). Variables related to feeding (mouth appendices), locomotion, and reproduction (legs and position of the genital pore) were also measured, as such variables have been shown to be under strong selection among host races of phytophagous insects (e.g. Pappers *et al*., 2002; Diegisser *et al*., 2004). In a few cases, it was not possible to measure certain variables because of tick malformations or problems with tick preservation. We thus had some missing data in our data set. Voucher specimens of each tick stage were deposited at the US National Tick Collection, Georgia Southern University, Statesboro, GA, USA (USNTC RML accession numbers: 124422–124441).

Genotyping

After morphometric measures were taken, all ticks were genotyped at eight previously described microsatellite loci (i.e. T3, T5, T22, T35, T38, T39, T44, and T47; McCoy & Tirard, 2000). Full details on DNA extraction, polymerase chain reaction (PCR), and genotyping procedures can be found in Kempf *et al*. (2009). Genotypes were visualized using an ABI PRISM 3130xl Genetic Analyser, and allele sizes were assigned using GeneMapper 4 (Applied Biosystems, Foster City, CA, USA).

Statistical analysis

Morphological variation

Separate analyses were conducted for individuals of each life stage (females, males and nymphs). Variables with more than 10% missing values were removed from the data set (see Table S1). For the remaining variables, the few missing data were replaced by values obtained via a linear regression, where the predictor variable was the idiosoma length for females and nymphs, and the idiosoma width for males (i.e. variables with no missing data). Coefficients of determination (R^2) from these regressions ranged from 0.01 to 0.83. Prior to analyses, raw measurements were log-transformed to better meet the assumptions of normality and homogeneity of variance. This raw data set (i.e. log-raw data) includes both size and shape variation. To investigate size variation, we calculated the geometric mean of each individual (i.e. log-size data) and differences among host races were determined by ANOVA and Tukey's honestly significant difference (HSD) post-hoc tests. In order to build shape variables (i.e. log-shape data), measurements of each variable were regressed on the geometric mean for each individual using least-squares linear regression. Residuals from these regressions are by construction orthogonal to size. They therefore reflect 'size-corrected' measurements and were used as shape variables in the remaining analyses.

Morphological variables were first analysed with principal component analyses (PCAs). PCAs were conducted on variance–covariance matrices of both log-raw and log-shape variables to determine to what extent overall differences among individuals could be attributed to a combination of size and shape versus shape only (Darroch & Mosimann, 1985). We then retained the components that explained at least 70% of the total variance of log-shape variables and used them in a multivariate analysis of variance (MANOVA) to test the significance of pairwise differentiation among host races.

A linear discriminant analysis (LDA) was employed on log-shape data to investigate the ability to discriminate body shape among a priori host races (Manly, 2005). The LDA method works by finding the linear combination of variables that display the largest variance between groups relative to the variance within the groups (Quinn & Keough, 2002). The minimum volume ellipsoid (MVE) estimator of the

centroid and covariance matrix was used, and prior probabilities for the groups were assumed to be equal (Venables & Ripley, 2002). Posterior probabilities were used to examine the correct classification of individual ticks with each host race.

The contribution of the different morphological variables to the observed discrimination was determined by selecting those variables with the highest linear correlation coefficients on the first and second discriminant functions (i.e. LDA1 and LDA2). Patterns of variation among host races of the most discriminating variables were visualized by plotting the median values of the log-shape measurements, and significant differences between host races were tested using ANOVA and Tukey's HSD post-hoc tests. All statistical analyses were performed with R software (R-Developement Core Team, 2006) using the ade4 (Chessel, Dufour & Thioulouse, 2004), MASS (Venables & Ripley, 2002), and FactoMineR (Lê, Josse & Husson, 2008) packages.

Neutral genetic variation

To assess the independence of the markers employed, linkage disequilibrium was tested between all pairs of loci. Deviations from Hardy–Weinberg proportions (HWPs) were investigated within each host race, by calculating Weir & Cockerham's (1984) unbiased estimator of Wright's inbreeding coefficient of an individual, relative to the subpopulation, F_{IS} (Wright, 1965). The significance of this estimator was assessed by randomizing alleles among individuals within samples (480 permutations). These computations were performed with the software FSTAT 2.9.3.2 (Goudet, 2002).

To estimate tick genetic structure among host species, we estimated pairwise differentiation among host races using Weir & Cockerham's (1984) estimator of Wright's effect of subpopulations, compared with the total population, F_{ST} (Wright, 1965). The significance of the estimator was assessed using 60 permutations of individuals among populations (Goudet *et al*., 1996). To assess the robustness of host races, we then calculated the percentage of correct assignments of individuals to their host race of origin using the Bayesian method of Rannala & Mountain (1997) in GeneClass 2.0 (Cornuet *et al*., 1999).

For each life stage, we wanted to determine whether there was a direct relationship between morphological and genetic distances among individuals. This relationship was tested using distance matrices computed for individuals for both morphological and microsatellite data. The matrix of morphological distances was calculated based on Euclidean distance. The matrix of genetic distances was computed using the chord distance (Cavalli-Sforza & Edwards, 1967). A Mantel test (Mantel, 1967) was then performed between the two matrices with 1000 permutations using the package ade4 in R.

RESULTS

Morphological variation

Log-raw data

The PCAs on log-raw variables resulted in two principal components that accounted, respectively, for 70, 73, and 51% of the variance of female, male, and nymphal data (Fig. S2). The first principal component PCA1 (63, 66, and 41% of the total variance) showed 29 (94%), 28 (93%), and 18 variables (69%) with high or moderate positive loadings (≥ 0.6) (Table S1). The large number of variables involved suggests that PCA1 was mainly associated with overall size. Indeed, the standardized variable scatterplots showed that log-raw measurements contained a great proportion of size variation among tick individuals (Fig. S2). Only partial separation among tick races occurred when visualizing PCA1 and PCA2, and no further separation occurred with subsequent components. It is interesting to note that, for each tick life stage, the highest variance along the PCA1 axis was observed among KT tick individuals (Fig. S2).

Log-size data

The size analyses (ANOVA) based on the geometric mean of morphological variables for each individual tick showed significant differences among host races for female ($F_{2,77} = 0.211$, $P < 0.001$, Fig. 1A) and male ticks ($F_{2,91} = 0.474$, $P < 0.001$, Fig. 1B), but not for nymphs ($F_{2,78} = 0.011$, $P = 0.760$, Fig. 1C). In particular, PF adult ticks were larger in size than the two other races (Fig. 1A, B). As noted for the log-raw data, KT ticks exhibited the highest variation in size across the three life stages.

Log-shape data

The first two PCA components of log-shape variables accounted for 29, 33, and 27% of the total variance for females, males, and nymphs, respectively (Fig. S3). These values are lower than the corresponding values in the log-raw data PCA because that analysis was dominated by a 'size axis' strongly correlated with the first component. The combination of PCA1 and PCA2 of log-shape variables separated host races to the same degree as log-raw data (Fig. S3). However, in this case, fewer variables (seven for females; three for males and nymphs) had high loading coefficients (≥ 0.6) (see correlation circles in Fig. S3; Table S1). Moreover, respective PCA1 loadings in both log-raw and log-shape analyses were not correlated (Pearson's correlation: $r_F = -0.131$, $r_M = 0.062$, $r_N = -0.132$;

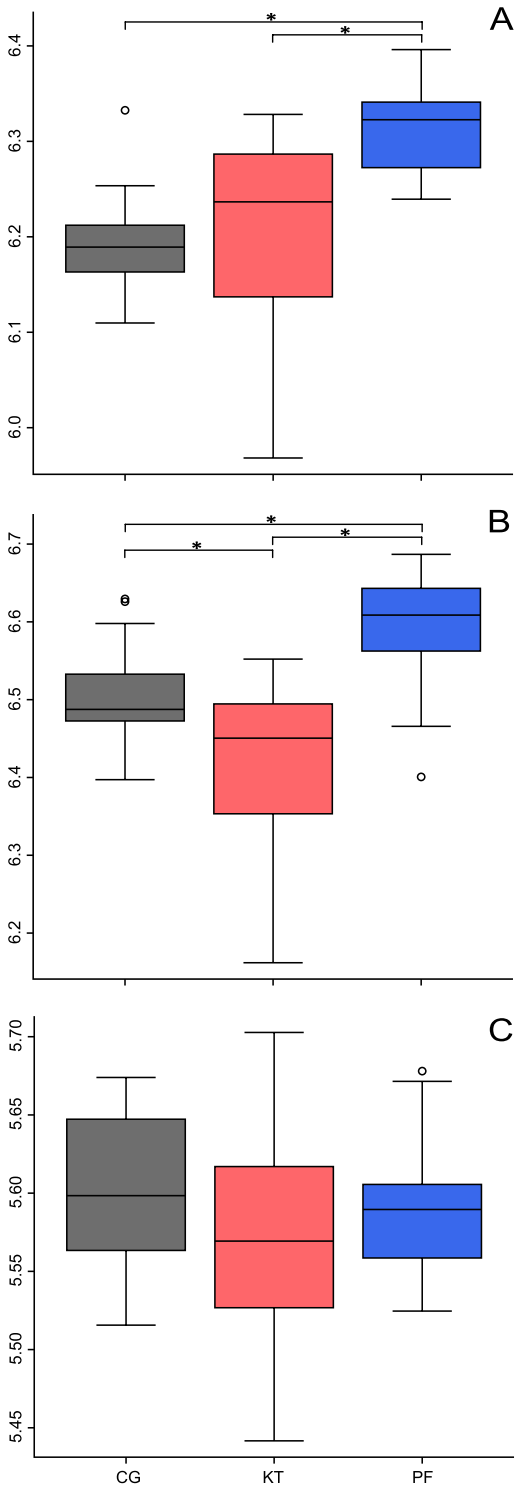

Figure 1. Size variation among *Ixodes uriae* host races for (A) females, (B) males, and (C) nymphs. The size of tick individuals was calculated as the geometric mean of all log-transformed variables. Median values, and the lower and upper quartiles, are shown. *Significant pairwise differences (Tukey's honestly significant difference test, $P < 0.05$). See the legend of Table 1 for host race labels.

Table 2. MANOVA results (Pillai's statistic) based on the principal components of the PCA on log-shape data

	Females	Males	Nymphs
CG –PF	0.703***	0.799***	0.700***
CG–KT	0.655***	0.712***	0.623***
KT–PF	0.440**	0.265*	0.711***

Analyses were conducted separately for each tick life stage. P values are: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. See Table 1 for host species labels.

$P \geq 0.1$), demonstrating that shape results are independent of size.

From the PCA of log-shape data, we extracted the principal components that explained at least 70% of the total variance, and used them in the MANOVA procedure (i.e. ten components for females, nine for males, and ten for nymphs). For all tick stages, highly significant pairwise differences among host races were observed, except between males of KT and PF ticks (Table 2).

The LDA on log-shape data showed three morphologically distinct clusters for all tick life stages, corresponding to each host race (Fig. 2). For females and males, the separation was mainly based on the first linear discriminant axis (LDA1) that explained most of the variance (i.e. 82 and 85%, respectively). For males, the separation between KT and PF ticks was reduced (Fig. 2B). For nymphs, a more even combination of the first and second linear discriminant axes contributed to the separation of the three host races (LDA1, 67%; LDA2, 33%; Fig. 2C). Finally, the overall average percentage of correctly classified individuals of females, males, and nymphs was relatively high (i.e. 89.0%; Table 3). The percentage of correct assignments using morphological variables was particularly high for CG ticks. In contrast, there was a relatively high number of misassignments between KT and PF ticks (Table 3).

Shape variation among host races is characterized predominantly by changes in the intercoxae IV width (ICW) and spiraculate plate length (SPL) for females, the ICW and scutum width (SCW) for males, and the anus–head basis length (AHL) and interspiraculate plate width (IPW) for nymphs (i.e. variables with the highest LDA1 and LDA2 correlation coefficients in Table 4). These characters are mostly associated with the tick body form, and thus suggest that each host race is characterized by a different body shape. Conversely, variables related to feeding (palp length PAL and width PAW, chelicerae length CHL and width CHW, hypostome length HYL and width HYW), locomotion (S1L, T1L, S4L, and T4L), and reproduction (GHL and GAL) were less involved in the morphological discrimination of host races.

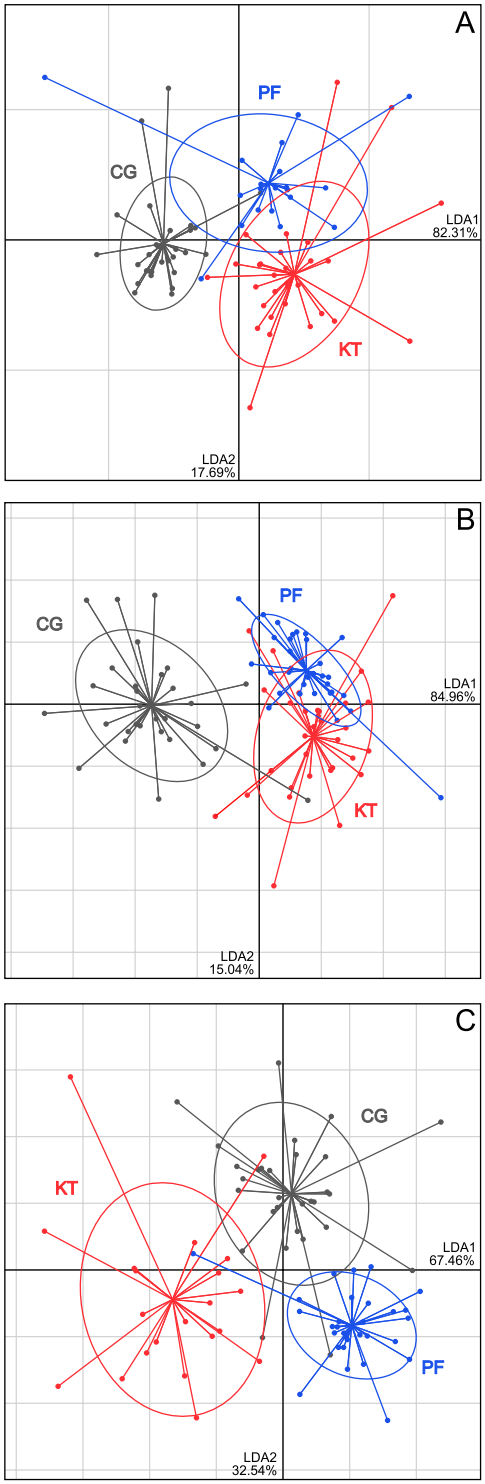

Figure 2. Scatter plots of the linear discriminant analysis based on morphological shape variables of different *Ixodes uriae* stages: A, females; B, males; and C, nymphs. Ellipses on scatter plots represent the distribution of 67% of individuals around the population barycentre. Each tick (dot) is connected to the barycentre of the group by a line. See the legend of Table 1 for host race labels.

Table 3. Percentage of tick assignments to different host-related groups based on morphological and microsatellite data

	Assigned to		
Host race	CG	KT	PF
Morphological data			
CG	**95.6 (1.9)**	1.1 (1.9)	3.3 (0.0)
KT	2.6 (2.4)	**84.0 (8.1)**	13.3 (8.6)
PF	3.8 (1.0)	7.9 (9.7)	**88.2 (9.4)**
Microsatellite data			
CG	**97.8**	1.1	1.1
KT	2.4	**91.6**	6.0
PF	0.0	1.2	**98.8**

For morphological data, assignment scores were computed for each host stage separately and then averaged. Standard errors are indicated in parentheses. See Table 1 for host species labels.

For female ticks, shape mainly differed between PF and CG ticks, with PF ticks having a relatively wider ICW but a shorter SPL than CG ticks (Fig. 3A, B). KT ticks showed an intermediate shape between those of the two other races. For males, the most discriminant variables (i.e. ICW and SCW) were both relative to the body width, and showed similar patterns of variation among host races as in females (Fig. 3C, data only shown for ICW). When we plotted a character more related to body length, i.e. the length of the coxa II (C2L, with the third highest correlation coefficient on LDA2), we observed the same pattern of variation as for SPL in females (Fig. 3D). For nymphs, IPW showed a similar pattern of variation as ICW in adults (Fig. 3E). However, AHL was longer in KT ticks compared with the two other host races (Fig. 3F). Therefore, to summarize across life stages, after correcting for overall size, PF ticks tend to be wider and shorter compared with CG ticks, with KT ticks being intermediate in shape.

Genetic variation

All possible pairs of loci were in linkage equilibrium. Significant departures from HWP were detected for CG ticks, and were caused by two loci (i.e. T39 and T47) that were found to have heterozygote deficits in a previous study in the North Atlantic (Kempf *et al.*, 2009). We ran the genetic analyses with and without these two loci, but results were unchanged, so we present the results with the eight loci.

Values of pairwise F_{ST} showed that host-related tick groups were highly divergent and confirmed the presence of host races ($F_{ST\ CG–PF} = 0.32$, $F_{ST\ CG–KT} = 0.20$,

Table 4. Correlation coefficients from the linear discriminant analysis of log-shape data

		Females		Males		Nymphs	
Variables	Code	LDA1	LDA2	LDA1	LDA2	LDA1	LDA2
Idiosoma length	IDL	0.17	–0.33	–0.06	0.21	–0.54	0.03
Idiosoma width	IDW	0.39	0.50	0.28	0.48	–0.07	–0.45
Basis capitulum length	BCL	0.34	0.30	0.21	–0.12	removed	
Basis capitulum width	BCW	–0.18	0.30	0.22	0.14	0.09	0.28
Palp length	PAL	0.04	0.11	–0.18	0.32	0.24	0.15
Palp width	PAW	–0.22	0.23	0.02	–0.02	0.24	–0.44
Chelicerae length	CHL	0.04	–0.12	NA		removed	
Chelicerae width	CHW	–0.04	0.28	NA		removed	
Scutum length	SCL	–0.27	–0.47	0.06	0.15	0.22	0.54
Scutum width	SCW	–0.23	0.52	0.38	**0.50**	–0.07	–0.38
Genital pore–head basis length	GHL	0.16	–0.17	–0.21	–0.18	NA	
Genital pore–anus length	GAL	0.30	–0.37	0.10	0.34	NA	
Anus–back length	ABL	–0.14	–0.10	–0.04	–0.08	–0.28	0.05
Spiracular plate length	SPL	–0.09	**–0.59**	removed		–0.26	0.64
Interspiraculate plate width	IPW	0.58	0.37	0.56	0.18	0.18	**–0.73**
Intercoxae IV width	ICW	**0.63**	0.20	**0.79**	0.06	–0.02	–0.44
Anal plate width	APW	0.62	0.32	0.48	0.12	NA	
Anal plate length	APL	NA		0.23	0.06	NA	
Hypostome length	HYL	–0.07	0.26	NA		0.38	–0.01
Hypostome width	HYW	–0.46	0.15	NA		0.13	–0.40
Coxa-I length	C1L	–0.28	0.02	–0.08	–0.17	0.52	0.13
Coxa-I width	C1W	–0.31	–0.04	–0.44	0.03	0.04	0.18
Coxa-II length	C2L	–0.23	–0.18	–0.35	–0.41	–0.03	0.18
Coxa-II width	C2W	–0.15	–0.04	–0.42	0.07	–0.46	0.01
Coxa-III length	C3L	–0.35	0.29	–0.51	–0.34	–0.06	0.07
Coxa-III width	C3W	–0.22	0.10	–0.32	–0.08	–0.40	0.01
Coxa-IV length	C4L	0.15	0.25	–0.37	–0.24	–0.07	0.14
Coxa-IV width	C4W	–0.19	0.03	–0.30	–0.09	–0.28	0.05
Tarsus-I length	S1L	–0.03	–0.13	–0.52	0.17	0.04	0.39
Tibia-I length	T1L	–0.17	–0.41	–0.06	0.11	0.15	0.33
Tarsus-IV length	S4L	–0.27	–0.43	–0.26	–0.24	0.02	0.10
Tibia-IV length	T4L	–0.21	–0.58	–0.01	–0.36	0.35	0.33
Interscapulae length	ISL	NA		< 0.01	0.39	NA	
Adanal plate length	DPL	NA		0.10	0.12	NA	
Adanal plate width	DPW	NA		0.77	0.16	NA	
Anus–head basis length	AHL	NA		NA		**–0.64**	–0.17
% total of variance		82.31	17.69	84.96	15.04	67.46	32.54

The highest coefficients of both first (LDA1) and second (LDA2) discriminant functions are set in bold. 'NA' means the variable was not measured, whereas 'removed' refers to situations where the variable was measured but then removed from the analysis because of missing data.

$F_{ST\ KT–PF} = 0.09$, $P < 0.02$). Assignment tests revealed a high probability of correctly assigning ticks to their host race of origin (Table 3). The PF ticks showed the highest score, with more than 98% correctly assigned individuals, and KT ticks showed the lowest score (i.e. 91.6%; Table 3). Finally, there was a significant correlation between morphological (log-shape) and microsatellite data, as shown by the positive and significant values of the Mantel tests for the three tick life stages (females, $r = 0.11$; males, $r = 0.14$; nymphs, $r = 0.18$, $P < 0.01$).

DISCUSSION

The results of this study revealed the existence of strong size variation in the morphology of *I. uriae*. First, size variation differed greatly among individual ticks. This may be explained by phenotypic plasticity

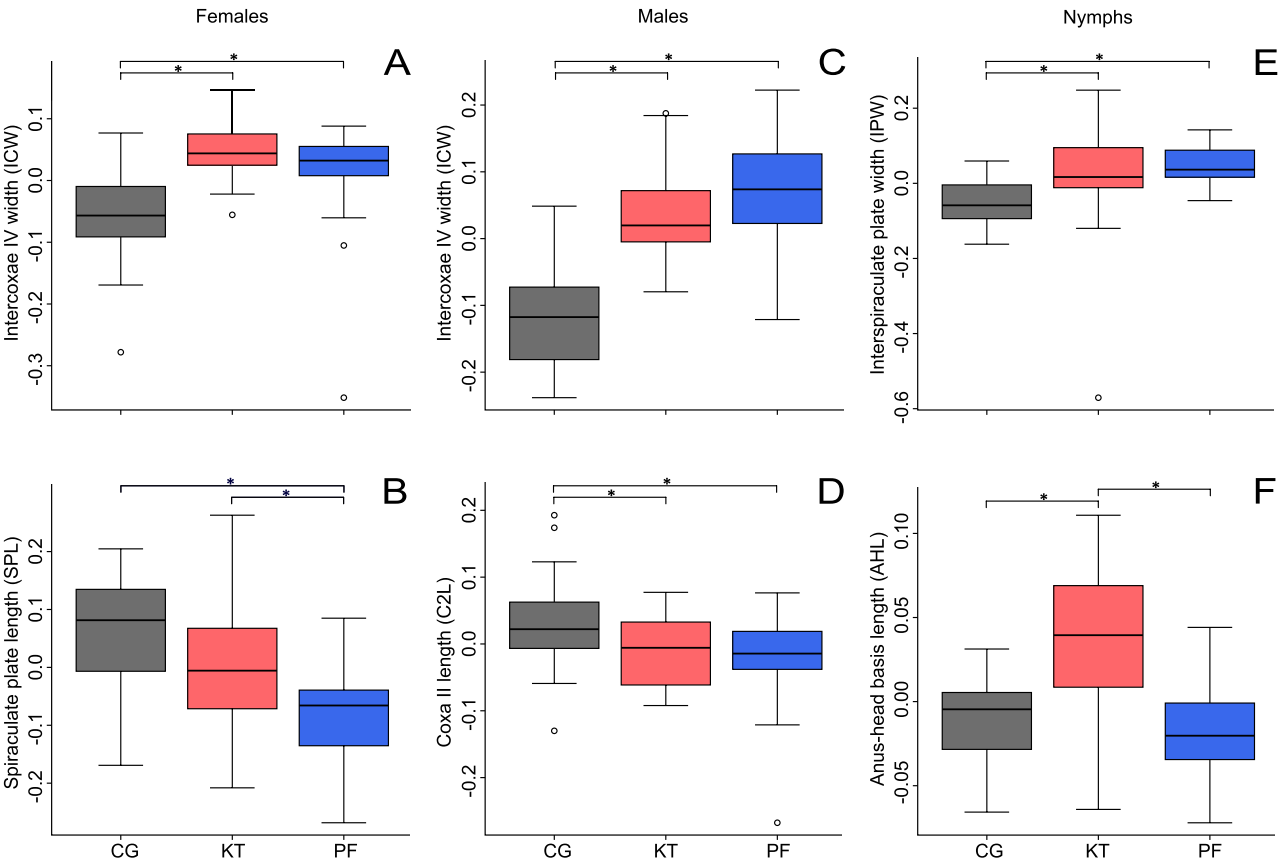

Figure 3. Comparison of morphometric variation among *Ixodes uriae* host races for (A, B) females, (C, D) males, and (E, F) nymphs. *Significant pairwise differences (Tukey's honestly significant difference test, $P < 0.05$). All measurements are expressed as relative measurements with no units (log-shape transformed). Median values, and the lower and upper quartiles, are shown. See the legend of Table 1 for host race labels.

associated with variation in engorgement success. Indeed, a relationship between feeding and tick body size has been observed in several other tick species. For example, incomplete feeding of immature stages has been shown to result in smaller adults in *Ixodes ricinus*, *Dermacentor variabilis*, *Hyalomma asiaticum*, and *Amblyomma americanum* (Amin & Sonenshine, 1970; Koch, 1986). Individual variation of the seabird host, i.e. in physiology, immunity, etc., could lead to differential engorgement success among ticks, and may explain some of the variation in tick size that we observed. The sampling method we used for KT ticks may also have contributed to the high size variation in this group; KT ticks were indeed collected directly from the host during the last stage of engorgement, and this may have led to a slightly reduced engorgement size and smaller tick size after moult for some individuals.

Tick body size also varied among host races within the studied colony, but to a lesser extent than among individuals, and only in adults. Adult PF ticks were always larger than the other races. In contrast, female CG ticks were the smallest, and differences between CG and KT ticks were sex dependent. As for individual size variation, size differences among host races may reflect differences in the nutritional value and composition of the host blood, and thus be a consequence of phenotypic plasticity (Galun, 1976). These differences may also result from the evolution of different capacities of each tick race to efficiently use host blood, independent of its composition. In particular, the larger size of PF ticks might reflect the greater adaptation of this race to exploit puffin blood, and could be linked to its higher specificity (McCoy *et al.*, 2005; Kempf *et al.*, 2009; see below). Indeed, blood digestion abilities have been shown to be linked to host specificity in other haematophagous parasites (e.g. Willadsen, Kemp & McKenna, 1984; Krasnov *et al.*, 2003; Sarfati *et al.*, 2005). This type of adaptation could be especially important in females, as blood digestion efficiency should increase repletion size, and thus have important consequences for fecundity; the size of females is a major component affecting egg numbers (Sonenshine, 1991). Based on this

hypothesis, we suggest that size variation among *I. uriae* host races may be at least partially genetically-based. Artificial feeding experiments will now be required to test this hypothesis.

When considering the shape of ticks, host races were clearly distinguishable, with a relatively high classification success rate, varying between 84 and 96%. The results obtained with morphometric methods are remarkably coherent with those acquired using molecular markers (Table 3); morphometric and genetic data were correlated for the three life stages and showed similar patterns of divergence among tick host races. Indeed, KT and PF ticks showed a high number of morphological misassignments (frequently assigned to the other host), which corroborates the low genetic differentiation between these two host races. In contrast, we observed the greatest differentiation between CG and PF ticks in both data sets. This last observation echoes previous genetic results in North Atlantic and North Pacific colonies, where local divergence was always strongest between ticks from puffins and guillemots (McCoy *et al*., 2005; Kempf *et al*., 2009; Dietrich *et al*., 2012).

Host-related shape variation could simply be the consequence of 'morphological drift' (i.e. genetically-based changes that do not affect individual fitness), correlated with reduced gene flow among host-associated tick groups. However, there are several potential arguments against this hypothesis. First, *I. uriae* host races are thought to have evolved relatively recently (Kempf *et al*., 2009), and there is little empirical evidence from laboratory studies or natural populations that drift alone will result in significant morphological differentiation over short ecological time scales (Rice & Hostert, 1993). In addition, we would not expect drift to produce the coherent patterns of morphological differences among races and life stages that we observed in the present study. Such elements suggest that the variation we observed among host species may be, at least in part, the result of selection imposed by the different host species, selection that seems to be independent of phylogenetic relationships. Indeed, we observed stronger shape-related differences between CG and PF ticks compared with those with KT ticks, even though *U. aalge* and *F. arctica* belong to one bird lineage (Alcidae), and *R. tridactyla* belongs to another (Laridae). This stronger pattern between Alcid ticks could result from stronger divergent selection imposed by these hosts and/or a more ancient divergence event between ticks exploiting CG and PF hosts. Data from additional colonies, where host-specific tick races have evolved independently (Dietrich *et al*., 2012), would help shed light on the robustness of these alternative hypotheses.

A possible adaptive interpretation of the morphological differences among *I. uriae* host races would be some type of mechanical selection pressure imposed by the host. For example, structures that provide shelter against grooming (e.g. underneath feathers) may represent different selective environments on different host species (e.g. density of the plumage). Beak morphology could also be important as the beak plays an essential role in preening, the first line of defence against harmful ectoparasites (Clayton *et al*., 2010). In addition to preening themselves, seabirds may also benefit from preening by conspecifics, notably their partner (i.e. allopreening). For example, solitary Macaroni penguins (*Eudyptes chrysolophus*), which could only self-preen, had two to three times more *I. uriae* ticks than paired birds (Brooke, 1985). Thus, a bird's ability to initially remove ticks when implanted in the skin may exert strong selection pressure on tick morphology. Other studies have further demonstrated how beak morphology, such as the maxillary overhang, can constraint the evolution of bird–ectoparasite interactions (Moyer, Townsend Peterson & Clayton, 2002; Clayton *et al*., 2005; Bush *et al*., 2012). For example, Clayton *et al*. (1999) showed experimentally that preening in feral pigeons has an impact on louse body size, but unfortunately this study did not analyse associated changes in body shape. Beak morphology may thus represent an important factor in the evolution of *I. uriae* morphology, potentially acting on both the size and shape of the tick body. For example, we could expect host species with larger maxillary overhangs to yield larger ticks. A comparative study correlating the morphometric traits of the host's beak with tick body size and shape would enable us to test this hypothesis.

According to the preening-mediated selection hypothesis, it is important to note that the sampling method we used for KT ticks may have led to increased shape variability in this group. Indeed, as previously mentioned, KT ticks were directly collected from the bodies of kittiwake nestlings, and may not have been through a full bout of preening-mediated selection. In addition, KT ticks were collected on nestlings that may not be as efficient as adults in preening themselves. As a consequence, the shape differences between KT ticks and the other two races could have been blurred by this potential sampling bias, and may have led to the non-significant differences we observed for some pairwise comparisons of shape (Fig. 3). The differences we observed should therefore be considered as conservative.

Morphological differences among host races may also be favoured by differences in the breeding habitats of the host species. Indeed, environmental characteristics may play a role in the evolution of ticks, as

the off-host environment represents the major habitat during the life cycle, and ticks can be highly sensitive to microhabitat conditions (i.e. variation in temperature and humidity; Klompen *et al*., 1996). Among the different races of *I. uriae*, PF ticks live in the dirt burrow of their host, KT ticks return deep into the fissures of the cliff, close to the nest site, and CG ticks hide under surface rocks within the breeding area. Temperature and humidity could differ substantially among these microhabitats, and thus may impose selection pressure, favouring different physiological adaptations (e.g. water retention), which could then result in morphological differences among host races. Data on the environmental characteristics of these microhabitats are now required to test this hypothesis.

The host-related morphological variation present in *I. uriae* suggests that natural selection on morphological traits may play a role during host-race formation. Additional data (e.g. rearing experiments) will now be required to test the adaptive component of *I. uriae* morphological variation. One approach would be to attempt removing environmentally induced morphological variation by rearing ticks from the three host species under the same conditions and on the same host (i.e. a common garden experiment). If the resulting adults from each population fail to show significant differences in morphology, a genetic component may be discounted. A complementary experiment would be to carry out a fully reciprocal crossing scheme with different combinations of tick parents. If body size and shape have a heritable component, offspring morphology should be influenced by cross type (for an example see Pappers *et al*., 2002). Unfortunately, these approaches are not yet feasible for *I. uriae* as this species is difficult to rear under laboratory conditions because of its high specificity for seabirds.

It remains speculative whether selection for morphological traits leads to restricted gene flow, or whether host-associated morphological differences evolved after races were already partially isolated. In the latter case, a positive feedback loop could have occurred where restricted gene flow facilitated morphological divergence, which in turn further reduced gene flow, and so on (Hendry, Taylor & McPhail, 2002). In our study, characteristics related to reproduction were not differentiated among host races, suggesting that morphological divergence is not linked to reproductive isolation. However, subtle characters related to reproduction were not examined here, and may play a role. It is also possible that the global difference in body size and shape among host races represents a barrier for inter-race copulation, thus favouring pre-mating reproductive isolation. This kind of adaptive differentiation should strongly reduce gene flow between host types, and eventually lead to speciation. Such pre-mating barriers have been shown to occur, for example, in reptile ticks; differences in body size and shape block copulation attempts among different tick species because males are either too far forward, or too far back, for their hypostome to fit into the female genital aperture (Andrews, 1982). As mating in *I. uriae* occurs off-host, a laboratory experiment could be envisaged to quantify the extent to which ticks of different host races succeed in mating together, and thus test whether mating success is lower between ticks of different host races as a result of physical constraints.

Our study sampled one locality within the North Atlantic where *I. uriae* host race formation is thought to have occurred relatively recently (Kempf *et al*., 2009). However, *I. uriae* is widely distributed in both the Northern and Southern hemispheres, with strong genetic isolation between different ocean basins (i.e. North Atlantic versus North Pacific basins), and recurrent evolution of host races within each ocean basin (McCoy *et al*., 2005; Dietrich *et al*., 2012). Therefore, additional morphological data from ticks collected on the same host species but in other regions would enable us to test the recurrent nature of host-related morphological variation in *I. uriae*, and thus would help support or refute the hypothesis that host-related morphological variation is adaptive. Moreover, we might expect stronger differences in morphology among tick populations in regions where this tick has had a longer history (i.e. Southern hemisphere, the presumed origin of *I. uriae*). An investigation of *I. uriae* morphological variation at larger spatial scales is therefore needed to investigate the relative roles of geographic isolation, host species, and evolutionary time in shaping morphology in this tick. This type of study would also enable us to clarify the current status of *I. uriae* populations, i.e. host races or cryptic species, and help predict future patterns of diversification in this system in relation to the changing distributions of polar seabirds.

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REFERENCES

Amin OM, Sonenshine DE. 1970. Development of the American dog tick, *Dermacentor variabilis*, following partial feeding by immatures. *Annals of the Entomological Society of America* **63:** 128–133.

Andrews RH. 1982. Mating behaviour and reproductive isolation of three species of reptile ticks. *Animal Behaviour* **30:** 514–524.

Bickford D, Lohman DJ, Sodhi NS, Ng PKL, Meier R, Winker K, Ingram KK, Das I. 2007. Cryptic species as a window on diversity and conservation. *Trends in Ecology and Evolution* **22:** 148–155.

Brooke MdeL. 1985. The effect of allopreening on tick burdens of molting *Eudyptid* penguins. *The Auk* **102:** 893–895.

Bush SE, Villa SM, Boves TJ, Brewer D, Belthoff JR. 2012. Influence of bill and foot morphology on the ectoparasites of barn owls. *Journal of Parasitology* **98:** 256–261.

Cavalli-Sforza L, Edwards W. 1967. Phylogenetic analysis: models and estimation procedures. *Evolution* **21:** 550–570.

Chessel D, Dufour AB, Thioulouse J. 2004. The ade4 package – I: one-table methods. *R-News* **4:** 5–10.

Clayton DH, Koop JAH, Harbison CW, Moyer BR, Bush SE. 2010. How birds combat ectoparasites? *The Open Ornithology Journal* **3:** 41–71.

Clayton DH, Lee LLM, Tompkins DM, Brodie, ED III. 1999. Reciprocal natural selection on host-parasite phenotypes. *The American Naturalist* **154:** 261–270.

Clayton DH, Moyer BR, Bush SE, Jones TG, Gardiner DW, Rhodes BB, Goller F. 2005. Adaptive significance of avian beak morphology for ectoparasite control. *Proceedings of the Royal Society of London Series B* **272:** 811–817.

Cornuet JM, Piry S, Luikart G, Estoup A, Solignac M. 1999. New methods employing multilocus genotypes to select or exclude populations as origins of individuals. *Genetics* **153:** 1989–2000.

Coulson J. 2001. Colonial breeding in seabirds. In: Schreiber B, Nurger J, eds. *Biology of marine birds*. Boca Raton, FL: CRC Press, 87–113.

Darroch JN, Mosimann JE. 1985. Canonical and principal components of shape. *Biometrika* **72:** 241–252.

De Meeûs T, Michalakis Y, Renaud F. 1998. Santa rosalia revisited: or why are there so many kinds of parasites in the garden of earthly delights? *Parasitology Today* **14:** 10–13.

Diegisser T, Johannesen J, Lehr C, Seitz A. 2004. Genetic and morphological differentiation in *Tephritis bardanae* (Diptera: Tephritidae): evidence for host-race formation. *Journal of Evolutionary Biology* **17:** 83–93.

Dietrich M, Gómez-Díaz E, McCoy KD. 2011. Worldwide distribution and diversity of seabird ticks: implications for the ecology and epidemiology of tick-borne pathogens. *Vector-Borne and Zoonotic Diseases* **5:** 453–470.

Dietrich M, Kempf F, Gómez-Díaz E, Kitaysky AS, Hipfner JM, Boulinier T, McCoy KD. 2012. Inter-oceanic variation in patterns of host-associated divergence in a seabird ectoparasite. *Journal of Biogeography* **39:** 545–555.

Edwards DD, Labhart M. 2000. Genetic differences among host-associated populations of water mites (Acari: Unionicolidae: *Unionicola*): allozyme variation supports morphological differentiation. *Journal of Parasitology* **86:** 1008–1011.

Fairbairn DJ. 1997. Allometry for sexual size dimorphism: pattern and process in the coevolution of body size in males and females. *Annual Review of Ecology, Evolution and Systematics* **28:** 659–687.

Galun R. 1976. The physiology of haematophagous insect/animal host relationships. *Proceedings of the 15th International Congress on Entomology* 257–265.

Goudet J. 2002. *Fstat: a program to estimate and test gene diversities and fixation indices*, ver. 2.9.3.2. Lausanne: Institute of Ecology, University of Lausanne. Available at: http://www2.unil.ch/popgen/softwares/fstat.htm

Goudet J, Raymond M, de Meeûs T, Rousset F. 1996. Testing differentiation in diploid populations. *Genetics* **144:** 1933–1940.

Hendry AP, Taylor EB, McPhail JD. 2002. Adaptive divergence and the balance between selection and gene flow: lake and stream stickleback in the misty system. *Evolution* **56:** 1199–1216.

Hutcheson HJ, Oliver JHJ. 1998. The question of fluctuating asymmetry in the blacklegged tick *Ixodes scapularis* (Acari: Ixodidae). *Experimental and Applied Acarology* **22:** 51–60.

Huyse T, Volckaert FAM. 2002. Identification of a host-associated species complex using molecular and morphometric analyses, with the description of *Gyrodactylus rugiensoides* n. sp. (Gyrodactylidae, Monogena). *International Journal for Parasitology* **32:** 907–919.

Kempf F, Boulinier T, de Meeûs T, Arnathau C, McCoy KD. 2009. Recent evolution of host-associated divergence in the seabird tick *Ixodes uriae*. *Molecular Ecology* **18:** 4450–4462.

Klimov PB, Bochkov AV, OConnor BM. 2006. Host specificity and multivariate diagnostics of cryptic species in predacious cheyletid mites of the genus *Cheletophyes* (Acari: Cheyletidae) associated with large carpenter bees. *Biological Journal of the Linnean Society* **87:** 45–58.

Klompen JS, Black IV WC, Keirans JE, Oliver, JH Jr. 1996. Evolution of ticks. *Annual Review of Entomology* **41:** 141–161.

Koch HG. 1986. Development of the lone star tick, *Amblyomma americanum* (Acari: Ixodidae), from immatures of different engorgement weights. *Journal of the Kansas Entomology Society* **59:** 309–313.

Krasnov BR, Sarfati M, Arakelyan MS, Khokhlova IS, Burdelova NV, Degen AA. 2003. Host specificity

and foraging efficiency in blood-sucking parasite: feeding patterns of the flea *Parapulex chephrenis* on two species of desert rodents. *Parasitology Research* **90:** 393–399.

Lê S, Josse J, Husson F. 2008. FactomineR: an R package for multivariate analysis. *Journal of Statistical Software* **25:** 1–18.

Malenke JR, Johnson KP, Clayton DH. 2009. Host specialization differentiates cryptic species of feather-feeding lice. *Evolution* **63:** 1427–1438.

Manly BFJ. 2005. *Multivariate statistical methods: a primer*. Boca Raton, FL: Chapman and Hall/CRC Press.

Mantel N. 1967. The detection of disease clustering and a generalized regression approach. *Cancer Research* **27:** 209–220.

McCoy KD, Boulinier T, Tirard C, Michalakis Y. 2001. Host specificity of a generalist parasite: genetic evidence of sympatric host races in the seabird tick *Ixodes uriae*. *Journal of Evolutionary Biology* **14:** 395–405.

McCoy KD, Chapuis E, Tirard C, Boulinier T, Michalakis Y, Le Bohec C, Le Maho Y, Gauthier-Clerc M. 2005. Recurrent evolution of host-specialized races in a globally distributed parasite. *Proceedings of the Royal Society of London Series B* **272:** 2389–2395.

McCoy KD, Tirard C. 2000. Isolation and characterization of microsatellites in the seabird ectoparasite *Ixodes uriae*. *Molecular Ecology* **9:** 2213–2214.

Moyer BR, Townsend Peterson A, Clayton DH. 2002. Influence of bill shape on ectoparasite load in western scrub-jays. *The Condor* **104:** 675–678.

Pappers SM, van der Velde G, Ouborg NJ, van Groenendael JM. 2002. Genetically based polymorphisms in morphology and life history associated with putative host races of the water lily leaf beetle, *Galerucella nymphaeae*. *Evolution* **56:** 1610–1621.

Poulin R. 2007. *Evolutionary ecology of parasites*, 2nd edn. Princeton, NJ: Princeton University Press.

Quinn GP, Keough MJ. 2002. *Experimental design and data analysis for biologists*. Cambridge: Cambridge University Press.

Rannala B, Mountain JL. 1997. Detecting immigration by using multilocus genotypes. *Proceedings of the National Academy of Sciences of the United States of America* **94:** 9197–9221.

R-Developement Core Team. 2006. *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.

Rice W, Hostert E. 1993. Laboratory experiments on speciation: what have we learned in 40 years? *Evolution* **47:** 1637–1653.

Sarfati M, Krasnov BR, Ghazaryan L, Khokhlova IS, Fielden LJ, Degen AA. 2005. Energy costs of blood digestion in a host-specific haematophagous parasite. *The Journal of Experimental Biology* **208:** 2489–2496.

Schmidt S, Walter GH, Moore CJ. 2000. Host plant adaptations in myrtaceous-feeding pergid sawflies: essential oils and the morphology and behaviour of *Pergagrapta* larvae (Hymenoptera, Symphyta, Pergidae). *Biological Journal of the Linnean Society* **70:** 15–26.

Smith TB, Thomassen HA, Freedman AH, Sehgal RNM, Buermann W, Saatchi S, Pollinger J, Milá B, Pires D, Valkiunas G, Wayne RK. 2011. Patterns of divergence in the olive sunbird *Cyanomitra olivacea* (Aves: Nectariniidae) across the African rainforest-savanna ecotone. *Biological Journal of the Linnean Society* **103:** 821–835.

Sonenshine DE. 1991. *Biology of ticks*, Vol. 1. New York, NY: Oxford Univ. Press.

Svensson GP, Althoff DM, Pellmyr O. 2005. Replicated host-race formation in bogus yucca moths: genetic and ecological divergence of *Prodoxus quinquepunctellus* on yucca hosts. *Evolutionary Ecology Research* **7:** 1139–1151.

Venables WN, Ripley BD. 2002. *Modern applied statistics with S-plus*, 4th edn. New York, NY: Springer.

Weir BS, Cockerham CC. 1984. Estimating f-statistics for the analysis of population structure. *Evolution* **38:** 1358–1370.

Willadsen P, Kemp DH, McKenna RV. 1984. Bloodmeal ingestion and utilization as a component of host specificity in the tick, *Boophilus microplus*. *Parasitology Research* **70:** 415–420.

Wright S. 1965. The interpretation of population structure by f-statistics with special regard to systems of mating. *Evolution* **19:** 395–420.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article:

Figure S1. Distances used in the morphometric analysis of *Ixodes uriae*. See Table S1 for variable abbreviations. Only female and male adults are presented. For nymph variables, see details in Table S1.

Figure S2. Principal component analysis scatter plots and variable correlation circles of log-raw data for (A) female, (B) male, and (C) nymphal ticks.

Figure S3. Principal component analysis scatter plots and variable correlation circles of log-shape data for (A) female, (B) male, and (C) nymphal ticks. See Figure S2 legend for details.

Table S1. PCA1 loadings of the principal component analysis of log-raw (PCA1-r) and log-shape data (PCA1-s).