Assortative Pairing in *Ixodes ricinus* (Acari: Ixodidae), the European Vector of Lyme Borreliosis

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J. Med. Entomol. 46(3): 471–474 (2009)

ABSTRACT In sexual organisms, the way in which gametes associate can greatly influence the maintenance of genetic variation, the structure of this variation in space, and ultimately organismal evolution. Based on patterns of genetic structure previously found, we explicitly tested whether adults of the sheep tick *Ixodes ricinus* pair according to their genetic relatedness. We sampled tick pairs from the vegetation in four natural populations and genotyped individual ticks at seven microsatellite loci. Based on this data, we observed highly significant assortative mating in two of the four locations, a pattern that could not be accounted for by a spatial autocorrelation in the distribution of related ticks. One explanation for these observations may be the existence of local host associations that develop independently in different populations. Assortative mating in *I. ricinus* will have clear consequences for its population dynamics and, through processes of adaptation and transmission, may significantly alter the epidemiological patterns of the pathogens it carries, including the Lyme disease agent *Borrelia burgdorferi* s.l. Future tests will now be required to examine the mechanisms leading to this pattern and its epidemiological consequences.

KEY WORDS assortative mating, genetic structure, host-associated divergence, vector-borne disease

Ticks are hematophagous ectoparasites and are second only to mosquitoes as major vectors of human and livestock disease (Troughton and Levin 2007). Despite their importance, we know relatively little about how their populations function under natural conditions or how different dynamics may alter pathogen transmission cycles. One tick of particular importance in Europe is *Ixodes ricinus*, a tick that transmits a variety of pathogenic agents, including Lyme disease bacteria *Borrelia burgdorferi* s.l.

In an innovating paper on *I. ricinus* population genetics, De Meeûs et al. (2002) found large heterozygote deficits that could not be entirely explained by technical biases in the data (De Meeûs et al. 2002, 2004). One hypothesis to explain these biases is the mode of pair formation. In particular, if these ticks show assortative mating for some genetically determined trait (i.e., a tendency to mate with similar individuals), within-population structure could occur, resulting in a dramatic decrease in population heterozygosity (i.e., inbred mating system). Such a mating pattern will have important consequences for the population dynamics of both the tick and the pathogens it carries because it will influence the maintenance of genetic variation, the structure of this variation in space, and ultimately organismal evolution (Kirkpatrick and Ravigné 2002, Bolnick and Fitzpatrick 2007).

In this paper, we test for a departure from pangamy (i.e., random pairing of sexual partners) in *I. ricinus* by examining mating patterns in four natural *I. ricinus* populations. In this species, paired adult individuals can be easily collected during their questing phase when they cluster on the tips of vegetation to ambush their hosts. If assortative mating occurs, we expect paired ticks to be more closely related than two randomly sampled individuals from the population. To rule out the possible effect of a spatial autocorrelation in genetic relatedness, we also tested if ticks aggregated locally according to their genotype (i.e., if tick broods remain associated after dispersal).

Materials and Methods

Sampling. During spring of 2006, 93 adult ticks were collected from the vegetation in four sites in northern France (Fig. 1), including 29 pairs and eight groups. In all but one case, pairs included males in direct contact with females, suggesting guarding behavior by the males (Kiszewski et al. 2001). In the *Ixodes* genus, mating ticks may remain in copula for several days. This mating frequently occurs before the bloodmeal, on the ground or on the vegetation, but may also take place on the host (Kiszewski et al. 2001). Females can mate with multiple, successive males. All collected ticks were stored in 90% ethanol until DNA extractions.

Genotyping. Conserved ticks were washed three times in distilled water to eliminate ethanol and were cut

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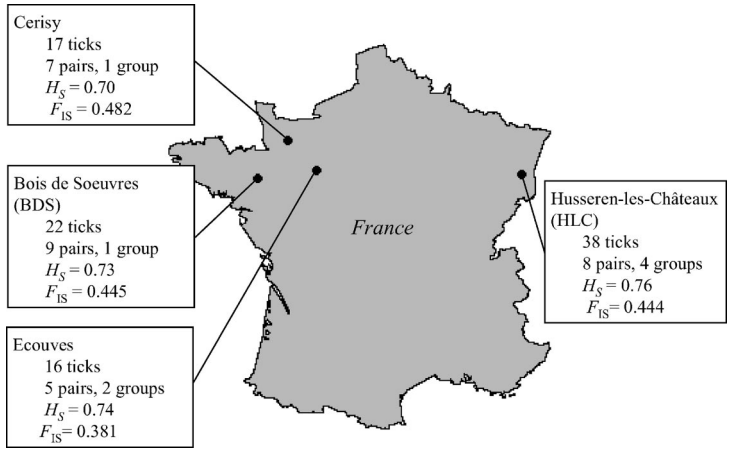

Fig. 1. Sampling locations of *I. ricinus*. The sample sizes (ticks, tick pairs, tick groups) are indicated for each location along with the mean unbiased expected genetic diversity, H_s (Nei 1987) and Weir and Cockerham's (1984) estimator of F_{IS}. In all cases, the F_{IS} was significant ($P < 0.001$).

in half. One half was ground with a mixer mill 301 (Retsch, Haan, Germany), and DNA was extracted using a Dneasy Tissue Kit (Qiagen, Valencia, CA). Ticks were genotyped at seven microsatellite loci: *IRN15*, *IRN37* (Roed et al. 2006), *IsaC4* (Fagerberg et al. 2001), *IR25*, *IR27*, *IR39*, and *IR32* (Delaye et al. 1998), following the polymerase chain reaction (PCR) protocols proposed by the authors. Genotypes were visualized using an automated sequencer (ABI Prism 310 Genetic Analyser; Applied Biosystems, Perkin-Elmer, France).

Genetic Analyses. We tested for the independence of the markers using the *G*-based test for linkage disequilibrium implemented in FSTAT 2.9.3.2 (Goudet 2001) with 15,000 randomizations of single locus genotypes among individuals within sites using all collected ticks (i.e., groups and pairs). Multiple testing was corrected by the Bonferroni method (Holm 1979), and the number of significant tests was compared with the expected proportion under the null hypothesis (0.05) with a unilateral binomial test (H_1: the number of significant tests is >5%). Genetic variability was assessed using Nei's unbiased estimator of genetic diversity (H_s, Nei 1987). We investigated departure from Hardy-Weinberg equilibrium by estimating Wright's F_{IS} (Wright 1965), using all collected ticks and Weir and Cockerham's (Weir and Cockerham 1984) estimator. The values of estimated F_{IS} were compared with the distribution obtained by randomizing alleles among individuals in each sample (15,000 permutations) (Goudet 2001).

We tested for nonrandom pairing in males and females by regressing the genetic relatedness in all potential male-female pairs against the mating status. Genetic relatedness was computed using Wang's estimator because of its robustness to small sample sizes (Wang 2002). The mating status of two ticks was coded as "1" when both ticks belonged to same mating pair and "0" when they were from different pairs. Under the hypothesis of assortative mating, we expected that mating status would significantly explain genetic relatedness, suggesting that ticks prefer kin as mates. We performed the computations with the freeware MER v. 3.0 (http://www.zoo.cam.ac.uk/ioz/software.htm#MER). This was tested by comparing the observed absolute regression coefficient to its chance distribution, calculated by randomizing the mating status 15,000 times. The analyses were carried out using the "Mantelize it" procedure of the program FSTAT v. 2.9.3.2 (Goudet 2001). To assess the power of our test to detect departure from pangamy, we performed the same analyses on a rearranged dataset that included the same ticks as the original data, but where the mating status was altered so that the most closely related individuals formed mating pairs.

We used the tick groups data to ensure that mating patterns were not simply a reflection of a spatial autocorrelation in genetic relatedness (i.e., individuals found together on vegetation are from the same brood). We compared the group membership and the genetic relatedness between all potential pairs of individuals (i.e., regardless of their sex) found as groups in each site. If both ticks of a given pair were found in the same group, their membership was coded as "1" and otherwise as "0." Under the hypothesis of a spatially autocorrelated relatedness, we expected that group membership would significantly explain genetic relatedness. As above, this was tested using FSTAT v. 2.9.3.2, and we performed a parallel analysis on rearranged data, containing the same number of groups, but where these groups were composed of the most closely related individuals within the sampled population.

Results and Discussion

All seven markers were polymorphic, and genetic diversities were high in each sample (>0.7; Fig. 1). Only one locus pair (*IRN15*, *IRN37*) showed significant linkage disequilibrium ($P = 0.03571$). However, this was no longer significant after sequential Bonferroni correction, and 1 significant test of 14 loci pairs is not significantly different from that expected under the null hypothesis ($P = 0.5123$).

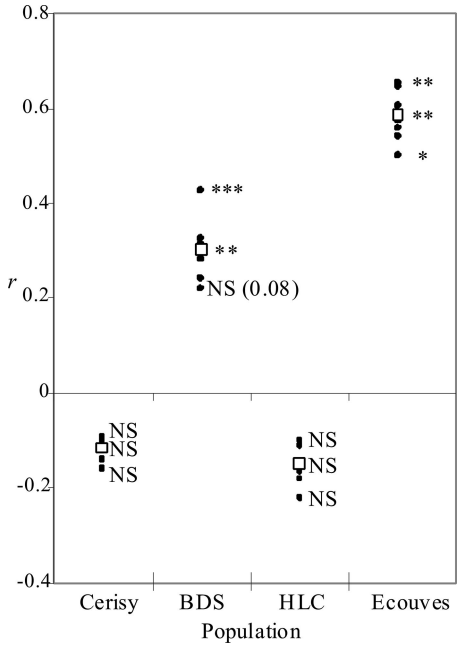

Fig. 2. Correlation coefficients (r) between relatedness and mating status among tick pairs. We distinguish correlation coefficients obtained after jackknifing over the seven loci (dots) and considering all loci (squares) in the four sampling locations. We also report the P values of the minimum and maximum correlations observed in the jackknifed datasets and for the complete dataset. We used the following annotations: NS, $P > 0.05$; ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$. The correlations and P values found for the rearranged datasets that maximized the relatedness between pairs within each population were, respectively, $r = 0.74$ (Cerisy), 0.69 (BDS), 0.67 (HLC), and 0.78 (Ecouves). The r's were all highly significant ($P < 0.001$).

Strong and significant heterozygote deficiencies were observed within all samples and at all loci (average $F_{IS} = 0.452$, $P = 0.00007$; Fig. 1). In a previous study, De Meeûs et al. (2002) found high heterozygote deficiencies at each locus examined that were explained in part by the presence of technical biases, i.e., null alleles or a process of short allele dominance (De Meeûs et al. 2002, 2004, Roed et al. 2006). Such technical biases are likely to increase the variance of parameter estimates. However, despite this variance, we observed a highly significant positive correlation between the mating status and the relatedness of pairs in two of the four populations studied (BDS and Ecouves; Fig. 2). In Ecouves, this value was close to the highest possible value (Fig. 2). For Cerisy and HLC, correlations were negative and nonsignificant ($P = 0.4069$ and 0.2317, respectively). The lack of significance in these two populations was not caused by the power of the test; in the rearranged datasets, we were able to detect highly significant departures from pangamy in all populations (Fig. 2). Similarly, we confirmed by jackknifing that these results did not rely on any one locus (Fig. 2), and we obtained similar results when the same analysis was performed with another relatedness measure, the shared allelic distance (D_{sa}, Bowcock et al. 1994) (results not shown). Finally, there was no evidence of spatial autocorrelation in relatedness that may have explained results. Indeed, group membership could not explain the genetic relatedness among tick pairs (mean correlation over all populations: $r = -0.254$; combined P value using Fisher method (Manly 1985) over all populations: $P = 0.32$), despite the fact that we were able to detect highly significant relationships in the rearranged dataset (mean $r = 0.790$, combined $P = 7 \times 10^{-10}$). In a different approach, we obtained similar results using hierarchical F-statistics (Goudet 2005): the $F_{Group/Site}$ (i.e., differentiation between groups within sites) was not significant ($P = 0.2262$, 10,000 permutations). The presence of tick groups is therefore likely associated with constraints on questing individuals that cluster at opportune sites without regard to sex or genotype (Healy and Bourke 2008, Medlock et al. 2008), whereas observed pairs may correspond to preprandial mating with assortment by relatedness.

Based on these results, it seems that the differences in mating patterns observed among populations must be caused by biological differences among these populations. If we hypothesize, for example, that within-population structure in *I. ricinus* is partially caused by local host-associated divergences and that observed patterns of assortative mating correspond to mating preferences for ticks of the same race, the differences among populations may be caused by a variable presence of different races. Indeed, many observations potentially support the existence of cryptic host-related subgroups in tick species and the dynamic nature of such divergences (McCoy et al. 2005, Magalhaes et al. 2007). There were noticeable differences in the distribution of the sampled ticks in the different populations considered here. Ticks of BDS and Ecouves were dispersed across an area of ~1 km^2, whereas those of Cerisy and HLC were aggregated in a limited area (i.e., along a transect of ≈100 m). Krasnov et al. (2007) have shown that a higher host species richness is empirically related to a lower aggregation of *I. ricinus*, at least in larval and nymphal stages. It may therefore be that differences among populations are caused by differences in host species richness. Indeed, the populations of Ecouves and BDS are much more anthropized (i.e., leisure activities) than the two other locations, which could result in significant differences in the range of potential host species that may use the sites. We therefore could have sampled several host races in BDS and Ecouves and only a single pangamic race in Cerisy and HLC. More detailed tests, where ticks are directly sampled from their different local host species, will now be required to test this hypothesis in *I. ricinus*.

Overall, the results obtained during this initial study show that *I. ricinus* may mate according to relatedness in natural populations, at least under certain conditions. There are at least three major implications of this finding. First, in a population at equilibrium, assortative mating will maintain high numbers of homozygotes and thus high variance among genotypes. Although rarely considered, the distribution of genetic variance within vector populations could be a key factor determining the transmission of associated

pathogens (Lambrechts et al. 2005). In the case of *I. ricinus*, this noticeably includes *Borrelia burgdorferi* s.l., the agent of human Lyme borreliosis. Second, at an evolutionary scale, assortative pairing can help to shift to a new assemblage of favorable mutations (Williams and Sarkar 1994). This may affect the dynamics of *I. ricinus* populations by leading to faster rates of host adaptation and as a consequence to higher transmission success for pathogens. Finally, assortative mating has been recognized has a major mechanism of prezygotic isolation in the context sympatric divergence (Bolnick and Fitzpatrick 2007). If confirmed, the existence of genetically isolated subgroups within *I. ricinus* may require the reassessment of our epidemiological perception of diseases transmitted by this tick. In particular, sympatric divergence may imply the existence of independent (or semi-independent) pathogen transmission cycles. To delve into the origin, pervasiveness, and potential consequences of the mating pattern shown in this study for the ecology and evolution of both the tick and the pathogens it carries, a concerted effort will now be needed to test for local population structure throughout the range of this tick.

Acknowledgments

We thank C. Chevillon for discussions, D. Kempf and P. Lambert for sampling assistance. This study was supported by the French Ministry for National Education, the Centre National de la Recherche Scientifique (CNRS), the Institut de Recherche pour le Développement (IRD), the University of Rennes 1, the Bureau des Ressources Génétiques, the Agence National de la Recherche (ANR-06-JCJC-0095–01), and the working group 'Tiques et Maladies à Tiques' of the Réseau Ecologique des Interactions Durables (REID).

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Received 21 May 2008; accepted 10 August 2008.