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Population Genetic Structure of *Ixodes ricinus* in Switzerland from Allozymic Data: No Evidence of Divergence Between Nearby Sites

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Abstract—Delaye C., Béati L., Aeschlimann A., Renaud F. & de Meeûs T. 1997. Population genetic structure of *Ixodes ricinus* in Switzerland from allozymic data: no evidence of divergence between nearby sites. *International Journal for Parasitology* 27: 769–773. *Ixodes ricinus* is a vector and reservoir of numerous infectious agents, especially *Borrelia burgdorferi*, the agent of Lyme disease. In Switzerland, its ecology and physiology have been well studied. Moreover, the foci of some infectious agents transmitted by this tick are identified. They can display relatively to extremely small geographical size depending on the diseases considered. In order to understand how the gene flows occur and to characterise the genetic structure of *Ixodes ricinus* populations, we used an indirect method based on genetic markers: allozymes. The sampling was carried out in 5 localities. Eighteen loci were analysed and 2 appeared polymorphic. This shows the low allozymic variability displayed by *Ixodes ricinus*. Based on these 2 loci, the populations appeared panmictic in Switzerland. This may be explained by the wide range of vertebrate species this tick can infest, especially birds. However, the result is surprising if we consider the extreme localisation of the foci of some infectious agents. We conclude that more powerful genetic markers could be used in order to better understand the epidemiology of tick-borne diseases in Switzerland. © 1997 Australian Society for Parasitology. Published by Elsevier Science Ltd.

Key words: *Ixodes ricinus*; vector; population genetics; allozymes; Switzerland.

INTRODUCTION

Ixodes ricinus is a widely distributed tick in Europe (e.g., Healy, 1979a). It lacks host specificity, since its potential hosts range from reptiles to birds and mammals (Hoogstraal & Aeschlimann, 1982). Its distribution is limited by altitude and environmental relative humidity. Strong hygrometric affinity confines it to forest undergrowth and it is rarely found above 1000-m altitude (e.g., Aeschlimann, 1972). Consequently, its distribution is discontinuous. *Ixodes ricinus* is a veterinary and medically important species, and is the vector and reservoir of numerous infectious agents. It can transmit especially the tick-borne encephalitis virus, rickettsiae, piroplasmosis and *Borrelia burgdorferi* (Lyme disease agent), the latter being a subject of much current research (e.g., Humair *et al.*, 1995; Suk *et al.*, 1995; Balmelli & Piffaretti, 1996; Montgomery *et al.*, 1996). In Switzerland, the geographical size of foci of the infectious agents transmitted by *Ixodes ricinus* can be relatively to extremely small, depending on the diseases considered (Aeschlimann, 1981). For example, the natural foci of tick-borne encephalitis in Switzerland are restricted to 4

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areas of the "Plateau Suisse" and mainly in the north and north-west of the Zurich canton (de Marval, 1995). For many years, the ecology and physiology of *Ixodes ricinus* have been well studied (e.g., Needham *et al.*, 1989; Aeschlimann, 1972, 1981; Mermod *et al.*, 1973). However, the population structure and migration capabilities of *I. ricinus* remain obscure because direct methods, such as capture–release–recapture, are difficult to use with these organisms (but see Falco & Fish, 1991). Alternatively, indirect methods, based on genetic markers, allow inferences to be made about the historical levels and/or patterns of gene-flow that have given rise to the observed pattern of genetic variation (Slatkin, 1985).

The population genetics of *I. ricinus* has attracted little attention (i.e. Healy, 1979a,b). In this paper we present results obtained using allozymic markers on 5 samples of *I. ricinus* from Switzerland. We show that this species, which displays a low level of allozymic variability, appears panmictic in the area investigated (about 3000 km^2). We then discuss our results in the light of *I. ricinus* ecology and compare them to those obtained in Ireland by Healy (1979a,b).

MATERIALS AND METHODS

Ticks were caught by the flag technique (Aeschlimann, 1972) and were stored in aerated boxes where humidity was ensured by a wet plaster bottom. The sampling was done during June 1994 in 5 localities: Montmollin, Neuchâtel, Staatswald wood (2 samples), Berne and Eclepens (Fig. 1). Eclepens and Berne are the 2 localities furthest apart (about 60 km), whereas the 2 samples from the Staatswald wood were separated only by about 200 m. This site has been identified as a tick-borne encephalitis focus (de Marval, 1995) and rickettsiae of the crimson fever group have also been reported there (Peter *et al.*, 1981). It is also a biotope of *Borrelia burgdorferi* (Lyme disease agent) (Aeschlimann *et al.*, 1986).

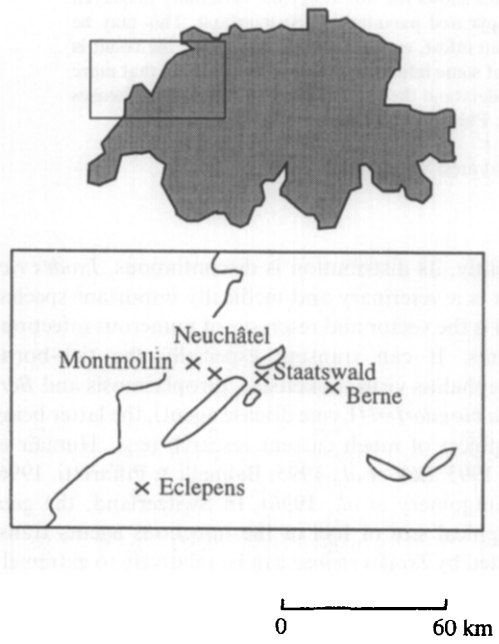

Fig. 1. Localisation of the sampling sites of *Ixodes ricinus*.

Electrophoresis was carried out using 10% starch gels according to the method described by Pasteur *et al.* (1987). Each individual was homogenised in 10-μl distilled water. The homogenate was then absorbed by 2 (for the males) or 3 (for the females) 2 × 10-mm pieces of Wattman No. 5 paper. The sample papers were then stored at −80°C and used as enzyme sources. Twenty-seven enzymatic systems were tested using 10 different buffers, and 18 loci appeared interpretable. They are aspartate-amino-transferase (*AAT*; EC 2.6.1.1), fumarase (*FUM*; EC 4.2.1.2), α-glycero-phosphate-dehydrogenase (*αGPD*; EC 1.1.1.8), hexokinase (*HK*; EC 2.7.1.1), isocitrate dehydrogenase (*IDH*; EC 1.1.1.42), malate dehydrogenase (*MDH*; EC 1.1.1.37), malic-enzyme (*ME*; EC 1.1.1.0), mannose-phosphate-isomerase (*MPI*; EC 5.3.1.8), purine-nucleoside-phosphorylase (*NP*; EC 2.4.2.1), peptidases (*PEPA*; EC 3.4.13.), 6-phosphogluconic-dehydrogenase (*6PGD*; EC 1.1.1.43), phosphoglucose-isomerase (*PGI*; EC 5.3.1.9), pyruvate-kinase (*PK*; EC 2.7.1.40), phospho-gluco-mutase (*PGM*; EC 2.7.5.1) and sorbitol-dehydrogenase (*SDH*; EC 1.1.1.14).

The presence of null alleles is very difficult to show, because the number of extracts per individuals is low and the enzymes appeared extremely sensitive to thawing.

Linkage disequilibria between pairs of loci were tested using the program Genepop V 1.2 (Raymond & Rousset, 1995) which computes unbiased estimates, with the Markov chain method (Guo & Thomson, 1992) of resampling, of the exact probabilities of random associations between pairs of loci, for all contingency tables corresponding to all possible pairs of loci in each population. All Markov chains were of at least 50 000 steps.

The genetic structure of the populations investigated was analysed using Wright's F statistics (Wright, 1951, 1965). The F_{is} measures the relative heterozygote deficit found in each sample. Its value ranges between −1 (all individuals heterozygotes), 0 (random association of alleles) and 1 (all individuals homozygotes). The F_{st} measures the genetic differentiation between the different samples. Its value ranges from 0 (random distribution of individuals) to 1 (no allele in common between each considered sample). These parameters were estimated by the Weir & Cockerham (1984) unbiased estimators f (for F_{is}) and θ (for F_{st}). These estimators were computed with the program Fstat V 1.2 (Goudet, 1996). This program also performs permutation procedures that we used to test the departure from 0 of these estimators. After a sufficient number of resamplings of the data ($n = 5000$), a distribution of the different possible values, under the null hypothesis, is obtained for the estimator. The comparison of the observed value to this distribution provides an unbiased estimate of the exact probability of obtaining by chance a value as large or larger than the one observed (type I error). The tests are thus one tailed. Absence of heterozygote deficiency (i.e. F_{is} not >0) is tested by permuting alleles within sites. Population structuring (i.e. null hypothesis: F_{st} not >0) is tested by permuting individuals between sites. The tests can be carried out over the loci and the populations. Note that despite the fact that the parameter F_{st} cannot be negative, its estimator θ can. When that is the case, it can be interpreted as a similarity between samples higher than expected by sampling errors.

Multiplying the number of tests enhances type I error. For

example, if panmixia is tested at the 5% level through 100 loci, then we expect, by definition, to reject the null hypothesis for 5 loci if the null hypothesis is indeed realised by the sampled population. To eliminate this bias, Holm (1979) proposed the sequential Bonferroni procedure (see Rice, 1989). We have first to select a significance level α (e.g., 0.05). Then, the P values of the k tests are ranked. The lowest one P_1 is compared to α/k. If P_1 is higher than α/k, then no test is considered significant at the α level. On the contrary, if it is lower, this test is considered significant. Consecutively the second lowest probability P_2 is compared to $\alpha/(k-1)$, and so on until $P_i > \alpha/(k-i+1)$. All tests with a P value lower than the corresponding Bonferroni level are considered significant. We used this procedure each time a multiple testing was carried out.

RESULTS

Among the 18 loci studied, only 2 are polymorphic, *αGPD* (4 alleles) in the Tris–maleate–EDTA, pH 7.4 buffer and *PGM* (5 alleles interpreted) in the Tris–maleate–EDTA, pH 6.9 buffer. For *PGM* additional rare alleles could be seen. Because the quantity of material provided by each tick only allowed 3 tests at most, these additional alleles were discarded (as in Healy, 1979a) in order to prevent interpretation mistakes.

Allelic frequencies are given in Table 1. Linkage disequilibrium was not significant between *α-GPD* and *PGM* in any of the sites (Table 1).

The F_{is} estimator f was -0.005 for *α-GPD*, 0.026 for *PGM* and 0.014 overall. The significance of these values was tested permuting alleles within samples. The probabilities of obtaining by chance values of f as large or larger than those observed were not significant ($P = 0.596$ for *α-GPD*, $P = 0.412$ for *PGM* and $P = 0.410$ overall), given the same allele frequencies. Thus, panmixia cannot be rejected.

We cannot see any significant θ (which measures the difference between populations) over all populations ($\theta = -0.004$, $P = 0.597$) or even between the most distant ones (i.e. between Berne and Eclepens: 60 km, $\theta = 0.019$, $P = 0.1795$) (Table 2). Healy (1979b) found a genetic differentiation between males and females for the locus *α-GPD*. This is not the case in our data either for *α-GPD* ($P = 0.32068$) or for *PGM* ($P = 0.1091$).

Table 2—Values obtained for θ (F_{st} estimator) and probabilities (P) that θ is not significantly above 0 (homogeneity between samples), when all sampling sites are considered and when only the 2 most distant ones (Berne–Eclepens) are

	PGM	α-GPD	Both loci
All sites	−0.005	−0.003	−0.004
P	0.54	0.52	0.597
Bern–Eclepens	0.048	−0.025	0.019
P	0.07	0.8	0.179

DISCUSSION

The first result of our study is the confirmation of the low variability of allozymes displayed by *I. ricinus*, or at least of the difficulty in inferring population genetic conclusions from allozymes in this species. The studies of Healy (1979a,b) concern the same polymorphic loci as in the present paper. He did not provide, however, the number of enzymatic systems he tested. Low allozymic variability is not the rule within the Ixodidae family. For example, Hilburn & Sattler (1986) studied *Amblyomma americanum*, which has a life-cycle similar to that of *Ixodes ricinus*. Out of the 21 loci studied, 17 were polymorphic. Bull *et al.* (1984) studied other ticks from the Ixodidae family parasitising reptiles (3 species of *Aponoma* genus and 3 of *Amblyomma*). The level of allozymic polymorphism varies with the species studied. In the genus *Aponoma*, 1 species displayed 5 polymorphic loci out of the 24 analysed, while the 2 other species were virtually monomorphic for all the loci studied. In the genus *Amblyomma*, 2 species were highly polymorphic (20

Table 1—Allelic frequencies observed and exact probability (P) for linkage equilibrium between the 2 loci in each sampling site of *Ixodes ricinus* from Switzerland. The different alleles are labelled following their anodal mobility)

Sample size:		Berne 22	Staatswald 1 31	Staatswald 2 27	Neuchâtel 19	Montmollin 25	Eclepens 14
α-GPD	1	0.023	0.000	0.000	0.000	0.000	0.036
	2	0.159	0.194	0.148	0.237	0.14	0.214
	3	0.795	0.726	0.852	0.737	0.860	0.750
	4	0.023	0.081	0.000	0.026	0.000	0.000
PGM	1	0.000	0.000	—	0.023	0.036	0.000
	2	0.109	0.063	—	0.068	0.071	0.038
	3	0.348	0.375	—	0.227	0.268	0.192
	4	0.543	0.563	—	0.659	0.607	0.769
	5	0.000	0.000	—	0.023	0.018	0.000
	P	0.900	0.163	—	0.937	1.000	0.642

polymorphic loci out of 20, 15 polymorphic loci out of 20) and the third one was only polymorphic for 1 locus. To our knowledge there is no obvious correlation between level of polymorphism and ecological characteristics of the different species.

As in the Irish samples studied by Healy (1979a,b), panmixia cannot be rejected in Swiss *I. ricinus* populations. Indeed, the few deviations from panmixia at the *αGPD* locus found by Healy (1979b) are no longer significant when the required Bonferroni procedure is applied. In a single sample, Healy (1979b) found slightly significant differences between males and females at the *αGPD* locus. However, in our results no differences could be seen. In our data, there is an apparent lack of differentiation between the localities. This is in agreement with Healy (1979a,b) where significant differences involved only 1 sample for *αGPD* and another 1 for *PGM*, when compared to the other samples, and not correlated with geographical distances.

Bull *et al.* (1984) studied ixodid ticks in southern Australia and Hilburn & Sattler (1986) sampled *A. americanum* in the south-eastern U.S.A. They each found only a weak genetic differentiation in all the species studied. Thus, at least for allozymic data within ixodid ticks, genetic homogeneity seems the rule.

In Switzerland, *Ixodes ricinus* parasitises a wide range of vertebrate species: 24 mammals, 9 birds, 2 reptiles (Aeschlimann, 1972). Birds may promote gene flow and thus decrease the level of differentiation between *I. ricinus* populations. However, epidemiological studies of diseases transmitted by *I. ricinus* (Aeschlimann, 1981) sometimes show extreme localisation of the infectious agents transmitted by this vector in Switzerland. This cannot be explained by the genetic structure of the tick populations as inferred from our present results. This may be because our samples are relatively small (due to a considerable loss of material during transport and conditioning) and the weaknes of genetic variation (only 2 polymorphic loci with 90% of the variation explained by 2 alleles for each locus). These factors can enhance type II error. Nevertheless, other factors, such as the distribution of vertebrate reservoir hosts, might influence disease focality. Moreover, as suggested by recent advances in the genetics of insect vectors (e.g., Lanzaro *et al.*, 1995; Lehmann *et al.*, 1996), further studies, using more powerful genetic markers as microsatellites, have to be considered in order to better understand the epidemiology of tick-borne diseases in Switzerland and in other countries.

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