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ISOLATION AND CHARACTERIZATION OF NEW POLYMORPHIC MICROSATellite MARKERS FOR THE TICK IXODES RICINUS (ACARI: IXODIDAE)

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ABSTRACT — Nine microsatellite markers were isolated from unfed larvae of *Ixodes ricinus* and were tested on two populations of nymphs collected on roe deer (N=21) and birds (N=39) in a French suburban forest. All markers were polymorphic, with limited evidence for deviations from linkage equilibrium. In accordance with previous markers developed for this species, we found large heterozygote deficits for six of the nine loci. Deficits were of the same order of magnitude within a tick infrapopulation, suggesting that population-level estimates were not due to a Wahlund effect among individual hosts, but more likely to technical problems (i.e., null alleles due to mutations in the flanking regions of the microsatellites). Although micro-geographic substructure (e.g., homogamy within infrapopulations) can not be ruled out, it is possible that null alleles could be an inherent problem associated with this tick species and specific genome-level studies are called for. Despite the possible presence of null alleles, the precision of population genetic estimates was improved by the addition of the newly-developed markers making them a useful addition for studying the population ecology of *I. ricinus*.

KEYWORDS — Ectoparasite; Genetic markers; Population genetics; Tick-borne disease

Ticks are haematophagous ectoparasites of major importance as vectors of human disease (Parola and Raoult 2001). *Ixodes ricinus* (Arthropoda, Acari, Ixodidae) is the main vector species in Europe, transmitting numerous human and livestock diseases including Lyme disease, tick-borne encephalitis, anaplasmosis and babesiosis (e.g., Stanek 2009). Efforts to understand the ecology of this tick in relation to disease transmission is difficult under natural conditions. This is particularly true for estimating patterns of dispersal and host use, two essential factors for understanding disease risk (McCoy 2008). Indirect methods that employ genetic markers are currently one of the best options to overcome the inherent difficulty in studying parasitic organisms, but require certain assumptions in order to make robust inferences (De Meuws et al. 2007). Microsatellite markers have been previously described and applied to populations of *I. ricinus* (Delaye et al. 1998, De Meuws et al. 2002, 2004a, 2004b, Røed
However, analyses using these markers have revealed significant deviations from Hardy-Weinberg proportions within populations. Hypotheses to explain these heterozygote deficits are numerous and not mutually exclusive: null alleles, short allele dominance, Wahlund effects or homogamy (Kempf et al. 2009). From previous studies, it is clear that technical problems are frequent (De Meeûs et al. 2004a). However, even after accounting for these problems, deficits are still apparent within populations suggesting the presence of population substructure (De Meeûs et al. 2004a, Kempf et al. 2010). Here, we outline the development of additional microsatellite markers for *I. ricinus* in an attempt to improve the precision of population genetic estimates used to study the biological factors that may be behind these patterns.

New microsatellite loci were isolated from a microsatellite-enriched library according to Billotte et al. (1999). We extracted Genomic DNA from unfed larvae with DNeasy Blood and Tissue Kit (Qiagen) following manufacturer’s instructions. DNA was restricted by HaeIII and fragments were ligated to Rsa21 and Rsa25 self-complementary primers (5'-CTCTTGCTTACCGTGACTA-3' and 5'-TAGTCCACCCGTAAAGACGCACA-3') and amplified by Polymerase Chain Reaction (PCR). Products were hybridized to a biotin-labelled I$_3$(GA)$_8$ probe and Streptavidin MagnetSphere Paramagnetic Particles (Promega). Enriched fragments were amplified by PCR, cloned in pGEM-T (Promega) and transformed in XL1-Blue competent cells (Stratagene). Recombinant colonies were randomly selected and amplified by PCR with Rsa21 primers. PCR products were run on a 1.1% agarose gel and transferred onto a Hybond N+ membrane (Amersham) which were hybridized with $[^{32}$P]dATP end-labelled (GA)$_{15}$ and (GT)$_{15}$ probes to verify amplification and improve fragment selection. Positive clones of differing fragment size were sent for sequencing (Beckman Coulter Ge-

| Locus | Genbank Accession No. | Repeat motif | Primer sequence (5’-3’) | Size range | $A_R$ |
|-------|-----------------------|--------------|--------------------------|------------|-------|
| IRic04 | JF724082 | (AC)6(CA)7 | F : ACGGGATGTAAAATTTGG  
R : GATCGACGAATGATCTCTG |
| IRic05 | JF724083 | (GA)8  | F : CTTTACCAACCTGTGCAC  
R : GAGCCAATTTITATGCAC |
| IRic07 | JQ349034 | (CA)6(AC)7/(ACA)5 (ACACAA)3  | F : TATTTCTTCTTCTGTG  
R : TGTATCTTCCAACAACGA |
| IRic08 | JF724084 | (TG)9  | F : TCAATGCTCCCTCCAGTACC  
R : AGAAAATAAGCCGGCCAGAAA |
| IRic09 | JQ349035 | (CT)10 | F : AAAAAACCCCAAGAAAACAA  
R : GGGAAGGAAAAATATGCTAA |
| IRic11 | JF724085 | (AC)8  | F : AGCTACGAGACTACATCAAAA  
R : TCAAAGACAGTGGAGGCTTA |
| IRic13 | JQ349036 | (AC)8  | F : ATGACGCGAGCCGAGATAAT  
R : TCTATATAGGGGTTGGCCGGAAT |
| IRic17 | JQ349037 | (CA)10 | F : ATAGTGAGCTTGTGAGCAAT  
R : CTGCCGTTTTAAATGAAAGTG |
| IRic18 | JQ349038 | (CT)11 | F : GTCCAGTCCTTTGACTCT  
R : GGAAAAAGGACCAAGAAA |

$A_R$: allelic richness based on 19 diploid individuals
nomics). Sequences were analysed and primers were designed using the SAT software (Dereeper et al. 2007).

We chose 19 loci for preliminary tests after checking that they differed from those described in previous studies. We performed PCR amplifications following a M13 protocol where each forward primer is 5’-tagged with the M13 sequence (5’-CACGACGTGTTAAAACGAC-3’) and a 5’-dye labelled M13 is added to the reaction mix. The 10 µL PCR mixture contained 20–50 ng of genomic DNA, 25 µM of each dNTP (Roche Diagnostics), 0.15 µM of each primer, 0.15 µM of labelled M13, 1 µL of 10x PCR buffer (Roche Diagnostics) and 0.25 U of Taq DNA polymerase (Roche Diagnostics). Amplifications were performed using a “touch down” PCR procedure consisting of an initial 2 min denaturation step at 94 °C, followed by 16 cycles with 45 s at 94 °C, 45 s at 60 °C with this annealing temperature decreasing by 0.5 °C at each cycle, 30 s at 72 °C, then 35 cycles with 45 s at 94 °C, 45 s at 52 °C, 30 s at 72 °C (25 cycles for IRic04, IRic05 and IRic18) and a final extension step of 10 mins at 72 °C. For genotyping, 0.5 µL of PCR products were pooled with 13 µL of Hi-Di Formamide and 0.25 µL of the GeneScan-500LIZ Size Standard (Applied Biosystems) and analysed on an ABI Prism 3130XL Genetic Analyser (Applied Biosystems). Raw data was sized using the associated GENEMAPPER software V4.0.

Of the 19 loci, we selected nine polymorphic loci that displayed good amplification results. These microsatellite loci were tested on two populations of nymphs from a suburban forest (Forêt de Sénart, Ile-de-France), one collected from five roe deer (N=21) and the other from twenty passerine birds (N=39). We considered these samples as representing potentially independent populations based on previous work that indicated the presence of host-associated races in this tick in some populations (Kempf et al. 2011). Data were analysed using GENEPOP 4.0.10 (Raymond and Rousset 1995) and FSTAT 2.9.3.2 (Goudet 1995). All markers were tested for independence using exact probability tests and for Hardy-Weinberg proportions by calculating Weir and Cocke...
TABLE 2: Tests of Hardy-Weinberg proportions for 14 microsatellite loci (nine new markers, IR25, IR27, IR32 and IR39 from Delaye et al. 1998, and IRN37 from Røed et al. 2006) in two nymphal populations of *I. ricinus* sampled respectively from birds and roe deer and in a tick infrapopulation from a single roe deer host.

| Locus | Host                  | N  | Ho   | Hs   | Fis  | P value |
|-------|-----------------------|----|------|------|------|--------|
| IRic04 | Bird                  | 36 | 0.389 | 0.947 | 0.589 | 0.0000* |
|        | Roe deer              | 20 | 0.650 | 0.963 | 0.325 | 0.0000* |
|        | Roe deer infrapopulation | 12 | 0.500 | 0.977 | 0.488 | 0.0000* |
| IRic05 | Bird                  | 33 | 0.727 | 0.813 | 0.105 | 0.1002 |
|        | Roe deer              | 20 | 0.850 | 0.836 | -0.017 | 0.8174 |
|        | Roe deer infrapopulation | 12 | 0.917 | 0.845 | -0.085 | 0.9493 |
| IRic07 | Bird                  | 38 | 0.684 | 0.862 | 0.207 | 0.0071 |
|        | Roe deer              | 20 | 0.650 | 0.795 | 0.182 | 0.4144 |
|        | Roe deer infrapopulation | 11 | 0.545 | 0.723 | 0.245 | 0.3944 |
| IRic08 | Bird                  | 36 | 0.667 | 0.856 | 0.221 | 0.0176 |
|        | Roe deer              | 21 | 0.619 | 0.886 | 0.301 | 0.0163 |
|        | Roe deer infrapopulation | 12 | 0.500 | 0.822 | 0.392 | 0.0845 |
| IRic09 | Bird                  | 39 | 0.615 | 0.923 | 0.334 | 0.0000* |
|        | Roe deer              | 21 | 0.810 | 0.907 | 0.108 | 0.1134 |
|        | Roe deer infrapopulation | 12 | 0.833 | 0.898 | 0.072 | 0.6401 |
| IRic11 | Bird                  | 39 | 0.385 | 0.891 | 0.568 | 0.0000* |
|        | Roe deer              | 19 | 0.263 | 0.943 | 0.721 | 0.0000* |
|        | Roe deer infrapopulation | 10 | 0.300 | 0.933 | 0.679 | 0.0000* |
| IRic13 | Bird                  | 33 | 0.273 | 0.718 | 0.620 | 0.0000* |
|        | Roe deer              | 21 | 0.286 | 0.815 | 0.650 | 0.0000* |
|        | Roe deer infrapopulation | 12 | 0.250 | 0.864 | 0.711 | 0.0000* |
| IRic17 | Bird                  | 32 | 0.063 | 0.518 | 0.879 | 0.0000* |
|        | Roe deer              | 19 | 0.053 | 0.585 | 0.910 | 0.0000* |
|        | Roe deer infrapopulation | 12 | 0.000 | 0.621 | 1.000 | 0.0000* |
| IRic18 | Bird                  | 37 | 0.432 | 0.922 | 0.531 | 0.0000* |
|        | Roe deer              | 21 | 0.381 | 0.842 | 0.547 | 0.0000* |
|        | Roe deer infrapopulation | 12 | 0.417 | 0.883 | 0.528 | 0.0002* |
| IR25   | Bird                  | 33 | 0.455 | 0.895 | 0.492 | 0.0000* |
|        | Roe deer              | 18 | 0.500 | 0.884 | 0.434 | 0.0000* |
|        | Roe deer infrapopulation | 11 | 0.545 | 0.864 | 0.368 | 0.0087 |

N: number of genotyped individuals
Ho: observed heterozygosity
Hs: expected heterozygosity
Fis: Weir and Cockerham’s (1984) estimator
P-value: Fis exact probability estimated by the Markov chain method
*: significant test for deviation from Hardy-Weinberg proportions after Bonferroni correction
Null alleles therefore seem to be common in this species and will require genome-level information in order to further understand their source. However, despite these technical issues, our new markers slightly improve the precision of previous population genetic estimates (Global $F_{IS}$ estimate across loci and populations for pre-existing markers $F_{IS} = 0.549 \pm 0.066$, for new markers $F_{IS} = 0.418 \pm 0.078$, for all markers $F_{IS} = 0.464 \pm 0.057$), with the addition of one marker that presented no indication of null alleles in either of the examined populations (IRic05). Thus, in tandem with appropriate sampling strategies, these markers should represent useful additional tools for studying the ecology of I. ricinus populations and their role as disease vectors.

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REFERENCES

Billotte N., Lagoda P.J.L., Risterucci A.-M., Baurens F.C. 1999 — Microsatellite-enriched libraries: applied methodology for the development of SSR markers in tropical crops — Fruits, 54: 277-288.

Delaye C., Aeschlimann A., Renaud F., Rosenthal B., De Meeus T. 1998 — Isolation and characterization of microsatellite markers in the Ixodes ricinus complex (Acar: Ixodidae) — Mol. Ecol., 7: 360-361.

De Meeus T., Béati L., Delaye C., Aeschlimann A., Renaud F. 2002 — Sex-biased genetic structure in the vector of
Lyme disease, *Ixodes ricinus* — Evolution, 56: 1802-1807.

De Meeûs T., Humair P.F., Grunau C., Delaye C., Renaud F. 2004a — Non-Mendelian transmission of alleles at microsatellite loci: an example in *Ixodes ricinus*, the vector of Lyme disease — Int. J. Parasitol. 34: 943-950. doi:10.1016/j.ijpara.2004.04.006

De Meeûs T., Lorimier Y., Renaud F. 2004b — Lyme borreliosis agents and the genetics and sex of their vector, *Ixodes ricinus* — Microbes Infect., 6: 299-304. doi:10.1016/j.micinf.2003.12.005

De Meeûs T., McCoy K.D., Prugnolle F., Chevillon C., Du-rand P., Hurtrez-Bousses S., Renaud F. 2007 — Population genetics and molecular epidemiology or how to "débusquer la bête" — Infect. Genet. Evol., 7: 308-332.

De Meeûs T., Guegan J.F., Teriokhin A.T. 2009 — Multi-Test V.1.2, a program to binomially combine independent tests and performance comparison with other related methods on proportional data — Bmc Bioinformatics, 10.

Dereeper A., Argout X., Billot C., Rami J.F., Ruiz M. 2007 — SAT, a flexible and optimized Web application for SSR marker development — BMC Bioinfor., 8: 465. doi:10.1186/1471-2105-8-465

Goudet J. 1995 — FSTAT (Version 1.2): A computer program to calculate F-statistics — Journal of Heredity, 86: 485-486.

Kempf F., De Meeus T., Arnathau C., Degelh B., McCoy K.D. 2009 — Assortative Pairing in *Ixodes ricinus* (Acar: Ixodidae), the European Vector of Lyme Borreliosis — J. Med. Entomol., 46: 471-474. doi:10.1603/033.046.0309

Kempf F., McCoy K.D., De Meeus T. 2010 — Wahlund effects and sex-biased dispersal in *Ixodes ricinus*, the European vector of Lyme borreliosis: New tools for old data — Infect. Genet. Evol., 10: 989-997. doi:10.1016/j.meegid.2010.06.003

Kempf F., De Meeus T., Vaumourin E., Noel V., Tarageľová V., Plantard O., Heylen D., Eyraud C., Chevillon C., McCoy, K.D. 2011 — Host races in *Ixodes ricinus*, the European vector of Lyme borreliosis — Infect. Genet. Evol., 11: 2043-2048. doi:10.1016/j.meegid.2011.09.016

McCoy K.D. 2008 — The population genetic structure of vectors and our understanding of disease epidemiology — Parasite, 15: 444-448.

Parola P., Raoult D. 2001 — Ticks and tickborne bacterial diseases in humans: an emerging infectious threat. (vol 32, pg 897, 2001) — Clinical Infectious Diseases, 33: 749-749.

Roed K.H., Hasle G., Midthjell V., Skretting G., Leinaas H.P. 2006 — Identification and characterization of 17 microsatellite primers for the tick, *Ixodes ricinus*, using enriched genomic libraries — Mol. Ecol. Notes, 6: 1165-1167. doi:10.1111/j.1471-8286.2006.01475.x

Raymond M., Rousset F. 1995 — GenePop (Version-1.2) — Population-Genetics Software for Exact Tests and Ecumenicism — J. Heredity, 86: 248-249.

Stanek G. 2009 — Pandora’s Box: pathogens in *Ixodes ricinus* ticks in Central Europe — Wiener Klinische Wochenschrift, 121: 673-683. doi:10.1007/s00508-009-1281-9

Van Oosterhout C., Hutchinson W.F., Wills D.P.M., Shipley P. 2004 — MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data — Mol. Ecol. Notes, 4: 535-538. doi:10.1111/j.1471-8286.2004.00684.x

Weir B.S., Cockerham C.C. 1984 — Estimating F-Statistics for the Analysis of Population-Structure — Evolution, 38: 1358-1370.

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