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PRIMER NOTE

Characterization of polymorphic microsatellite loci within a young *Boophilus microplus* metapopulation

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Abstract

Nine microsatellite loci from the cattle tick *Boophilus microplus* were isolated and characterized within New Caledonia. This Pacific island was invaded by the cattle tick from a few immigration events dating from mid-20th century. A population survey involving 94 adult ticks indicated monomorphism at one locus, presence of null alleles at another, and high genetic diversity (0.61–0.72) at seven loci apparently suitable for population genetics studies. This opens the opportunity to dissect the populational mechanisms involved in the spectacular capacity of *B. microplus* to cope with local environmental heterogeneity during its recurrent invasions of tropical areas.

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Boophilus microplus causes large economic loss in tropical agrosystems because of explosive demography on bovine herds, vector competence for diverse pathogens and recurrent development of acaricide resistances (Frisch 1999; Foil *et al*. 2004; Oliveira *et al*. 2005; and references therein). Another remarkable trait of *B. microplus* has not received attention yet: its ability to quickly adapt to new environmental heterogeneity, as noticed along its short history in New Caledonia. *Boophilus microplus* immigrated there around 1942 (Ragau 1966), before drastic quarantine isolated the island. Since then, the local evolution of this tick has been characterized by invasion of all herds, resistance developments towards all chemicals used in control (Bianchi *et al*. 2003; Ducornez *et al*. 2005) and apparent adaptation to a new invasive host (Barré *et al*. 2001). Neutral markers with rapidly evolving polymorphism are required to analyse the evolutionary determinants of these traits, but published microsatellite markers (Chigagure *et al*. 2000) only lead to null alleles on New Caledonian samples. We thus isolated new microsatellite markers from New Caledonian samples and investigated their potential to characterize polymorphism structure within this isolated and young (*c*. 200 generations old) metapopulation.

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DNA was extracted with DNeasy Tissue Kit (QIAGEN) onto a pool (*c*. 1 g) of unfed larvae. Five micrograms of DNA were used to build a microsatellite-enriched library as described in Billotte *et al*. (1999). The enriched microsatellite fragments were amplified by polymerase chain reaction (PCR) into 20 cycles with Rsa21 self-complementary primer (5′-CTCTTGCTTACGCGTGGACTA-3′), cloned into pGEM-T (Promega) and used to transform XL1-Blue MRF supercompetent cells (Stratagene). Randomly picked recombinant colonies lead to 192 inserts that were PCR amplified with Rsa21 primer. PCR products were transferred on Hybond-N+ nylon membranes (Amersham), which were hybridized at 56 °C with [γ^{32}P] dATP 5′end-labelled $(GA)_{15}$ and $(GT)_{15}$. Forty-eight positive clones were sent for sequencing (Genome Express). Designing primers with PRIMER 3 (Rozen & Skaletsky 2000; http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi), we screened sequences to obtain amplicons sizing between 100 and 220 bp, and retained 25 loci for individual genotyping.

Female blood-meal content was removed to avoid blood PCR-inhibitory effects (Wilson 1997), and individual DNA was extracted with DNeasy Tissue Kit (QIAGEN). Amplifications were performed in 25 μL containing 2.5 μL of 10× buffer (Promega), 10 pmol of each primer, 20 pmol of dNTPs (Invitrogen), 2.5 μL $MgCl_2$ (25 mM, Promega), 2.5 U

Table 1 Description of loci (name, GenBank access to reference clone, primers' sequences, fluorescent dye, structure of repeated array), thermocycling conditions (annealing temperature, T_a; number of cycles, N_c) and genetic variation (alleles number, N_a; allele size range; observed heterozygosity H_O; Nei's (1987) average estimate of within-sample gene diversity computed by FSTAT as $H_S = [\tilde{n}/(1-\tilde{n})] \cdot [1 - \Sigma p_{ik}^2 - H_O/2\tilde{n}]$ where $\tilde{n}$ is the harmonic mean of sample sizes)

Locus name	GenBank Accession no.	Primers (5′–3′)	Dye	Repeated array	T_a (°C)	N_c	N_a	Size range (bp)	H_O	H_S
BmA05	DQ001903	F: GGCGTGCAGGTTTAGCATA R: ATAGATGAGGGCTTGCCTTG	PET	$(CA)_{5+10}$	54	35	3	[110; 114]	0.12	0.27
BmA12	DQ001904	F: CCCCTATAGTGTGCCCTTGA R: GCAGTGCGTAATACAGGACAA	6-FAM	$(CA)_{3+7}(CG)_4$	52	35	4	[195; 199]	0.73	0.70
BmA06	DQ001905	F: AATCTGTGCATGGAGGTGTG R: AACCATTAAACAAGAAATGCAGTG	NED	$(GT)_{8+2}$	54	38	1	101	—	—
BmB12	DQ001906	F: CTGCTCACGGCTGGTTATTT R: GCACTTTTTCATCTCCGCTTC	VIC	$(TA)_4(TG)_9$	51	30	4	[192; 200]	0.69	0.66
BmC03	DQ001907	F: ACGACTTGTGAGGCGTAACC R: AGATACAGCGCAGCATTTCG	PET	$(CA)_{10+9}$	52	35	8	[146; 168]	0.70	0.72
BmC04	DQ001908	F: GAAAAATTGTCCGTCGCATT R: CACGAGTCACTCTTCGAGACTTT	VIC	$(TG)_8$	57	30	3	[110; 112]	0.69	0.62
BmC07	DQ001909	F: TTTGCAGGTTTGTGCCATAG R: TCAGCCATATGTTCAACCAGA	NED	$(GT)_{17}$	56	35	6	[145; 192]	0.70	0.73
BmD10	DQ001910	F: ACGTGGCGTTTACTGAGTCC R: GGCATATTTTCCCACCTCCT	6-FAM	$(GT)_{13}$	55	35	5	[151; 155]	0.65	0.65
BmD12	DQ001911	F: CGGGCCTCATAATCGTAAAA R: TGGCCACTGGTGACTATGAC	6-FAM	$(CA)_{10+5}$	50	28	10	[92; 118]	0.65	0.67

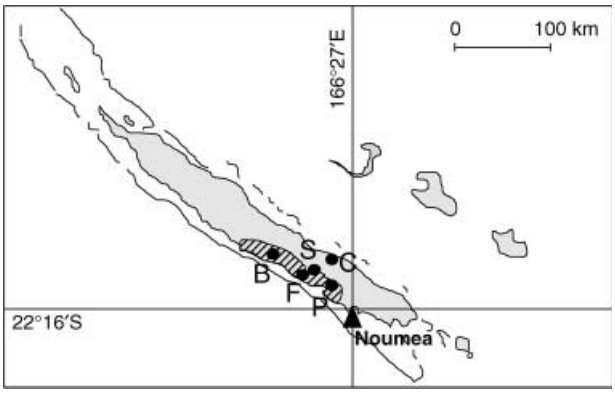

Fig. 1 Tick sampling. Cattle and cattle tick are omnipresent on the main island except the south of Noumea. The stripped area indicates the highest density of both cattle and cattle tick, and letters indicate the sampling locations. Sample sizes were $N = 24$ in B and C, $N = 22$ in F and $N = 12$ in S and P.

Taq polymerase (Promega) and 2–5 μL of DNA extracts. Thermocycling consisted in a denaturation step at 94 °C for 3 min followed by 35 cycles of 30 s at 94 °C, 30 s at annealing temperature (T_a), and 30 s at 72 °C. Forward primers were purchased as fluorescently labelled in 5′ end (Applera) for use on ABI 310 sequencer. PCR optimization was achievable for nine loci, requiring the thermocycing adjustments detailed in Table 1 and a reduction to 2 μL $MgCl_2$ (25 mM, Promega) for BmA05 amplification. For a given DNA template, electrophoresis was performed by pooling 0.5 μL of internal size standard (GeneScan-500 LIZ, Applera), Hi-Di formamide (20 μL QSP) and 1 μL of PCR product of each of three loci differing in fluorescent dye and amplicon size. Allele lengths were measured with GENESCAN (Applied Biosystem).

Consistency in individual genotypic determination among repeated assays was verified, and polymorphism was analysed among five representative samples (Fig. 1). BmA06 was the only monomorphic locus (Table 1). Using MICRO-CHECKER (van Oosterhout *et al*. 2004), we verified the absence of stutter-inducing errors at BmA12, BmC04 and BmD10 loci where single base-pair differences in allele sizes were recorded. MICRO-CHECKER detected heterozygote deficits at BmA05 locus within samples B, C and F, and at BmC04 locus in sample B. These deficits may indicate the presence of null alleles or may result from *B. microplus* biology: development is synchronous within large brotherhoods ($N \geq 100$), eggs are patchily distributed within mother cadavers, neighbour larvae regroup in clusters when seeking an individual host onto develop and mate, and females mate only once. Standard population genetic analyses were performed with FSTAT (Goudet 1995) to settle this point. A low average estimate of gene diversity ($H_S = 0.27$) and a non-null F_{IS} estimate ($F_{IS} = 0.65$, $P < 10^{-4}$) discriminated BmA05 from all other polymorphic loci ($0.62 \leq H_S \leq 0.73$ and acceptation of the hypothesis that 'F_{IS} is not different from 0' with $P > 0.10$). These particularities lead to remove BmA05 polymorphism to investigate further the tick population structure. The

polymorphism recorded at the seven remaining loci involved significant differentiation among samples (F_{ST} = 0.014, P = 0.00005). Meanwhile, genotypic disequilibrium was detected neither between any two polymorphic loci, nor between sex and either polymorphic locus. Therefore, the seven remaining loci look suitable for population genetics investigation of the local evolutionary determinants of *B. microplus*, at least among New Caledonian and genetically related populations.

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