References

Brown ED, Norcross BL, Short JW (1996) An introduction to studies on the effects of the Exxon Valdes oil spill on early life-history stages of pacific herring (*Clupea pallasi*) in Prince William Sound Alaska. *Canadian Journal of Fisheries and Aquatic Sciences*, **53**, 2337–2342.

Grant WS, Utter FM (1984) Biochemical population genetics of Pacific herring (*Clupea pallasi*). *Canadian Journal of Fisheries and Aquatic Sciences*, **41**, 856–864.

Nei M (1987) *Molecular Evolutionary Genetics*. Columbia University Press, New York.

O'Connell M, Wright JM, Farid A (1996) Development of PCR primers for nine polymorphic American mink, *Mustela vison*, microsatellite loci. *Molecular Ecology*, **5**, 311–312.

Taylor AC, Sherwin WB, Wayne RK (1994) Genetic variation of simple sequence loci in a bottlenecked species: the decline of the hairy-nosed wombat (*Lasiorhinus krefftii*). *Molecular Ecology*, **3**, 277–290.

Isolation and characterization of microsatellite markers in the *Ixodes ricinus* complex (Acari: Ixodidae)

C. DELAYE,* A. AESCHLIMANN,† F. RENAUD,* B. ROSENTHAL,‡ and T. DE MEEÛS,*

**Laboratoire de Parasitologie comparée, UMR 5555 CNRS Université Montpellier II, CC 105, Place E. Bataillon, 34095 Montpellier CEDEX 05, France, †Institut de Zoologie de Neuchâtel, Université de Neuchâtel, Chemin de Chantemerle, 22.2007 Neuchâtel, Switzerland, ‡Department of Tropical Public Health, Harvard School of Public Health, 665 Huntington Avenue, Boston, MA 02115, USA*

Keywords: Ixodes ricinus complex, *Ixodes ricinus*, microsatellite, primer, tick, vector

Received 8 August 1997; accepted 2 October 1997

Correspondence: C. Delaye. Tel. +33-467-14-47-24; Fax: +33-467-14-46-46; E-mail: parasito@univ-montp2.fr

The European tick *Ixodes ricinus* is a species of great medical and veterinary importance, serving as the vector of tick-borne encephalitis, rickettsiosis, piroplasmosis and Lyme disease. Nonetheless, its migratory capabilities are poorly understood. Indirect methods based on genetic markers show great potential utility to evaluate the historical levels and patterns of gene flow that have given rise to the observed pattern of genetic variation (Slatkin 1985). Allozyme markers have revealed little polymorphism in *I. ricinus* (Healy 1979a,b; Delaye *et al.* 1997). To obtain more information, we considered microsatellites because they have been useful in many species including insect vectors such as *Anopheles gambiae* (Lanzaro *et al.* 1995; Lehmann *et al.* 1996). Moreover, studies (see, for example, Hughes & Queller 1993) show that highly polymorphic microsatellites have been found in species with little allozymic variation polymorphism. Here we report the development of primers for PCR amplification of six microsatellite loci in the tick *I. ricinus*.

Two genomic libraries were constructed successively as described in Estoup *et al.* (1993). For each library, 30 µg of genomic DNA from 20 individuals was restricted with the enzyme *Sau*3AI. The fragments were separated on a 1% low-melting-point agarose gel and the 400–600 fragments isolated and ligated into a pBluescript vector (Stratagene). The ligation products were transformed into XL1-Blue MRF' Supercompetents cells (Stratagene) and the resulting colonies were blotted on Hybond-N+ membranes which were hybridized with a mixture of two probes $(CA)_n$ and $(GA)_n$. Two thousand clones from each library gave four and 34 positively hybridizing clones, respectively, of which 29 clones were sequenced. The majority of the microsatellites had no more than 10 repeats. Most had a mononucleotide repeat (A or C) adjacent to the dinucleotide (CA or GA) repeat region. After removing almost all of the clones which either had associated mononucleotide repeats or which were truncated by the end of the insert, 12 loci were subsequently targeted for PCR amplification. The primers were designed using OSP software (L. Hiller Washington, URL: infobiogen.fr). Six gave clear PCR products of the expected size. The PCR primers designed for the six loci are described in Table 1. The GenBank accession numbers for the nucleotide sequences of these six microsatellite loci are AFO24667 to AFO24671 and AFO25851.

The genomic DNA for genotyping was prepared using a phenol–isopropanol extraction method. Initially, one primer from each pair was end-labelled with T4 polynucleotide kinase and [γ-^{33}P]-ATP. The PCR amplifications were carried out in 20 µL of a mixture containing 10–50 ng of template DNA, 1× *Taq* polymerase buffer, $MgCl_2$ (concentration in Table 1), 200 µM each dNTP, 3 pmol of each primer and 0.5 U of *Taq* polymerase (Goldstar, Eurogentec SA). Initial denaturation was 4 min at 94 °C followed by 30 cycles (94 °C for 30 s, 30 s at annealing temperature as specified in Table 1 and 72 °C for 30 s) and 10 min at 72 °C in a thermocycler (Crocodile II, Appligene). PCR products were resolved in 5% acrylamide–bisacrylamide and 8 M urea sequencing gels. Clones already sequenced served for size control.

In a sample of 50 individuals, five of these six loci were polymorphic, with more than 16 alleles identified in each of three loci leading to high expected heterozygosities (Table 1). We note that the least polymorphic locus, IR27, has the most often interrupted repeats.

We have begun evaluating these primers on *I. dammini*, the Lyme disease vector prevalent in northeastern and upper midwestern North America. With two individuals and the PCR conditions used in *I. ricinus*, we succeeded in amplifying five out of six loci (IR8, IR18, IR27, IR32 and IR39). These first tests are encouraging in the possibility of using these primer sets with other members of the *I. ricinus* complex.

Acknowledgements

We thank Patrice Bouvagnet for his help for designing the primers. This work has been supported by a PICS (Programme International de Collaboration Scientifique) of the CNRS (PICS no. 290) and the FNRS (Request no. 31–42919.95).

References

Delaye C, Beati 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.

Table 1 Characterization of six *Ixodes ricinus* microsatellite primer sets

Locus	Repeat array	Primer sequences (5′→3′)	$MgCl_2$ (mM)	Annealing temp. (°C)	*n*	Size range (bp)*	Expected heterozygosities
IR8	$(AC)_9$ $(C)_{15}$	AGTCCATATGCTTATATACCCG	0.6	56	17	164–192 (182)	92.3
		CTGTATCTACAACCGGTCGT					
IR18	$(GT)_5$ $(GTCT)_2$	GAGAGGCTAAAGTTCTATTC	1.5	56	1	127 (127)	–
	$(GT)_2$	TACGCTAAAGCAAGTGTG					
IR25	$(CA)_{10}$	CGTAAGAGCCCATAGTTC	1.5	58	18	128–152 (135)	89.3
		ACGTGTAAAGCTGGTAAA					
IR27	$(AC)_9$	CATCGCTAGTGGCTAGAG	1.5	57	5	117–127 (121)	39
		TTATAACCCGAGGTCGTAAAA					
IR32	$(AG)_{12}$	TCGACAAGTGCAGTGGAGAC	1.5	60	7	≈ 233–250** (233)	68.2
		GTTTCCTACCACAGATTCTCC					
IR39	$(AG)_9$	ATACCCGTAGAACGAGAG	1.5	55	18	121–149 (129)	91
		GTTTTTCAAGATTTCCGCC					

n = No. of alleles (for 50 individuals).
*Cloned insert size in parentheses.
†Approximative absolute length.

Estoup A, Solignac M, Harry M, Cornuet JM (1993) Characterisation of $(CG)_n$ and $(CT)_n$ microsatellites in two insect species: *Apis mellifera* and *Bombus terrestris*. *Nuclear Acids Research*, **21**, 1427–1431.

Healy J (1979a) Phosphoglucomutase polymorphism in the tick *Ixodes ricinus*. *Parasitology*, **78**, 7–17.

Healy J (1979b) Analysis of α-glycerophosphate deshydrogenase variability in the tick *Ixodes ricinus* (Acari: Ixodidae). *Genetica*, **50**, 19–30.

Hughes C, Queller D (1993) Detection of highly polymorphic microsatellite loci in a species with little allozyme polymorphism. *Molecular Ecology*, **2**, 131–137.

Lanzaro G, Zheng L, Toure Y *et al.* (1995) Microsatellite DNA and isozyme variability in a West African population of *Anopheles gambiae*. *Insect Molecular Biology*, **4**, 105–112.

Lehmann T, Hawley W, Kamau L *et al.* (1996) Genetic differentiation of *Anopheles gambiae* populations from East and West Africa: comparison of microsatellite and allozyme loci. *Heredity*, **77**, 192–208.

Slatkin M (1985) Gene flow in natural populations. *Annual Review of Ecology and Systematics*, **16**, 393–430.

Isolation and characterization of microsatellite loci from the Gila topminnow (*Poeciliopsis o. occidentalis*) and their utility in guppies (*Poecilia reticulata*)

K. M. PARKER,* K. HUGHES,† T. J. KIM* and P. W. HEDRICK*

*Department of Biology, Arizona State University, Tempe AZ 85287–1501, USA, †Department of Life Sciences, Arizona State University West, Glendale AZ 85069–7100, USA

Correspondence: K. Parker. Tel.: +1-602-9654556; Fax: +1-602-9652519; E-mail: karen@hedricklab.la.asu.edu

Keywords: guppy, microsatellite, *Poecilia*, *Poeciliopsis*, topminnow

Received 7 August 1997; accepted 29 September 1997

The endangered Gila topminnow (*Poeciliopsis o. occidentalis*) is a small, live-bearing fish inhabitating four isolated watersheds in Arizona (Sheffer *et al.* 1997). Factors contributing to the decline of what was once the most abundant fish in the Gila River drainage include habitat destruction and/or fragmentation and the introduction of non-native central mosquitofish (Meffe *et al.* 1983). Previous genetic studies have shown little variation with one haplotype based on mitochondrial DNA restriction site variation (Quattro *et al.* 1996) and only two polymorphic allozyme loci out of 25 (Vrijenhoek *et al.* 1985). A recent study of a highly polymorphic MHC class II gene characterized nine different alleles among the four populations (P. W. Hedrick & K. M. Parker, 1998). Here we provide information on the microsatellite loci we have developed to examine neutral variation in Gila topminnows and give data showing their applicability in guppies, *Poecilia reticulata*.

DNA was isolated from tail and fin tissue of a single topminnow, digested with *Sau* 3AI and size-fractionated on a 1% agarose gel. The 300–800 bp size range was ligated into dephosphorylated pUC18 digested with *Bam*HI. Ligation products were transformed with competent cells (Invitrogen One Shot) and plated overnight on selective media. Approximately 3100 clones were streaked on nylon membranes (Hybond-N+, Amersham) and screened with Quick-Light genome mapping $(GA)_n$ and $(CA)_n$ probes and Quick-Light hybridization kit (both FMC BioProducts). Eighteen positives were obtained with two failing to amplify with plasmid primers. The remaining 16 positives were sequenced on both strands. Two subclones contained unusable microsatellites with seven or less repeat units. Primers were designed for the remaining 14 positive clones with four of the primer pairs failing to amplify in either the clone or genomic samples. The remaining 10 primer pairs were used to screen topminnow samples.