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PRIMER NOTE

Identification of 10 microsatellite loci in the earwig *Labidura riparia* (Dermaptera, Labiduridae)

MAREIKE GUETH* and WALTER DURKA†

**Brandenburg Technical University, Chair General Ecology, Siemens-Halske-Ring 8, D-03046 Cottbus, Germany, †UFZ Centre for Environmental Research Leipzig-Halle GmbH, Department of Community Ecology (BZF), Theodor-Lieser-Strasse 4, D-06110 Halle, Germany*

Abstract

Ten microsatellite loci were isolated from the earwig *Labidura riparia* (Pallas, 1773). The polymorphism of the loci was assessed in 24 individuals from one population. The number of alleles ranged from four to 11 alleles and observed and expected heterozygosities from 0.250 to 0.833 and from 0.551 to 0.861, respectively.

Keywords: Dermaptera, earwig, *Labidura riparia*, microsatellites

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The earwig *Labidura riparia* (Pallas, 1773) inhabits sandy but damp sites free of vegetation or with sparse vegetation (Beier 1953, 1959; Klausnitzer 2001). It has an almost cosmopolitic distribution (Albouy & Caussanel 1990; Steinmann 1993). In Western, Central and Eastern Europe, the species occurs naturally along the sea coasts and near rivers or waters. First observations of *L. riparia* in Lusatia (Germany) were made in the middle of last century in secondary habitats like open cast mines or sand and gravel pits (Jordan 1957; Höregott 1959; Donath 1988).

Labidura riparia is used as a model organism for physiological studies (Vancassel *et al*. 1984; Sayah *et al*. 1998). Wing-dimorphism has been detected, but flying individuals were rarely observed (Kleinow 1971; Matzke 1995). However, colonization of new habitats seems to take place rather rapidly (Gross & Spink 1971; Matzke & Klaus 1996). Rivers with their open sandy banks were postulated to have served as colonization routes of Central Europe (Harz 1957; Müller-Motzfeld *et al*. 1990; Adis & Junk 2002).

For further analysis of the genetic population structure of *L. riparia*, and patterns of colonization of new habitats, we developed microsatellite markers.

For library construction, we used a composite sample of DNA from 30 individuals from one population live trapped in Lower Lusatia (51°46′56.7′′N, 13°46′13.0′′E) and stored in liquid nitrogen. To avoid contamination of DNA of the zoophagous insects, only heads and legs were used for DNA extraction. The tissue was mashed with a plastic pestle, and the DNA was extracted using DNeasy Tissue Kit (QIAGEN) following the manufacturer's instructions for insects applying 50 μL H_2O at the final step. A microsatellite-enriched genomic library was made, from which recombinant colonies were sequenced by ecogenics GmbH (Zurich, Switzerland). Size-selected DNA was ligated into SAULA/SAULAB-linker (5′-GCGGTACCCGGGAAGCTTGG/5′-GATCCCAAGCTTCCCGGGTACCGC, Armour *et al*. 1994), and enriched by magnetic bead selection with biotin-labelled $(CA)_{13}$ and $(GA)_{13}$ oligonucleotide repeats (Gautschi *et al*. 2000a, b). From 384 recombinant colonies screened, 106 gave a positive signal after hybridization. Plasmids from 50 positive clones were sequenced using the M13/pUC sequencing primer. Primers were designed for 20 microsatellite inserts using PRIMER 3 (Rozen & Skaletsky 2000) and tested for polymorphism.

For analysis of genetic variability, we analysed 24 individuals (9 males, 15 females) from the same population used for library construction. This site is a secondary, sandy habitat with sparse vegetation, which developed spontaneously after dumping of the open cast mine Schlabendorf-Süd. DNA was extracted from one leg per individual using the Chelex resin extraction protocol described by Estroup *et al*. (1996). Microsatellite DNA amplification reactions were performed in a 20 μL volume containing approximately 20 ng DNA, 0.8 U *Taq* polymerase (MBI Fermentas), 200 μM dNTPs, 1.5 mM $MgCl_2$, 5 pmol of fluorescent-labelled forward primer and unlabelled reverse primer and 2 μL 10× polymerase chain reaction

Correspondence: Mareike Gueth, Fax: +49 (0)355692225; E-mail: Mareike.Gueth@tu-cottbus.de

Table 1 Characterization of 10 microsatellite loci based on a sample of 24 individuals of *Labidura riparia*

Locus	Repeat type	Primer sequence (5′–3′)	Size of cloned fragment	Size range (bp)	No. of alleles	H_O	H_E	F_{IS}	EMBL Accession no.
Lari77	CA_{15}	F: GCGCACCGAGAATACTAAAGAG	114	100–109	4	0.625	0.731	0.145	AM231137
		R: GTGTGTGTTCAAAGGATTATTTCAG						$P = 0.177$	
Lari10	CT_{18}	F: CCCCCTATAAATCACTAACGATG	182	178–206	7	0.667	0.734	0.091	AM231128
		R: ATTTAAGGGACGACGACGATAC						$P = 0.285$	
Lari18	CT_{21}	F: AAGGCTTGCCTACTTGGTCAC	125	110–126	7	0.542	0.736	0.264	AM231129
		R: GGTTCACGAGAAGGGCTTTATG						$P = 0.033$	
Lari51	CA_{29}	F: TCTGTCTGTCTGTTTTGAAATGG	175	171–177	4	0.625	0.551	−0.135	AM231130
		R: GGTAGGTTTATCGGTCGATTG						$P = 0.825$	
Lari17	CT_{21}	F: CGGAATGTATTGCAATGTTCG	126	116–140	8	0.250	0.691	0.638	AM231131
		R: GCCCAACCTTAATGAAGAGAATATC						$P = 0.001$	
Lari05	GA_{19}	L: GAATGTTCAATTTGTAACTTGTAATGG	133	116–138	9	0.583	0.766	0.239	AM231132
		R: GCCGTCCTGTTTTTTCAGAAG						$P = 0.026$	
Lari14	GA_{19}	F: GTATGATTGGTGAGAGCTGCTG	126	99–137	7	0.542	0.679	0.203	AM231133
		R: AGGAGTCTCGCTGGTCTTCTTC						$P = 0.081$	
Lari39b	CT_{22}	F: AGCTGCCTCCTTTCTCTATACATTC	180	169–181	6	0.826	0.754	−0.096	AM231134
		R: TATTTAAATTAACGCTTAGCGCTTTC						$P = 0.851$	
Lari37b	CT_{19}	F: CAACATCGGCAAGGAGGTAG	273	272–286	6	0.833	0.775	−0.075	AM231135
		R: AACAAAACAAATGGCGGATG						$P = 0.811$	
Lari33	$CT_4(CC)CT_{29}$	F: TCAAATGCAAATGAACAAAAGC	180	139–189	11	0.792	0.861	0.081	AM231136
		R: ATAATCTAATGGCCGGAAATATACTG						$P = 0.250$	

H_O, observed heterozygosity; H_E, expected heterozygosity.

(PCR) buffer with $(NH_4)_2SO_4$ (MBI Fermentas). The latter contains 750 mM Tris-HCl (pH 8.8), 200 mM $(NH_4)_2SO_4$ and 0.1% Tween 20. PCR amplifications were carried out using a Primus 96 thermocycler (MWG Biotech) programmed for 92 °C, 3 min, followed by 35 cycles of 92 °C, 15 s, 60 °C, 45 s, 72 °C, 1 min, and 72 °C, 10 min. The amplification products were scored on an ABI PRISM 310 analyser (Applied Biosystems) and product sizes were determined by comparison with GENESCAN 500 ROX standard. Of 20 primer pairs tested, 10 gave clear polymorphic banding patterns. Observed and expected heterozygosities were calculated using FSTAT version 2.8 (Goudet 1995) which was also used to perform exact tests for deviations from Hardy–Weinberg equilibrium. Linkage disequilibrium was tested using GENEPOP (Raymond & Rousset 1995).

The number of alleles at each polymorphic locus, their size range, and observed and expected heterozygosities together with inbreeding coefficients are shown in Table 1. The number of alleles for the 10 loci ranged from four to 11 (mean over loci was 6.9) and the mean expected heterozygosity was 0.728. Significant deviations from Hardy–Weinberg equilibrium were found for three loci (see Table 1). Four of the 45 pairs of loci compared exhibited significant genotypic disequilibria, mostly including loci (Lari18, Lari17, Lari05, Lari10, Lari37) that also showed deviations from Hardy–Weinberg equilibrium. These deviations might be results of null alleles, nonrandom sampling, mating systems or inbreeding.

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