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TECHNICAL NOTE

Microsatellite markers developed for a Swedish population of sand lizard (*Lacerta agilis*)

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Abstract Populations of sand lizards (*Lacerta agilis*) are declining throughout its north-western range. Here we characterize fifteen new microsatellite markers developed specifically for parentage analysis in a small Swedish population of sand lizards. These loci were screened in the Asketunnan population and a much larger and genetically diverse Hungarian population, with heterozygosities ranging from (0.217–0.875) and (0.400–0.974), respectively. All loci were in Hardy-Weinberg Equilibrium in the Swedish population but eight loci had significant heterozygote deficiencies in the Hungarian population. Two loci were significantly linked in both populations. These microsatellite loci are likely to be applicable in research on other sand lizard populations throughout Europe.

Keywords DNA markers · Microsatellite · Simple tandom repeat · Paternity · Lacertid

Introduction

The sand lizard (*Lacerta agilis*) is widely distributed throughout Europe with its northern most range in south central Sweden. While wide-spread, this species is suffering declines throughout its northwestern range, including Sweden, due to habitat degradation and fragmentation (Corbett 1989). Previous studies have characterized the Swedish populations as small with low genetic diversity and high reproductive isolation (Gullberg et al. 1999). Research on a small coastal population of sand lizards in southwest Sweden, Asketunnan, over the past 20 years has provided evidence for inbreeding depression, making it an ideal population to study various aspects of conservation genetics (Gullberg et al. 1999; Madsen et al. 2000; Olsson et al. 1996). The low genetic diversity of the Asketunnan population causes parentage analysis to be challenging with the few previously described microsatellite markers (Gullberg et al. 1997). Here we characterize fifteen additional microsatellite markers that were developed specifically from the population in Asketunnan, Sweden.

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Methods

Ten samples from the Asketunnan population were sent to ECOGENICS GmbH (Zurich, Switzerland) for the development of the microsatellite library. During the library development genomic DNA was digested, size selected, and ligated to a TSPAD-linker (Tenzer et al. 1999). Magnetic bead separation and biotin-labeled probes containing $(CA)_{13}$ and $(ACAG)_7$ repeats were used to enrich the library for microsatellite regions (Gautschi et al. 2000a, b). Fluorescently labeled probes $(ACAG)_{30}$ and $(CA)_{63}$ were used to screen 384 recombinant clones, of which 52 (13.5%) were positive for a hybridization signal and 48 of these were sequenced. Primers were designed for 21 loci that were tested for amplification and polymorphism. Fifteen of the loci amplified consistently and were polymorphic in the Asketunnan population. Primers for these loci were fluorescently labeled (6-FAM, VIC,

Table 1 Characteristics of the microsatellite loci: primer sequence with fluorescent label, GenBank accession number, and repeat type. Amplification conditions are described by the annealing temperature of the primers and the final concentration (mM) of the $MgCl_2$ in the PCR reaction. The loci can be multiplexed in three PCR reactions as grouped in the Multiplexed PCR column with the conditions footnoted. The number of alleles at each locus and their size range in base pairs, along with the observed (H_O) and expected (H_E) heterozytosities are described for the two populations screened: Asketunnan, Sweden N = 46 and Hungary N = 40.

Locus	Primer sequence (5'–3') and fluorescent label	Accession Number	Repeat Type	Annealing: $MgCl_2$	Multiplex PCR	Sweden Alleles Ho: He	Hungary Alleles Ho: He
LA01	VIC-AACGGAGGTAGAATGTCATAGC CTTGAAGGGAAAGAGCTACTGC	DQ860187	$(GT)_2AT(GT)_{15}$	56°C: 1.5 mM	1	3 (110–116) 0.343 : 0.381	10 (87–123) 0.472 : 0.883*
LA02	FAM-TGCCTGCAAGACTATAATCCAAG GGAATGGCATGAGATATGGTG	DQ60188	$(GT)_{23}$	56°C: 1.5 mM	1	6 (222–240) 0.826 : 0.809	13 (216–244) 0.900 : 0.898
LA3E	VIC-AAAGTTGGTCTGCACTGACG CAATTCAAAATGCACACAACG	DQ60189	$(GT)_{13}AT(GT)_{10}$	58°C: 2.5 mM	2	2 (243–245) 0.087 : 0.144	12 (220–258) 0.550 : 0.822*
LA04	VIC-CTAGGCATGGAGAATGGATGTG AGCCACTTCCCTAAGTGTGTCC	DQ60190	$(CA)_{20}$	56°C: 1.5 mM	3	3 (141–149) 0.348 : 0.443	14 (113–149) 0.925 : 0.901
LA10	VIC-TAATAAAGCAGGCGCAAACC TGCAGCTAATCTTCATTTAGGATG	EF028088	$(CA)_5(GA)_4GGGA$ $(GACA)_9(CA)_9(GA)_{17}$	54°C: 1.5 mM	3	9 (174–223) 0.875 : 0.766	11 (170–215) 0.621 : 0.855*
LA12	CAGAGTTCATGGAAAGTGAAGG FAM-GGAGACTCTGCTGGTCATTC	DQ60191	$(CA)_{18}$	58°C: 2.5 mM	3	4 (203–221) 0.500 : 0.475	10 (195–225) 0.625 : 0.775
LA27	VIC-AAATGCAAGCGAGCAACAAT ATCTGGCGGAGGGATGAG	DQ60192	$(GT)_{11}(AG)_{26}$	58°C: 2.5 mM	1	7 (137–155) 0.370 : 0.392	19 (112–160) 0.846 : 0.920*
LA37	PET-TTTGCTTGGAGCTTCTGTCC GATGCAGGACGGAGAGTAGC	DQ60194	$(GT)_{19}$	58°C: 2.5 mM	3	2 (110–128) 0.217 : 0.215	11 (98–128) 0.974 : 0.866
LA40	GGGAACCGTTGTACTAAGTTTGG VIC-ATGCATTCAGATGTCTCCCAAG	DQ60195	$(CA)_{19}$	56°C: 1.5 mM	1	4 (191–203) 0.565 : 0.602	10 (187–209) 0.625 : 0.652
LA45	NED-CAGAGTTCATGGAAAGTGAAGG AAGGAGACTCTGCTGGTCATTC	DQ860196	$(CA)_{18}$	56°C: 1.5 mM	2	6 (203–221) 0.609: 0.568	10 (195–225) 0.750 : 0.763
LA47	PET-CCCACTAGAGAAATGAGCTTCTG CAAACAAGGAGGGTAAGGAATG	DQ60197	$(GT)_{18}$	60°C: 1.5 mM	2	4 (111–119) 0.783 : 0.646	14 (95–127) 0.667 : 0.847*
LA50	FAM-AGGTAGCCCAGGTGTCATACAG TGGGTCTTACATGAGCTGAATC	DQ60198	$(GT)_{21}$	60°C: 1.5 mM	2	5 (115–123) 0.695 : 0.688	10 (99–123) 0.590 : 0.852*
LA55	NED-TCCCTCATTACAGGCATAGGAG TCTGAACAAAACATGGGACTTG	DQ60199	$(CA)_{19}$	56°C: 1.5 mM	1	4 (126–140) 0.652: 0.665	9 (124–142) 0.400 : 0.814*
LA58	FAM-CAGTTCTGGGGATTTTCTCCTAC CATTGTAATTGGAGCACAAAGC	DQ60200	$(CA)_{18}$	56°C: 1.5 mM	2	4 (165–175) 0.587 : 0.622	8 (159–173) 0.406 : 0.853*
LA64	PET-AGATGCTGAACTACCAGCTTGC GCTATCCTGGCTGACCATTAAG	DQ60201	$(CA)_{16}$	56°C: 1.5 mM	3	2 (186–192) 0.311 : 0.314	7 (184–195) 0.800 : 0.720*

* Deviation from Hardy-Weinberg Equilibrium, $p \leq 0.05$ after Sequential Bonferroni Corrections

[1] Multiplex 1: Annealing Temperature of 56°C: 1.65 mM $MgCl_2$

[2] Multiplex 2: Annealing Temperature of 56°C: 3.15 mM $MgCl_2$

[3] Multiplex 3: Annealing Temperature of 56°C: 2.65 mM $MgCl_2$

NED, or PET) and used to genotype 46 samples from the Asketunnan population and 40 samples from a larger Hungarian population. Samples were PCR amplified, electrophoresed on an ABI 3130xl (Applied Biosystems), and scored with the assistance of GeneMapper (Applied Biosystems).

Amplification of a single locus occurred in 7 μl reactions containing 0.05 U Hot Start Taq (Qiagen); 0.22 mM dNTPs; 250–850 pmols of primer; 1 × Qiagen PCR buffer containing Tris-Cl, KCL, $(NH_4)_2SO_4$, 15 mM $MgCl_2$, pH 8.7; 20 ng of DNA; and varying amounts of additional $MgCl_2$. Cycling conditions included an initial hot start denaturation at 95°C for 15 min, followed by 34 cycles of 95°C for 30 s, annealing temperature for 30 s, 72°C for 1 min 15 s; and a final extension at 72°C for 1 h. Primers were also tested for multiplexing in the PCR reactions (Table 1).

Tests for linkage disequilibrium were conducted in *Genepop* using the following Markov chain parameters: 1,000 dememorization steps, 100 batches, and 1,000 iterations per batch (Raymond and Rousset 1995), and significance values were sequential Bonferronii corrected for multiple tests (Rice 1989). *Arlequin 3.01* was used to calculate observed and expected heterozygosities and exact tests for Hardy-Weinberg equilibrium using 10,000 Markov chains, and 100 dememorization steps (Excoffier et al. 2005).

Results and discussion

The number of alleles for these loci ranged from 2–9 in the Swedish population and 7–19 in the Hungarian population. Observed heterozygosities ranged from 0.217–0.875 in the Swedish population and 0.400–0.974 in the Hungarian population. One loci pair, LA45 and LA12, was significantly linked in both populations. None of the loci were out of Hardy-Weinberg Equilibrium in the Swedish population from which the primers were designed, but eight of the loci had significant heterozygosity deficits in the Hungarian population, likely due to null alleles (Table 1).

These loci, combined with previously described loci within the genus (Bohme et al. 2005; Boudjemadi et al. 1999; Gullberg et al. 1997) provide sufficient power for paternity analysis in the genetically depauparate Asketunnan. Additionally, many of these loci are likely to be applicable to other populations throughout Europe, although caution must be taken to screen for null alleles.

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