Table 1 Motifs and primer sequences of eight microsatellite loci of *Nectomys squamipes*. F, Forward primer; R, Reverse primer. T_a °C, annealing temperature

Locus	Repeat motif	Primer sequences (5′ → 3′)	T_a °C	GenBank accession no.
Nec12	$(CA)_4T(CA)_{19}$	F: CTCCCTTCCCTCAATTTGCTGAGT	61	AF283417
		R: ACATGTGCAAAGCATGAAAATGGA		
Nec14	$(CA)_{24}$	F: CAGGCGATTTACACAAAAGAAT	57	AF283420
		R: CACTGAGCCATCTATCCAGTTC		AF283419
Nec15	$(AC)_{24}T(CA)_6$	F: AGGAAATGCTTATCTGGAGGAG	58	AF283422
		R: GACTCCTGATGTTGAACTGACC		AF283421
Nec18	$(CA)_{34}$	F: CTCTTTGAGGCCACTTCATTAA	58	AF283426
		R: GAACTAACATTTGCATCCTCCAG		AF283424
Nec28	$(CA)_{19}$	F: AGGAGAAAACCTGTATGCCATG	59	AF283428
		R: GTTTCTTCTTGCTGACCATGAGG		

Table 2 Genetic variation of eight microsatellite loci in *Nectomys squamipes*. *N*, number of examined animals; *A*, number of alleles per loci; Freq., frequency of the most common allele; H_O, observed heterozygosity; H_E, expected heterozygosity

Locus	*N*	*A*	Freq.	H_E	H_O	Allele range (bp)
Nec12	110	26	0.15	0.93	0.72*	206–242
Nec14	110	16	0.16	0.90	0.73***	204–236
Nec15	100	19	0.17	0.90	0.68***	171–213
Nec18	109	21	0.11	0.93	0.80**	128–170
Nec28	110	12	0.24	0.85	0.82	133–155

*$P < 0.01$, **$P < 0.001$, ***$P < 0.0001$. *P*-values obtained with Fisher's exact test for difference between H_E and H_O considering the null hypothesis of heterozygote deficiency.

3.2 (Raymond & Rousset 1995). Expected heterozygozity was significantly higher than observed heterozygosity for all but one locus (Table 2). Although this was probably a result of the Wahlund effect (Hartl & Clark 1997), and since samples were collected in eight different localities, the existence of null alleles cannot be ruled out until a more detailed population study can be performed.

The five polymorphic microsatellites loci, the first known for *Nectomys*, will be useful for assessing genetic variability within and among water-rat populations as well as for detecting differentiation and migration.

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A set of CA repeat microsatellite markers derived from the pool frog, *Rana lessonae*

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The pool frog, *Rana lessonae*, is broadly distributed in central Europe and often forms hybridogenetic, hemiclonal hybrids with the lake frog, *Rana ridibunda* (Blankenhorn 1977). These hybrids, known as *Rana* klepton *esculenta*, sexually parasitize either one or the other of the parental species, the most common form of which is the L-E system (*R. lessonae* LL × *R. esculenta* LR) (Graf & Polls-Pelaz 1989). In this system, hybrids transmit a clonal *R. ridibunda* haplotype by mating with a syntopic *R. lessonae*, while hybrid by hybrid crosses result in inviable offspring (Graf & Müller 1979; Uzzell *et al*.

1980). Hybrid lineages, therefore, represent frozen lineages that are assumed to be subject to an accumulation of deleterious mutations; mutations that are expressed when a hybrid by hybrid cross occurs and are suppressed when backcrosses with the parental species occur (Uzzell *et al*. 1980). The obvious lack of fitness benefits for *R. lessonae* individuals involved in LL × LR pairings make investigations of mate choice and sexual selection in this complex of great interest (Abt & Reyer 1993; Reyer *et al*. 1999). As well, pure *R. esculenta* populations have been detected, while theoretical investigations show that such pure populations cannot persist in isolation (Som *et al*. 2000). Even when a few *R. lessonae* are present, these generally are involved in hybrid matings due to the predominance of hybrids, which suggests that immigration by *R. lessonae* into such populations is required for population maintenance (Som *et al*. 2000; Hellriegel & Reyer in press). In these cases, management of pure or almost pure hybrid populations also requires identifying and managing *R. lessonae* source ponds.

With these and other applications in mind, we identified and characterized a suite of CA repeat microsatellite loci derived from *R. lessonae*. We constructed a highly enriched subgenomic library following standard protocols (Tenzer *et al*. 1999). A brief outline follows: genomic DNA isolated from a single male *R. lessonae* was digested to completion with *Tsp*509I (New England Biolabs) and the 500–1000 bp size fraction was isolated from LM-MP agarose (Boehringer Mannheim) using freezer phenol extraction. This size fraction was ligated to TSPADSHORT/TSPADLONG linkers (Tenzer *et al*. 1999) and amplified using TSPADSHORT and the polymerase chain reaction (PCR) as follows; total reaction volume was 25 μL and included 100 ng DNA, 1 U *Taq* polymerase (Quantum-Appligene), 10 mM Tris-HCl, pH 9.0, 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% TritonX100, 0.2 mg BSA (Quantum-Appligene), 100 μM of each dNTP (Promega), and 1 μM of TSPADSHORT. PCR was performed on a Techne Genius thermocycler (Techne Ltd) using the following thermotreatment: 2 min at 72 °C, followed by 25 cycles of 1 min at 94 °C, 1 min at 55 °C, and 1 min at 72 °. A total of 32 PCRs were carried out, pooled, cleaned and concentrated to minimize the likelihood of redundant products being detected during screening for positive clones (B. Gautschi *et al*. submitted). PCR products were hybridized to biotinylated CA(20) probes bonded to streptavidin-coated magnetic beads (Dynabeads M-280 Streptavadin, DYNAL, France) and amplified again. These final PCR products were cloned following the Original TA Cloning® Kit (Invitrogen) protocol. White colonies were dot-blotted onto nylon membranes (Hybond™-N^+, Amersham Pharmacia) and screened for CA repeats using the ECL 3′-oligolabelling and detection system (Amersham Pharmacia) and a 40mer CA oligonucleotide. All positive clones were sequenced using M13 forward and reverse primers, following the ABI Prism® BigDye™ Terminator Cycle Sequencing Ready Reaction Kit protocol, version 2.0 (PE Biosystems) and using the ABI 377 automated sequencing system (PE Biosystems). Primer design was carried out using primer 3 software (Rozen & Skaletsky 1998) and oligonucleotides were synthesized by Microsynth GmbH (Switzerland). Initial tests for amplification and polymorphism were done at 55 °C and electrophoresed on 8%, nondenaturing, 14.5 cm by 17 cm acrylamide gels at 80 V overnight. Those primers amplifying polymorphic products using five test templates (Table 1) were used for subsequent analyses reported below.

PCR amplification of frog DNA isolated from a sample of *R. lessonae* and *R. esculenta* adults captured and toe-clipped at a pond near Hellberg, north of Zürich, Switzerland was performed as follows. Reactions were 10 μL total volume and contained 50–100 ng template DNA, 0.5 U *Taq* polymerase (Quantum-Appligene), buffer components and dNTPs as listed above, and 0.5 μM of both forward and reverse primer. All PCR was performed using the following conditions: 3 min at 94 °C, followed by 25 cycles of 30 s at 94 °C, 30 s at 57 or 58 °C, and 30 s at 72 °C, followed by a final step of 2 min at 72 °C. Products were electrophoresed on Spreadex™ gels, either EL-300 or EL-500 (Elchrom Scientific AG, Switzerland), depending on the size of the alleles generated. All electrophoresis was performed using the SEA 2000™ advanced submerged gel electrophoresis apparatus (Elchrom Scientific AG, Switzerland) at 100 V for 60–120 min, depending on allele size, then scored against the M3 Marker ladder (Elchrom Scientific AG, Switzerland) and a 20-bp ladder (Bio-Rad). Expected and observed counts for homozygotes/heterozygotes were determined using GENEPOP, version 3x (Raymond & Rousset 1995) and tested for significant deviations using Chi-square analysis (null hypothesis rejected at $P < 0.05$).

All 10 loci were variable in *R. lessonae* and as well in *R. esculenta* (data not shown). Locus RlCA1b5 amplified an allele 137 bp in length only in *R. esculenta* individuals and is most likely the clonally transmitted *R. ridibunda* allele (data not shown). Loci RlCA1b17, RlCA1b20, RlCA1b27, RlCA18, RlCA19 and RlCA31 all appear to amplify only a single allele in a sample of *R. esculenta* tested in two other populations not reported here. Only *R. lessonae* frogs were used to test for homozygote excess, for obvious reasons. Loci RlCA5, RlCA1b17, RlCA1b20 and RlCA2a49 all exhibited homozygote excess ($P \geq 0.05$), which may indicate the presence of at least one null allele at each of these loci. Considering the bizarre nature of the LE complex, these homozygote excesses may instead be indicative of a departure from Hardy–Weinberg due to a violation of the assumption of random mating.

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Table 1 10 CA repeat microsatellite loci developed for *Rana lessonae*. All data based on PCR analysis of 25 *R. lessonae* individuals. T_a, annealing temperature; H_O, observed number of homozygotes; H_E, unbiased average heterozygosity estimate (Nei 1978). Both size and repeat motif are based on that detected in the original sequenced clone (GenBank Accession nos: AF286384–93)

Locus	Primer Sequences (5′–3′)	Repeat motif	T_a (°C)	No. alleles	Size (bp)	H_O	H_E
RlCA1	AAATGCAAGCGTCCCAATAC GGACGCAGTTTCTGGATTTG	$(CA)_{16}$	58	10	110	0.20	0.832
RlCA5	CTTCCACTTTGCCCATCAAG ATGTGTCGGCAGCTATGTTC	$(CA)_{17}$	58	6	250	0.52	0.678
RlCA18	CTCTGCTCCCTCAGCTATGC AAAAAGTGGTCCTTTCATTTTGAG	$(CA)_{22}$	57	5	177	0.48	0.573
RlCA19	GTCTGTCCGTGTGCAGAGAG CAAGTGATTGAGAGCCTCAGC	$(CA)_{15}$	57	2	129	0.52	0.490
RlCA1b5	CCCAGTGACAGTGAGTACCG CCCAACTGGAGGACCAAAAG	$(CA)_{17}$	58	3*	145	0.56	0.476
RlCA1b17	TAAACCTTAAAAGTGGTTATAAAAACC GTAAGTGTTAGGGATGCTGAGG	$(A)_8(CAAA)_2(CA)_{16}$	57	8	134	0.44	0.742
RlCA1b20	GGGCAGGTATTGTACTCAATATCAC CAACACAAGGACTCCACTGC	$(CA)_8(C)_{13}$	57	4	87	0.72	0.506
RlCA2a49	TGTCCACATTAAGGAACTTTTGC TTCAGAGATCAGGGGTCTCC	$(C)_8(A)_2(CA)_{15}CG(CA)_4$	57	5	200	0.48	0.710
RlCA1b27	GTAAGTGTTAGGGATGCTGAGG TAAACCTTAAAAGTGGTTATAAAAAGG	$(CA)_{15}(CAAA)_3(A)_5$	58	6	134	0.48	0.644
RlCA31	GAAGCTTAAACCACTTGACCAAC TCCCTTTTTCAGGTCTTTGG	$(C)_4A(C)_5GACAAA$ $CATA(CA)_6TA(CA)_5$	58	3	98	0.44	0.640

*Third allele detected at this locus only amplifies in *R. esculenta* and is not included in enumerations of H_O, and calculations of H_E and homozygote excess (see text for last).

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Characterization of microsatellite and minisatellite loci in Atlantic salmon (*Salmo salar* L.) and cross-species amplification in other salmonids

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The Atlantic salmon (*Salmo salar*) is a salmonid fish species which naturally inhabits cool rivers and oceans of the Northern hemisphere. It is of considerable economic importance, both for recreational fishing and as a major aquaculture species. Novel polymorphic genetic markers are in continual demand to extend familial and population genetic studies in this species. We report here on the identification of 'higher order' (tri- and tetranucleotide) Atlantic salmon microsatellites.

A number of different size-selected Atlantic salmon genomic DNA libraries were constructed, employing a microsatellite enrichment methodology (comprehensively described by Kijas *et al*. 1994). This protocol uses biotinylated microsatellite motif