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MICROSATELLITE LETTERS

# Characterization of microsatellite loci for a threatened species, the King Rail, *Rallus elegans*, using a next-generation sequencing protocol

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**Abstract** The King Rail *Rallus elegans* (Audubon) has experienced population declines of 4.6 % per year on average since the 1960s. Wetland loss, most severely affecting inland marshes, has significantly reduced this species' distribution to the coastal margins of its historic range. Polymorphic microsatellite markers were generated by 454 pyrosequencing of genomic DNA from King Rails, and Clapper Rails *R. longirostris* from Louisiana after AFLP enrichment and barcoding of restriction fragment cut sites across individuals. Of 1,419 microsatellite-containing sequences, 20 hypervariable microsatellite loci with up to 20 different alleles were identified at the alignment stage. We characterized nine loci, tested variability in 45 Atlantic coast King Rail samples, and detected 4–19 alleles per locus. Cross-species amplification revealed variability in the Virginia Rail, *R. limicola*, and Sora, *Porzana carolina*. These loci will be useful for studying secretive marsh rails, many of which are threatened or endangered.

**Keywords** King rail · Clapper rail · *Rallus elegans* · *Rallus longirostris* · Marshbird · Microsatellite · Pyrosequencing · AFLP enrichment · Barcoding

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Genomic DNA was isolated from ten unrelated King Rails and ten unrelated Clapper Rails from southern Louisiana (Maley 2012). We generated AFLPs to reduce the genome and enrich markers. Each individual was barcoded for segments adjacent to restriction fragment cut sites. Each sample of genomic DNA was initially digested with *Eco*RI and *Mse*I cutters, and then ligated with a primer sequence. Digests were separated by electrophoresis. DNA fragments (350–450 bp) were cut from the gel, isolated and used in two rounds of PCR extending the primer by one base the first round, size selecting again, then adding two more bases and size selecting again. These steps were followed to obtain sequences from identical loci from all 20 individuals for the original purpose of identifying single nucleotide polymorphisms to distinguish King from Clapper Rails (Maley 2012).

Barcoded fragments were pyrosequenced on a Roche 454 using standard protocols (see McCormack et al. 2012). MSAT commander (Faircloth 2008) was used to screen the concatenated sequence data for microsatellite repeats. The selection process produced 1,419 DNA sequence fragments between 48 and 477 base pairs long containing di-, tetra- and pentamer repeat microsatellites.

Alignments of 2–62 sequences (mean = 16.45) containing the same microsatellite motif revealed 20 different loci with repeat number variability of up to 20 alleles per locus among 20 individuals. Primer pairs were designed in unique flanking sequence for 15 loci. For another five, only one primer could be designed due to inadequate flanking sequence.

Primer pairs for each of the 15 loci were tested for amplification on King Rail samples collected from Mackay Island NWR, North Carolina (Brackett 2013). PCR was performed in 5 μL total volume with 15–50 ng template DNA, 20 mM Tris–HCL (pH 8.4), 50 mM KCL,

**Table 1** Characterization of polymorphic microsatellite loci for King Rails, including forward and reverse primers, specific annealing temperature $T_a$ (°C), number of individuals tested (*No*), variability of alleles ($N_A$), observed heterozygosity ($H_O$), and expected heterozygosity ($H_E$)

| Locus | GenBank accession no. | Repeat motif | Primers sequences (5′–3′) | Allele size range (bp) | $MgCl_2$ (mM) | $T_a$ (°C) | *No* | $N_A$ | $H_O$ | $H_E$ |
|---|---|---|---|---|---|---|---|---|---|---|
| KiRa1a | KC990034 | CACAT | F: AAGTGCTGGAGTGTGTCC | 189–277 | 10 | 59 | 42 | 14 | 0.881 | 0.884 |
| | | | R: ACTGTACCTCATCAACACAGAG | | | | | | | |
| KiRa5 | KC990035 | TC | F: TGCTGCACTGAGACAACATCT | 304–331 | 30 | 57 | 45 | 12 | 0.844 | 0.846 |
| | | | R: TGATCATGAGTAGAAGGAATAACCA | | | | | | | |
| KiRa6 | KC990036 | TC | F: CCTGCTGGAGGTACAAGGAG | 243–249 | 30 | 59 | 45 | 4 | 0.600 | 0.602 |
| | | | R: ACAACGCAGGAGAAGGTGTT | | | | | | | |
| KiRa7 | KC990037 | TAGA | F: TACTCGTATGCCAGTGTTG | 144–192 | 40 | 54 | 44 | 14 | 0.864 | 0.889 |
| | | | R: CAGAGATTATGTTCTCAATGACT | | | | | | | |
| KiRa8a | KC990038 | ATGG | F: GGCTGTGCAGAGAGGAAG | 264–334 | 40 | 60 | 42 | 19 | 0.952 | 0.919 |
| | | | R: GTGACACTGATACAGTGTGCCT | | | | | | | |
| KiRa9 | KC990039 | GT | F: TGATCTGGGCAGGCTTCTAC | 165–209 | 10 | 60 | 44 | 18 | 0.886 | 0.919 |
| | | | R: GTCGAATAATGGCAGCAATG | | | | | | | |
| KiRa10 | KC990040 | CA | F: CCAAGTACCATCTGCGAAGC | 124–146 | 40 | 59 | 44 | 10 | 0.795 | 0.783 |
| | | | R: AACCCGAACGAGAGATGTGA | | | | | | | |
| KiRa16 | KC990041 | TG | F: CCAGGTGGAAACTCTGCATT | 265–304 | 40 | 59 | 45 | 12 | 0.756 | 0.849 |
| | | | R: ACAGTTGTGATGTGGCTGGA | | | | | | | |
| KiRa17a | KC990042 | CA | F: TTACCAGCAGCCAACTGTGA | 234–268 | 20 | 60 | 45 | 19 | 0.867 | 0.907 |
| | | | R: AGTAGTGGTATCCTGGTGAGAGG | | | | | | | |

10–40 mM $MgCl_2$, 0.2 mM dNTPs, 0.5 pmol of each primer, and 0.05 Units of *Taq* DNA polymerase (Invitrogen). Reactions were carried out on a PTC-100 thermal cycler (MJ Research) using the following parameters: 94° for 2 min, 25 cycles of 94° for 30 s, specified annealing temperature for 30 s, and 72° for 1 min followed by a final extension of 72° for 5 min. One primer of each pair was labeled with a colored fluorophore for visualization on an ABI 3130*xl* Genetic Analyzer. Alleles were sized using the program GeneMapper® (Applied Biosystems).

Of 15 loci, nine were successfully optimized (Table 1). Among 45 unrelated Atlantic coast King Rails, no significant deviations from Hardy–Weinberg were detected. However, KiRa5 and KiRa10 showed significant linkage disequilibrium ($p = 0.04$). All loci were polymorphic in this population with a mean allele number of 13.56 and a mean observed heterozygosity of 0.8233. Collectively, this panel of markers gives an estimated parentage exclusion probability of $5.91 \times 10^{-4}$ for first parent and $3.72 \times 10^{-9}$ for both parents.

Using the same PCR conditions, the primers cross-amplified in the congeneric Virginia Rail and confamilial Sora (see Supplementary Materials). All but one locus were polymorphic in Virginia Rails; only one locus (KiRa6) failed to amplify in Sora. Alignments revealed variability also in Clapper Rails. Thus, these markers will be useful for studying a broad array of secretive rails, many of which are threatened or declining (Fleischer et al. 2009).

Next-generation sequencing using a mixture of genomic DNA from multiple individuals facilitated the rapid development of variable loci because we were able to detect variability at the alignment stage. Primer design could therefore be prioritized to those loci revealing high variability. Only 52.5 % of our 1,419 microsatellite-containing contigs were more than 250 bp in length, limiting our ability to design primer pairs for all microsatellite loci identified. Nevertheless, that variability could be assessed during the alignment stage significantly reduced the time and costs associated with purchasing and testing fluorescently labeled primers.

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## References

Brackett CL (2013) Reproductive ecology and population genetics of the King Rail (*Rallus elegans*). MS thesis, East Carolina University, Greenville, NC

Faircloth BC (2008) MSATCOMMANDER: detection of microsatellite repeat arrays and automated, locus-specific primer design. Mol Ecol Resour 8:92–94

Fleischer RC, Fuller G, Ledig DB (2009) Genetic structure of endangered Clapper Rail (*Rallus longirostris*) populations in southern California. Conserv Biol 9:1234–1243

Maley J (2012) Ecological speciation of King Rails (*Rallus elegans*) and Clapper Rails (*Rallus longirostris*). Ph.D. dissertation, Louisiana State University, Baton Rouge

McCormack JE, Maley JM, Hird SM, Derryberry EP, Graves GR, Brumfield RT (2012) Next-generation sequencing reveals phylogeographic structure and a species tree for recent bird divergences. Mol Phylogenet Evol 62:397–406