



Isolation and characterization of microsatellite loci in the globally endangered Corncrake, *Crex crex* Linné

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The Corncrake (*Crex crex*) is a trans-Saharan migrating rail species breeding exclusively in tall grasslands ranging from the Scottish islands to about 120° E in western Siberia (Green et al. 1997). Due to intensification of agricultural practices, population numbers have been declining in Western Europe by more than 50% during the last 50 years, resulting in a fragmented distribution of the Corncrake (Crockford et al. 1996). The Corncrake is now considered as a vulnerable species (Hilton-Taylor 2000). Recently, positive population trends have been observed in several regions. It is not clear however, whether this increase results from conservation management or immigration from eastern populations (Heer et al. 2000). To address this question and to resolve the population genetic structure of the Corncrake, we developed 9 microsatellite markers. Results obtained with these markers will help to evaluate the effectiveness of the taken conservation measures and facilitate further planning on a large international, biologically relevant geographical scale.

We constructed a partial genomic library enriched for CA and GA repeats using a slight modification of the procedures described by Tenzer et al. (1999) and Gautschi et al. (2000a, b). Briefly, total genomic DNA, isolated from blood samples of two birds using a standard phenol-chloroform extraction protocol (Sambrook et al. 1989), was digested with *Tsp509I* (New England Biolabs). Fragments of 200–700 bp were isolated and ligated to TSPADSHORT/TSPADLONG linker sequences (Tenzer et al. 1999). DNA-linker molecules were amplified according to Gautschi et al. (2000b) using TSPADSHORT as polymerase chain reaction (PCR) primer, and PCR products were hybridised to biotinylated (CA)₁₃ and (GA)₁₃ probes attached to streptavidin-coated magnetic beads (Dyna-

beads M-280 Streptavidin, DYNAL, France) (Tenzer et al. 1999). Enriched fragments were again amplified and products were cloned using the Original TA Cloning Kit (Invitrogen BV) following the manufacturer's instructions. After dot-plotting of recombinant colonies onto Nylon-Membranes (Hybond™-N⁺, Amersham Pharmacia), oligonucleotide probes labelled with ECL3'-oligolabelling and detection system (Amersham Pharmacia) were used to screen for inserts containing CA and GA repeats. The hybridisation was carried out in accordance with the manufacturer's instructions. Of 384 recombinant colonies screened, 168 gave a positive signal after hybridisation. Plasmids from 63 positive clones were sequenced (see Gautschi et al. 2000b). Primers were designed for 13 microsatellite inserts using primer3 software (Rozen and Skaletsky 1998) and oligonucleotides were synthesised by Microsynth GmbH (Switzerland). One primer of each successful pair was labelled with fluorescent dye (Table 1).

To assay the variation among individuals total genomic DNA was extracted from the tip of one wing feather using QIAamp tissue kit (Qiagen). We proceeded according to the manufacturer's instruction with the following slight modifications. The digestion step was carried out over night, and at each step we wrapped the tubes with Parafilm M[®] to avoid cross-contamination among samples. We eluted twice with 100 μl elution buffer which we preheated to 70 °C. PCR amplification was performed in a 10 μl reaction volume containing 2 μl of extracted DNA, 5 μl HotstarTaq master mix (Qiagen), 2 μl double distilled water, and 0.5 μM of each forward and reverse primer. We used the following thermotreatment on a PTC-100™ Programmable Thermal Con-

Table 1. Genetic characteristics of 9 Corncrake (*Crex crex*) microsatellite loci. Data on numbers of alleles and heterozygosities are based on genotypes of 15 Corncrake individuals. Ta, locus-specific annealing temperature, H_O, observed heterozygosity, H_E expected heterozygosity. Size range refers to the PCR product sizes at each locus. Significant departures from Hardy-Weinberg equilibrium were detected for locus Crex1 and Crex12. The characteristics of repeat motifs are based on the sequenced clones (GenBank Accession numbers AF458031–AF458039)

Locus	Primer sequence (5'-3')	Repeat motif	Ta (°C)	Size range (bp)	No. of alleles	H _O	H _E
Crex1	CACTGTTCCCTTTGGAACCTTCTC [§] TAACCCCAGGGATCATTTTG	(GT) ₉ AT (GT) ₈	55	135–176	10	0.54	0.87
Crex2	GTGTCTCAGGCAGCACAGAA [§] AGCAGGGCAGGACCCATT	(GT) ₅ AT (GT) ₆ CT (GT) ₇	55	81–117	15	0.93	0.91
Crex4	CACAGGCTGGCACAGTTG [§] GTGCGGTTGTTTCGATGTG	(CA) ₁₀ CTCATG (GA) ₂ GGC (GT) ₂ G (CA) ₂ (C) ₄ CATGGG (CA) ₃ GGCTGG (CA) ₂ (CG) ₂ (CA) ₁₃	57	124–167	13	0.92	0.90
Crex6	CGCCCAAGTTGTCTTCATC [§] ACAGTGCTGCAGGGGAAG	(AC) ₂₂	57	70–128	13	0.87	0.84
Crex7	TCTCTCCAAGGGAACAGCTC [§] TATTTGGCTGAGCTGCAA	(GA) ₂₃ GG (GA) ₁₀	57	109–154	13	0.87	0.92
Crex8	GAACCAGAGCAAAGGAGGAG [§] TCCACATCTTCCCATCACTG	(AC) ₁₂	57	180–204	17	1	0.96
Crex9	GGCAGGGAAAGATGGTTTTTC [§] AATGATGCTCCTGGAGATGG	(CA) ₁₁ TA (CA) ₄	57	76–116	13	0.93	0.92
Crex11	CACCTGGTCAAGTAAGCAACC [§] GCTTGCATAACCTGTGCTTG	(CA) ₂₇	60	77–134	14	0.86	0.93
Crex12	CTAATGGGGTTTTTGGTTGG [§] GACCCGATGATCTCTTGAGG	(CA) ₉	58	113–131	8	0.47	0.84

[§] fluorescent labelled primer

troller (MJ Research, Inc.): 30–35 cycles with 95 °C for 30 seconds, locus specific annealing temperature (Table 1) for 30 seconds, and 72 °C for 30 seconds. Before the first cycle, a prolonged denaturation step (95 °C for 15min) was included and the last cycle was followed by a 8 min extension. The amplified products were diluted, mixed with formamide containing Genescan-350 (ROX) Size Standard (Applied Biosystems), and genotyped on an ABI Prism310 Genetic Analyzer using GeneScanAnalysis[®] Software 2.1 and Genotyper[®] 2.1 Software (Applied Biosystems). Observed (H_O) and expected (H_E) heterozygosity at each locus was estimated using Genepop version 3.1c (Raymond and Rousset 1995).

All 9 microsatellite loci reported here were variable in *Crex crex* and we detected between 8 and 17 alleles in 15 birds originating from the Aggtelek region in northern Hungary. Exact tests for departure from Hardy-Weinberg equilibrium (HWE), per-

formed with Genepop version 3.1c (Raymond and Rousset 1995) indicated significant deviations at loci Crex1 and Crex12 and suggest the presence of null alleles at these loci. No significant genotypic linkage disequilibrium was found using Fisher's exact test implemented in Genepop version 3.1c (Raymond and Rousset 1995). The microsatellites markers presented here therefore provide a valuable tool for the conservation genetic analyses mentioned above.

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