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Identification of 24 polymorphic microsatellite markers for the double-crested cormorant (*Phalacrocorax auritus*)

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Abstract

Twenty-four polymorphic microsatellite markers were developed for the double-crested cormorant (*Phalacrocorax auritus*). The number of alleles ranged from two to 13 and observed heterozygosities ranged from 0.032 to 0.871. The use of these loci should enable researchers and biologists to learn more about the population structure and ecology of this species.

Keywords: colonial-nesting birds, double-crested cormorant, microsatellite, *Phalacrocorax auritus*

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Populations of double-crested cormorants (*Phalacrocorax auritus*), large, fish-eating, colonial-nesting waterbirds, increased dramatically from the mid-1970s until recently throughout the central and eastern USA (Hatch 1995; Weseloh *et al*. 1995). The increase in population sizes, coupled with an opportunistic foraging behaviour, has led to conflicts with commercial and recreational fishing (Rudstam *et al*. 2004) and aquaculture (Taylor & Dorr 2003). Furthermore, the emergence of large nesting colonies of double-crested cormorants has had adverse impacts on other colonial-nesting birds (Shieldcastle & Martin 1999) and sensitive vegetation (Hebert *et al*. 2005). As a result, cormorants are managed aggressively on their breeding and wintering grounds and during migration to alleviate biological damage and reduce conflicts with human interests (Bedard *et al*. 1999; DeVault *et al*. 2009). However, to more effectively manage cormorants, further information concerning population sizes, reproductive rates and migration patterns is needed. The availability of microsatellite markers for the double-crested cormorant will help researchers explore these questions and aid in the management of this species.

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Two genomic libraries enriched for either tetranucleotide [$(GATA)_7$, $(GATC)_7$, and $(GACA)_7$] or dinucleotide [$(GT)_{12}$ and $(CT)_{12}$] repeats were prepared using a microsatellite cloning protocol based on Hamilton *et al*. (1999) and Hauswaldt & Glenn (2003), modified as reported earlier (Beheler *et al*. 2004). To construct the library, we used a pooled sample of genomic DNA from two double-crested cormorants, which we extracted from muscle tissue using an ammonium acetate protocol (modified from the PUREGENE kit; Gentra Systems). Seven hundred and sixty-eight colonies were screened and sequenced. Sequence data were imported into Sequencher 4.1 (Gene Codes Corporation) for analysis.

We selected a subset (n = 51) of microsatellite-containing sequences and designed primers for polymerase chain reaction (PCR) amplification of these microsatellites using the software program Primer 3 (Rozen & Skaletsky 2000). We amplified these 51 microsatellite loci in 10-μL PCRs using a Mastercycler ep gradient (Eppendorf) and 20 ng of template DNA, 0.2 mM of each dNTP, 0.25 μM of each primer, 1 U of *Taq* DNA polymerase (NEB), 1.25 mM $MgCl_2$ and 1× reaction buffer (10 mM Tris-HCl, 50 mM KCl,

Table 1 Characterization of 24 polymorphic microsatellite loci developed for the double-crested cormorant

Locus	GenBank no.	Primer sequence	Motif	PCR product (bp)	*A*	H_E	H_O
COR 01	FJ477898	F: TCATGAACCAGCACCACATAG R: TTGATAGGCAGGTGACATGG	$(GATA)_{4,7}$	154–198	10	0.778	0.839
COR 03	FJ477899	F: TGCAGTTGCATTCCCTTTC R: CTTCCTAATGTGTTACACTGTCTGG	$(TATC)_{17}$	180–234	10	0.879	0.871
COR 05	FJ477900	F: AGGAACAACAACTGTGATTTGG R: CTTCAATGCCTTTCTCAAACC	$(GATA)_7$	233–265	9	0.797	0.677†
COR 06	FJ477901	F: GCCCACCATTCTTATTGCAC R: AGAAAGAATTGAGCCTAAGAAGTGA	$(TATC)_{15}$	128–184	13	0.860	0.871
COR 07	FJ477902	F: ACACCATCACAGTGCTTCACC R: CCCTTTGTCAATCTATGAACAACC	$(TGTC)_5$	131–135	2	0.032	0.032
COR 12	FJ477903	F: ACGGAGAACACATTTGTTAAGG R: CTTTCAGACACCCCATTGC	$(CT)_{10}(GT)_5$	217–219	2	0.094	0.097
COR 15	FJ477904	F: CACCCTTCACAGGTCAGTCA R: ACGGGGCTTGAACACTTTT	$(AC)_{13}$	167–177	4	0.517	0.419†
COR 17	FJ477905	F: CTGCCTCACTGATGTAAATGC R: ACACTCCAGTGACTGCCAAA	$(AC)_{12}$	228–234	3	0.539	0.516
COR 19	FJ477906	F: CTACAAGTTTCTACTAACGTGTGTGTC R: GCTGTAAGCTTTTCAGACTGC	$(GT)_{20}$	118–142	6	0.707	0.645
COR 20	FJ477907	F: CGGTTAACCCACTCAATGC R: GGGAGAAGTGATGTTGTTTGG	$(GT)_{6,10}$	168–178	4	0.533	0.484†
COR 21	FJ477908	F: GAACTTGGCAGCTTGCATC R: ACCAGAGAGTCCTCCGTGTG	$(AC)_{20}$	151–167	5	0.705	0.613†
COR 22	FJ477909	F: CCCCGCTTCCTCTTATATTTG R: TTGCAGATCCCCAAAATACTG	$(GT)_{15}$	394–398	3	0.476	0.581
COR 23	FJ477910	F: CCTGCAATTTCCTGGAGCTA R: TATTTGGCTGACCCTCTGCT	$(GAGT)_7$	263–275	3	0.386	0.367
COR 26	FJ477911	F: CACAGCAGTTCAGCATCAGTC R: CGGGATGAAAAGAAACCTAGAG	$(GT)_{11}$	177–181	2	0.178	0.129†
COR 28	FJ477912	F: ATGACAGATGGCGAAAGGTC R: AAACACATTGGCTCCTCCAG	$(AC)_{12}$	165–174	4	0.714	0.742
COR 30	FJ477913	F: ACGAAGTGAGCGTGTGAGTG R: CAGCTGTTGAACACAAGCAAC	$(GT)_8$	335–357	7	0.759	0.742
COR 31	FJ477914	F: TTGTTTCATTGACCTGGCTTC R: GCTGCCACTCTCCATATTCC	$(GT)_9$	222–226	2	0.275	0.323
COR 35	FJ477915	F: GATTCCACCCAGTCAAATTCC R: TAGGATCAGCTCCCAACAGC	$(GT)_9(AG)_4$	245–247	2	0.063	0.065
COR 38	FJ477916	F: GTGGAGCACTGGAACAAGC R: TTTGACAGGACACCAAGATCC	$(AC)_9$	301–307	4	0.690	0.645
COR 40	FJ477917	F: GAAACCTACCCTTCAAACTAGGC R: TTCTAGAAATATACAGGGAGGAGAGG	$(GT)_{10}$	160–166	5	0.711	0.581†
COR 41	FJ477918	F: TTCCTCTGCTGGATGGAC R: AACTTACAGCAGACAGGTAATGG	$(GT)_{10}$	168–182	6	0.527	0.516
COR 43	FJ477919	F: CAGCAGAAGGCAGATACAGC R: AGACCATGGTATTTGCAGAGG	$(AC)_{12}$	372–382	5	0.633	0.548†
COR 45	FJ477920	F: CAATTCATCCTGCTACACTGC R: TAGCAGCAGACATGCAAGG	$(GT)_{12}$	259–263	4	0.387	0.419
COR 47	FJ477921	F: AAGATTAGTGCAAGTCTTCTCTGC R: TCGCATCTTGGTGTTATGG	$(GT)_{20}$	167–181	6	0.560	0.548

The number of alleles (*A*), expected (H_E) and observed (H_O) heterozygosities are reported. Loci were screened in 31 individuals; †frequency of null alleles > 0.05.

0.05 mg/μL BSA). The amplification conditions were as follows: 94 °C for 2 min, then 94 °C for 30 s, 64 °C for 15 s, 72 °C for 15 s for 30 cycles, then 72 °C for 10 min and a final extension at 60 °C for 45 min. PCR products were initially screened on 2% agarose gels stained with ethidium bromide to verify amplification. Forty-six of the 51 loci consistently produced products of the expected size and subsequently were screened for polymorphism using a panel of 31 double-crested cormorant samples collected near Lake Guntersville, Alabama. The protocols for this polymorphism screening were the same as those given above, except that (i) in each reaction, the concentration of dTTP was reduced to 0.15 mM and 0.05 mM of chromatide rhodamine green 5dUTP (Molecular Probes) was added,

and (ii) PCR products were run on an ABI 3730 automated sequencer (Applied Biosystems) and genotypes were assigned using GeneMapper version 3.7 (Applied Biosystems). Of the 46 primer sets screened, 24 exhibited polymorphism.

For each polymorphic locus, we calculated observed heterozygosity, expected heterozygosity and null allele frequencies using Cervus 1.0 (Marshall *et al*. 1998). GenePop version 3.4 (Raymond & Rousset 2000) was used to test for evidence of linkage disequilibrium and deviations from Hardy–Weinberg equilibrium. The number of alleles per locus ranged from two to 13, and single locus heterozygosities ranged from 0.032 to 0.871 (Table 1). None of the 24 loci were found to be out of Hardy–Weinberg equilibrium ($P < 0.05$). Evidence of null alleles was detected at seven loci (Table 1). We tested 276 pairwise comparisons and found no evidence for genotypic linkage disequilibrium between any set of paired loci after a sequential Bonferroni correction was applied ($P < 0.00004$).

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Isolation and characterization of nine microsatellite loci in an ant-tended treehopper *Publilia concava*

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Abstract

***Publilia concava* is an eastern North American membracid commonly occurring in large but spatially patchy aggregations, primarily on the host plant *Solidago altissima*. Like other**

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