However, a consistent increase of 4 bp for SEB33, and a decrease of 2 bp for SEB30 were found with the fluorescent method. Allelic sizes were systematically corrected in order to adjust both methods.

In order to estimate microsatellite variation and polymorphism in redfish DNA, 30 individuals of each taxa were screened. DNA from each individual was extracted from muscle tissue using the Chelex extraction method (Walsh *et al.* 1991). All loci showed high variability. The total number of alleles per locus varied between 12 and 46 (Table 2). This also translated into high gene diversity, with a mean overall expected heterozygosity (H_E) varying between 0.500 and 0.960 depending on locus and taxa (Table 2). These values are comparable to those found in other marine fish (de García León *et al.* 1995; Rico *et al.* 1997; Ruzzante *et al.* 1996).

Acknowledgements

We are grateful to G. Naevdal and E. Parent for providing tissue samples, Sylvain Martin for technical assistance and to the Department of Fisheries and Oceans (Canada) for financial support. This is a contribution to the programme of GIROQ.

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Characterization of microsatellite loci for red-necked grebes *Podiceps grisegena*

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Keywords: Aves, coloniality, grebe, microsatellite, Podicipediformes, primers

Received 24 September 1998; revision accepted 2 November 1998

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A wide range of evolutionary forces have been implicated in the evolution of coloniality in birds, including reproductive competition, kin selection, sexual selection, and population structure (Alexander 1974; Wagner 1993). Thorough study of the evolutionary origin or maintenance of coloniality therefore requires that field study be augmented with genetic work (e.g. Hoi & Hoi-Leitner 1996). Microsatellite loci are expected to yield data that allow the roles of these evolutionary factors to be these assessed.

The red-necked grebe, *Podiceps grisegena*, typically nests territorially, while rarely nesting colonially (Cramp & Simmons 1977). Territorial and colonially nesting pairs co-occur at our study population in Minnesota, offering the opportunity of comparative analysis. We developed and characterized seven microsatellite loci in the red-necked grebe to help examine the evolutionary roles of the factors listed above. Additionally, we examined the polymorphism of these loci in five other nearctic grebe species and one hybrid form.

DNA was extracted from blood using a proteinase K, phenol–chloroform extraction procedure modified from Müllenbach *et al.* (1989). Size-selected genomic DNA digested with *Dpn*II was cloned into Lambda Zap Express, Stratagene, La Jolla, CA (Hughes & Moralez DeLoach 1997). We screened (125 000 clones with the oligo $(AAT)_{10}$ (Hughes & Queller 1993), sequenced 35 positives, and developed primers for all seven clones containing ≥ 8 uninterrupted repeats of the sequence AAT.

PCR reactions (5 μL) contained ≈ 5 ng of DNA, 50 mM KCl, 10 mM Tris/Cl pH 8.3, 1.5 mM $MgCl_2$, 0.1% NP40, 250 mM each dNTP, 0.25 U *Taq* DNA polymerase (Perkin-Elmer), 2.5 pmol each primer, and 0.05 μL of ^{35}S-dATP. Primer pairs 3 and 41 yielded scorable product only when *Taq* DNA polymerase was preincubated with Taqstart (Clontech); this produces a 'hot start' effect. Reactions were cycled using the 'tube-control' function of a Hybaid thermal cycler: 90 s at 92 °C, then 5 s at the optimal annealing temperature for each primer pair (Table 1), 5 s at 72 °C, 5 s at 92 °C, 30 times, and finally 90 s at 72 °C. Amplified fragments were resolved using 6% denaturing polyacrylamide gels. Allele length was determined by comparison to the sequencing products of M13.

All loci tested were found to be polymorphic in the red-necked grebe, having between seven and 18 alleles and heterozygosities ranging from 65 to 86% (Table 1).

The six polymorphic loci were tested on five other nearctic grebe species and one hybrid. PCR conditions for all loci

Table 1 Polymorphic AAT-repeat microsatellite loci developed for the red-necked grebe. H_O is based on the proportion of heterozygotes in a population sample of 87 individuals. GenBank accession numbers: AF080238–AF080244

Locus Name	Repeat Motif	Primer sequences 5'-3'	H_O	H_E	Product size range	No. of alleles	Optimal *Tm* (°C)
PgAAT1	AAT_{11}	CAAAGCTCGCGAGAATAG CCCGAGCGTTCCTCAGATA	0.65	0.84	180–204	9	55
PgAAT3	AAT_{14}	TTTCACAGCAACAGTAATG CAGTTCTCTCCGGTCTAC	0.68	0.77	120–150	11	60
PgAAT6	AAT_{11}	CTGCTGGCCACGATTCTTC TCACGTACTTTTGTCCGTATTGTA	0.53	0.78	153–171	8	60
PgAAT8	AAT_{15}	CAGCCACGGCATCATCTTAAT CTGCCTTTCAAGCCTGGTAAT	0.86	0.85	192–216	9	60
PgAAT25	AAT_{12}	AGGTTAGCAAAGGAAGAGATGATA TTGTGCATCACTGGGTTTGTAT	0.71	0.91	78–102	18	50
PgAAT34	AAT_{13}	ATCTGGAACCATCTGATAAGT GCACCTAAAGGAAACAATAAT	0.76	0.85	82–109	11	55
PgAAT41	AAT_{14}	AACCCAAACCATTATAT CTTCCCTGTAAAATTCT	0.83	0.83	151–172	11	45

Table 2 Number of alleles detected at each locus using samples of five individuals from each of six North American species of grebe. A dash indicates failure of amplification

	Locus						
Species	PgAAT1	PgAAT3	PgAAT6	PgAAT8	PgAAT25	PgAAT34	PgAAT41
Podiceps nigricollis	1	1	1	1	1	1	1
Podiceps auritus	5	4	5	3	4	7	3
Podilymbus podiceps	–	–	–	–	–	5	5
Aechmophorus occidentalis	–	1	1	1	2	3	1
Aechmophorus clarkii	–	1	2	1	2	2	2
Hybrid:*occidentalis/clarkii*	–	1	2	1	3	3	2

were as described above. Our primers had less utility in these species (Table 2). While all loci appear to be useful in the eared grebe, *P. auritus*, they are monomorphic in the closely related black-necked (horned) grebe *P. nigricollis*. This is consistent with effective population size being much lower in this, the rarer of the two species.

Acknowledgements

This work was supported by NSF grant no. OSR9452892 through ND-EPSCoR. We thank Gary Nuechterlein, Deborah Buitron, Bruce Eichhorst for kindly providing the samples, and Paul Klatt and the 1997 Molecular Techniques class at UND for help in the laboratory.

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