Conservation Genet Resour (2011) 3:13–15
DOI 10.1007/s12686-010-9267-5

TECHNICAL NOTE

Characterization of 12 polymorphic microsatellites for the Reddish Egret, *Egretta rufescens*

Austin Hill · M. Clay Green

Received: 4 June 2010 / Accepted: 16 June 2010 / Published online: 30 June 2010

Abstract The Reddish Egret is a rare, plumage dimorphic North American heron with disjunct colonies across its range due to habitat specialization. We isolated and characterized 12 novel polymorphic microsatellite loci for the Reddish Egret (*Egretta rufescens*) using 31 individuals from a single breeding colony in Texas. The number of alleles ranged from 2 to 10 among the loci within the colony. Observed heterozygosity ranged from 0.03 to 0.90 while expected heterozygosity ranged from 0.03 to 0.88. Two loci failed to meet Hardy–Weinberg expectations after correction while none of the loci exhibited significant linkage disequilibrium. Only 1 loci contained possible null alleles. These molecular markers will be used to assess genetic structure across the species range as well as examine potential differentiation between color morphs within and among populations.

Keywords Bird · Egret · *Egretta rufescens* · Heron · Microsatellite · Reddish Egret

The Reddish Egret (*Egretta rufescens*), with it's narrow habitat requirements and limited distribution, has an estimated global population of 5000–7000 adults (Paul 1991; Green 2006). Optimal habitat consists of broad shallow coastal mud flats conducive to its active foraging behavior (Paul 1991). It remains the rarest heron in North America, with populations still recovering from the plume trade that nearly extirpated the species from Florida and Texas in the early twentieth century. *Egretta rufescens* is listed as threatened in Texas and a species of special concern in Florida; as a coastal species it is also subjected to the many impacts associated with anthropogenic influence including habitat alteration, commercial ventures, recreational use, and pollution among others. The plumage dimorphic egret has disjunct populations spanning from Baja California, Mexico to Great Inagua, Bahamas with dispersal, structure, and genetic diversity within and among the populations mostly unknown. Three subspecies are recognized: *E. r. rufescens, E. r. dickeyi,* and *E. r. colorata* are suggested to represent populations in Texas/Florida/Caribbean, Baja California, and the Yucatan/Belize, respectively though the validity of these forms are not established and they appear to be weakly differentiated morphologically (Lowther and Paul 2002). Previous work with mtDNA for Reddish Egrets has shown no evidence for structure among colonies on the Texas coast (Bates et al. 2009). Highly polymorphic neutral markers like microsatellites are highly useful for answering ecological and population biology related questions (Selkoe and Toonen 2006) and will prove critical in appropriately managing this species. The objectives of this study were to isolate and characterize polymorphic microsatellite loci for examining population structure across the species range in addition to assessing potential differentiation between color morphs both within and among populations.

Genomic DNA was obtained by drawing 4 µl blood from the brachial vein in the wing of nestling egrets; all nestlings were returned to the nest immediately after sample collection. We performed DNA extraction using Puregene (Qiagen) DNA isolation protocol for avian whole blood. Approximately 100 µg samples from 13 individuals from different breeding colonies were sent to Genetic

A. Hill · M. C. Green (✉)
Department of Biology, Population and Conservation Biology Program, Texas State University, San Marcos, TX 78666, USA
e-mail: claygreen@txstate.edu

Identification Services (GIS, Chatsworth, CA, www.genetic-id-services.com) for development of 8 microsatellite enriched libraries of motifs CA, AAC, ATG, CATC, GA, AAG, TACA and TAGA. Microsatellite containing clones were sequenced using the ET Terminator Cycle Sequencing Kit (Amersham Biosciences) on an Applied BioSystems 377 DNA Sequencer. We then designed 78 PCR primers for the flanking regions for appropriate microsatellite sequences using DesignerPCR, version 1.03 (Research Genetics, Inc.). PCR amplifications were carried out in total volume of 10 µl containing 1 µl of 10× reaction buffer, 2 mM MgCl2, 0.2 mM of each DNTP, 0.3 µM of forward and reverse primers, 0.25 U *Taq* polymerase, and approx 0.2 ng/µl DNA template under the following PCR conditions: 3 min at 94°C; followed by 35 cycles of 40 s at 94°C, 40 s at 55°C to 57°C, 30 s at 72°C: followed by a final extension of 4 min at 72°C; then storage at 4°C. From the 78 primers developed, 13 primers were successfully amplified in 13 samples.

The 13 primer pairs were then optimized for PCR conditions using 8 individuals taken from different breeding colonies. One set of primers was excluded because it failed to amplify consistently across samples. The twelve remaining primers had the 5′ end of the forward primer fluorescently labeled with WellRED dye (Sigma-Proligo) for allele scoring carried out on a Beckman Coulter CEQ 8800. Arlequin 3.0 (Excoffier and Schneider 2005) was used to assess the deviation from Hardy–Weinberg and linkage disequilibrium in 31 individual nestlings from different nest on a single breeding colony located in the Laguna Madre in Texas (East Flat Spoils Island). We used the program MICROCHECKER to determine whether deviations from Hardy–Weinberg expectations were due to the presence of null alleles (van Oosterhout et al. 2004).

Table 1 12 polymorphic microsatellites for Reddish Egrets, *Egretta rufescens*

Primer	Primer Sequence (5′–3′)	Motif	T_a °C	Allele size range (bp)	N_A	H_o/H_e	GenBank accession no.
Er21	F: AGG-AAG-AGG-AGA-GGT-GAG-GT R: CCA-AGA-GCA-CTT-TTC-TAT-CAA-G	$GG(GT)_{11}(GC)_2$	56.8	141–147	2	0.06/0.06	HM368080
Er22	F: TTC-AGA-CAG-ACT-GGA-ATC-ACA-G R: AAG-TTT-ATT-GGC-AGC-AGA-TAG-C	$(CT)_{17}$	57.3	201–211	6	0.81/0.74	HM368081
Er41	F: AAT-ACG-CAT-CAA-GAC-AAA-TCA-G R: TCA-TGC-CTT-CTA-TGA-CAC-TAG-G	$(TATC)_5TATT(TATC)$ 6TATT$(TATC)_4$	57.7	286–298	2	0.16/0.43*[a]	HM368088
Er42	F: CTC-CAG-TGC-AGA-ACT-AGA-CCT-G R: CCT-GCT-GAT-TGA-AAC-ATG-AAT-T	$(GATT)_8$	57.8	209–221	4	0.74/0.68	HM368088
Er43	F: GCA-GGA-CCT-ACC-CTT-CAG R: GTG-CCT-TTC-ACA-TCT-CAC-TC	$(ATCT)5ATTT(ATCT)_5$	55.7	144–152	3	0.48/0.58	HM368087
Er51	F: AAC-AGG-CTT-CCC-AGA-GTG R: TGA-ACC-CTC-AGG-CAT-TTA-C	$(TTCTC)_6TTNTC(TTCTC)_4$	57.5	278–333	10	0.90/0.88	HM368079
Er23	F: AGA-GGT-TTC-CCT-TTC-CTA-GAT R: ATG-GCA-TTC-TGT-ATG-GGT-AG	$(TC)_{23}$	56.5	182–206	6	*0.58/0.62	HM368085
Er44	F: GAG-GGC-GAG-AAC-TTG-AGG R: CAA-AGC-AAC-AAA-ACA-TTC-AGC	$(TAGG)_5(TAGA)_6$	57.3	190–206	4	0.42/0.51	HM368086
Er31	F: GCA-AAA-GAT-TCA-AGT-CTG-ATG R: TAG-TTG-GGC-AGC-ATA-ATG-ATA	$(GTT)_5GCT(GTT)_3$	57.1	292–298	2	0.03/0.03	HM368082
Er45	F: AAG-GAA-ATA-ATG-GCG-ATA-GC R: TCT-ACC-AGG-GCA-GTA-AAC-TAA-A	$(TATC)_3TTTC(TATC)_5$	56.5	180–188	2	0.48/0.51	HM368089
Er24	F: CAT-TTG-CTT-TAT-CCA-AGA-CCT R: TAT-CCT-CAT-TTT-CCT-CAG-TGT-C	$(GA)_{22}$	56.3	203–221	5	0.68/0.76	HM368084
Er46	F: AGG-GAA-AGA-AAG-AGA-GGG-AC R: TGC-TAC-CAC-TTT-GAA-ACA-GAC	$(GAAG)_{17}$	56.2	130–166	6	0.61/0.69	HM368083

For each locus, we list the primer pair, the repeat motif from the original clone, the annealing temp (°C) and size range of alleles, the number of observed alleles and observed (H_o) and expected (H_e) heterozygosity

* Significant deviation from Hardy–Weinberg after correction

[a] Possible null alleles

The number of alleles per locus varied from 2 to 10 with a mean of 4.33 (Table 1). A deficiency in observed heterozygosity was found in 4 of the 12 primers (Er41, Er42, Er23, Er24) before bonferroni correction but in only 2 after correction (Er41, Er23). Observed heterozygosity ranged from 0.03 to 0.90 while expected heterozygosity ranged from 0.03 to 0.88. MICROCHECKER (van Oosterhout et al. 2004) detected the possibility of null alleles at only one locus (Er41). No linkage was found between pairs of loci after bonferroni correction.

The microsatellite primers described here are expected to be useful in studies concerning population structure and gene flow of Reddish Egrets throughout their range, in addition to other aspects of Reddish Egret ecology including color polymorphism and the occurrence of extra-pair copulations.

Acknowledgments The authors thank Zachary Holderby for his assistance in collecting samples. We thank Texas Parks and Wildlife, The Audubon Society, Coastal Bend Bays & Estuaries, Padre Island National Seashore, and the Texas General Land Office for their cooperation and assistance. We also thank Genetic Identification Services for assistance in developing markers. This research was funded by grants from United States Fish and Wildlife Service (Grant # No. 401817M112) to MCG and The Waterbird Society Kushlan Research Award in Ciconiiform Research and Conservation to AH.

References

Bates EM, Deyoung RW, Ballard BM (2009) Genetic diversity and population structure of Reddish Egrets along the Texas coast. Waterbirds 30:430–436

Excoffier LGL, Schneider S (2005) Arlequin ver. 3.0: an integrated software package for population genetics data analysis. Evol Bioinform Online 1:47–50

Green MC (2006) Status Report and survey recommendations on the Reddish Egret (*Egretta rufescens*). U.S. Fish and Wildlife Service, Atlanta, GA

Lowther PE, Paul RT (2002) Reddish Egret. The Birds of North America 633

Paul RT (1991) Status report–*Egretta rufescens* (GMELIN) Reddish Egret. U.S. Fish and Wildlife Service, Houston, Texas

Selkoe KA, Toonen RJ (2006) Micorsatellites for ecologists: a practical guide for using and evaluating microsateellite markers. Ecol Lett 9:615–629

van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) Microchecker: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 4:535–538