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PRIMER NOTE

Polymorphic microsatellites in the black-legged kittiwake *Rissa tridactyla*

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Abstract

We isolated seven microsatellite sequences from a library of recombinant clones in *Rissa tridactyla* (Laridae). We investigated their polymorphism in one population from France. Preliminary results indicate that these markers should prove valuable tools for the study of mating systems, population genetic structure and dispersal abilities in this seabird species. We present the results of cross-species amplification for two seabird species: *Uria aalge* (Alcidae) and *Stercorarius parasiticus* (Stercorariidae). Some of the microsatellites isolated in *R. tridactyla* might be useful for studies on other seabird species.

Keywords: extra-pair paternity, genetic variability, Kittiwake, microsatellite, *Rissa tridactyla*, seabird

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Rissa tridactyla is a well studied pelagic colonial sea bird with biparental care and a monogamous mating system. Populations are characterized by a high degree of heterogeneity among individuals in their survival probability and breeding success. This variation may have a intrinsic (genetic) basis (Coulson & Porter 1985; Cam & Monnat 2000). In this context, males and females may develop sexual strategies that maximize their fitness such as those that lead to extra-pair fertilizations (EPF). Colonial breeding provides open opportunities for extra-pair copulations (EPC) because there are many, easily accessible potential extra-pair partners. However, earlier work suggests that there is no universal relationship for seabirds (Hunter *et al*. 1992; Birkhead & Moller 1992).

The cryptic nature of EPCs make them difficult to observe in the field. In the particular case of *R. tridactyla*, copulations take place on the nests but EPCs could occur outside the colony. Paternity analyses based on DNA markers are powerful tools to detect EPFs, outcome of unobserved EPCs or to check whether observed EPCs lead to effective fertilization.

In this study we describe the development of microsatellite markers which should be useful for studying mating strategies in *R. tridactyla* populations and for adressing a wider range of questions related to population genetics and movement of the seabirds.

Using microsatellites, McCoy *et al*. (2001) have recently studied the migratory abilities of *R. tridactyla*'s major ectoparasite, *Ixodes uriae*. They have shown that this parasite has greater dispersal abilities than thought previously, suggesting that the within-season movement of seabirds may be more frequent and occur at larger spatial scales than predicted by ring returns. The use of microsatellites for both host and parasite could also enable the question of relative rates of gene flow of *R. tridactyla* and *I. uriae* to be addressed.

A genomic library was constructed following Estoup *et al*. (1993). Thirty ng of DNA extracted from the blood of one individual originating from a colony in Brittany (Cap-Sizun, Western France) were restricted with the enzyme *Sau*3A. Fragments between 400 and 800 bp were isolated on a low-melting-point agarose gel, purified using a Q1Aquick Gel Extraction Kit (Qiagen, Courtaboeuf, France) and ligated into a pBluescript vector II Sk + plasmid (Stratagene, Amsterdam, The Netherlands). Ligation products were then transformed into XL1-Blue MRF Supercompetent cells (Stratagene) and the resulting colonies were blotted on Hybond-N + membranes, which were hybridized with a mixture of two probes $(CT)_{10}$ and $(GT)_{10}$. A total of 51 positive clones were sequenced. We selected clones for which appropriate flanking sequences could be defined (Table 1). For the amplification of microsatellite loci,

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Table 1 Characteristics of the seven polymorphic loci of *R. tridactyla* from a single colony in Brittany, France

Locus	Repeat motif	*n*	Size range	N_A	H_O	H_E	Accession no.	Primer sequence (5′–3′)
K6	$(AC)_4T(TA)_{12}$	65	115–125	6	0.49	0.46	AY083596	F: AAAAAGAAAGCACCCTCTTC
								R: AAGTGGGATATGAAAGATGC
K16	$(TG)_4(TA)_8(GA)_{10}$	68	153–159	4	0.56	0.58	AY083597	F: TGCAATTTGTACAACCAGATTT
								R: GGGTTCCTGTTTGCAATGAA
K31	$(TG)_{13}$	63	160–184	6	0.63	0.67	AY083598	F: TTCTCGGGCACATAAACCTC
								R: CTCGCAGCATCTGGAAGG
K32	$(GA)_2(GT)_{12}$	67	117–178	18	0.90	0.90	AY083599	F: CATTGCACGAGTGTTAAGCTG
								R: AAGGGTGCCTGTCCTTGTC
K56	$TAATTAA\,(CA)_{10}$	67	148–156	7	0.65	0.64	AY083600	F: CCTGACTGCAGAGTTGGAG
								R: TGAGAACGGACTTTCTTTGG
K67	$(CA)_2(TA)_9$	63	137–141	2	0.40	0.36	AY083601	F: CACACCTGTATCCATCCATC
								R: TGGACGCACACATACATATT
K71	$(AC)_{11}$	67	147–153	3	0.54	0.53	AY083602	F: TAGTCTGGAGGTTGCAAATG
								R: AAACAACACCAAGAGGAAGG

n: Number of individuals; N_A: number of alleles; H_O and H_E: mean observed and expected heterozygosities. GenBank accession nos are given for each locus.

primers were designed using Primer 3 (http://www-genome.wi.mit.edu/cgi-bin/primer/primer3.cgi). Seven loci were polymorphic and gave clear polymerase chain reaction (PCR) results of expected size.

Genomic DNA from 68 adult individuals originating from the Brittany colony was extracted from blood samples using a Perfect gDNA blood isolation kit (Eppendorf, Hamburg, Germany) and was submitted to PCR amplification.

PCR amplifications were performed in a 10-μL mixture containing 1 μL of genomic DNA (about 50 ng), 75 μM of each dNTP, 0.2 μM of each primer, 1 μL of 10× *Taq* buffer, 0.25 U *Taq* DNA polymerase (Qiagen). The forward primer was end-labelled with one of the three fluorescent phosphoramidite dyes (FAM, HEX or NED) appropriate for ABI PRISM instruments. Amplifications were performed in an ABI thermocycler (Gene Amp PCR System 9700) using: an initial 12 min denaturation step at 95 °C, followed by 10 cycles with 15 s at 94 °C, 15 s at 53 °C, 30 s at 72 °C, then 20 cycles with 15 s at 89 °C, 15 s at 53 °C, 30 s at 72 °C and a final elongation step of 10 min at 72 °C.

PCR products were visualized and sized on an automated DNA sequencer (ABI PRISM 310) using GENESCAN version 3.1.2 (ABI) and ROX 400HD size standard (ABI).

Expected heterozygosities of loci were variable, ranging from 0.40 to 0.90 (Table 1) with an average of 0.56 (± 0.08). Our population did not show significant deviation from Hardy–Weinberg equilibrium after correcting for multiple tests (Rice 1989), nor was there evidence of linkage disequilibrium (GENEPOP, 3.1, Raymond & Rousset 1995).

Cross-species amplification was tested in other seabirds. Among the seven loci developped for *R. tridactyla*, only K6 and K67 failed to amplify in common murres *Uria aalge* (France). The PCR products of locus K32 and locus K67 proved polymorphic with eight and 2 alleles, respectively, in the 20 individuals tested. Loci K16, K31 and K32 were succesfully amplified in five individuals of Arctic skua *Stercorarius parasiticus* (Norway), with only locus K16 showing polymorphism (three alleles). In addition, five microsatellite loci developed for murres *U. aalge* and *U. lomvia* (Ibarguchi *et al*. 2000) were tested on *R. tridactyla*. All loci were amplified but only ulo12a12 (AF195180) was polymorphic with 2 alleles.

Results suggest that the microsatellite loci developed will be useful for investigating the mating system, population structure and dispersal abilities of *R. tridactyla*, and can be similarly employed for a variety of other seabird species.

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