PRIMER NOTE

Polymorphic microsatellites in the black-legged kittiwake
Rissa tridactyla

CLAIRE TIRARD,* FABRICE HELFENSTEIN† and ETIENNE DANCHIN†

*Laboratoire de Parasitologie Evolutive, Université Paris VI, CNRS-UMR 7103, BP 237, 75252 Paris Cedex 05, France, †Laboratoire d’Ecologie, Université Paris VI, CNRS UMR 7625, BP 237, 75252 Paris Cedex 05, France

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 (Aliciidae) 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 an 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 addressing 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 Smal. Fragments between 400 and 800 bp were isolated on a low-melting-point agarose gel, purified using a QIAquick Gel Extraction Kit (Qiagen, Courtaboeuf, France) and ligated into a plasmid vector pBluescript (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,
primes 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 10x 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 94 °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). 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 94 °C, 15 s at 53 °C, 30 s at 72 °C and a final elongation step of 10 min at 72 °C.

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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References


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