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SHORT NOTE

Polymorphic microsatellites identified by cross-species amplifications in the European Coot *Fulica atra*

Adeline Loyau · Dirk S. Schmeller

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Abstract We tested the cross-amplification of 26 microsatellites developed for passerines and an additional three developed for *Gallinula* species in eight European Coots from two populations. Sixteen microsatellite markers successfully amplified, of which nine were polymorphic with 2–6 alleles (mean 3.7 alleles) and an expected heterozygosity (H_e) ranging from 0.375 to 0.805 (mean $H_e = 0.589$). On average, we found 2.22 alleles/locus and a mean H_e of 0.440 in one nest, and 2.56 alleles/locus and a mean H_e of 0.494 in the other one. These nine polymorphic markers could be of potential use in studies of genetic variability, population structure and reproductive strategy of European Coots.

Keywords Coot · *Fulica atra* · Microsatellite · Cross-amplification

Introduction

The European Coot (*Fulica atra*) is a widely distributed waterbird species nesting on wetlands, ponds and rivers. Individuals usually aggregate in flocks and pairs are socially monogamous. Both parents build the nest, incubate the eggs, defend the breeding territory and care for the young that are semi-precocial.

In birds, alternative female reproductive behaviours such as extra-pair paternity and conspecific brood parasitism are widespread and are thought to be favoured by aggregation. Conspecific brood parasitism is commonly found in female American Coots (*F. americana*) (Lyon et al. 2002). Brood parasitism can be detected when there is a new egg two or more days after clutch completion or by counting the number of new eggs per day (e.g. Lyon et al. 2002). In the latter case, the variation in the pigmented egg shell is usually used as an indicator of maternal identity. The method, which cannot be used to detect extra-pair paternity, has been validated by fingerprinting in the American Coot (Lyon et al. 2002).

Fingerprinting, however, reveals complex multiple banding patterns in which it is not possible to assign alleles to loci. Hence, a practical, repeatable and reliable technique is needed. Polymorphic microsatellites are a useful tool to investigate mating strategies and also genetic variability, population structure and gene flow for a given species. Cross-amplification is widespread and is convenient to identify microsatellites in birds as it is less time consuming and cost intensive than developing species-specific markers (Primmer et al. 1996). Our aim was therefore to test for the cross-amplification of microsatellites in the European Coot *F. atra*.

Methods

We collected blood samples from eight individuals in the region of Angers (47°27′N, 00°32′W, Maine et Loire,

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A. Loyau · D. S. Schmeller
Department of Conservation Biology,
Helmholtz Centre for Environmental Research-UFZ,
Permoserstrasse 15, 04318 Leipzig, Germany

Present Address:
A. Loyau · D. S. Schmeller
Station d'Ecologie Expérimentale du CNRS à Moulis,
09200 Saint Girons, France
e-mail: dirk.schmeller@EcoEx-Moulis.cnrs.fr

A. Loyau (✉)
Laboratoire d'Ecologie, Université Pierre et Marie Curie-Paris 6,
75252 Paris, France
e-mail: al@adeline-loyau.net

France). The individuals stem from two nests from two ponds about 11 km apart. The chicks were caught on the nest by hand. We took blood samples from the right jugular vein and diluted them in 1 ml of PBS containing EDTA (2 mM). The samples were stored at 4°C until extraction. DNA was extracted using silica columns (QiaQuick 96 Kit, Qiagen) according to the procedure advised by the manufacturer. After extraction, DNA concentration was measured with a NanoDrop ND-1000 spectrophotometer (peQlab-Biotechnologie) and DNA was subsequently diluted to obtain samples of approximately 50 ng μl^{-1}.

We tested the cross-amplification of a set of 29 microsatellite markers by trial and error (Table 1). Twenty-six of them were originally developed for passerines. Three were isolated in the Tasmanian Native-hen *Gallinula mortierii* (Buchan 2000), a species belonging to the same family as the European Coot, as the probability of successful amplification and polymorphism is inversely related to the evolutionary distance between the two species (Primmer et al. 1996, 2005). One primer of each pair was labelled with a fluorescent dye at the 5′ end. We used a *Taq* PCR Core Kit (Qiagen) to perform Polymerase chain reactions (PCRs). Each 10-µl volume/sample contained: 1 µl of Qiagen PCR Buffer 10× (TrisCl, KCl, $(NH_4)_2SO_4$, 15 mM $MgCl_2$, pH 8.7), 0.75 µl of dNTP Mix (10 mM of each), 0.2 µl of each primer at 10 µM, 0.05 µl of Qiagen *Taq* DNA Polymerase 5U, 1 µl of DNA and H_2O. For Pca-8, Tm-27 and Tm-105 we added 1 µl of $MgCl_2$ (15 mM) and accordingly decreased the amount of H_2O. Samples were amplified in a DNA gradient cycler (PTC-200, Peltier Thermal Cycler) according to: 10 min of initial denaturation at 94°C; 35 cycles of 94°C for 30 s, corresponding annealing temperature (T_a) for 30 s and 72°C for 40 s; and a final extension of 72°C for 10 min. Five µl of the PCR products were controlled under UV light after electrophoresis on a 2.5% TAE-agarose gel stained with ethidium bromide. Thirteen of 26 passerine primers (50%) and all three Tasmanian hen primers successfully amplified (Table 1). Their amplification products were tested for polymorphism on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems) sequencer, except for Pca-7 that amplified a sequence longer than 1000 bp. We read the runs with GeneMapper v3.7 (Applied Biosystems).

Results and discussion

One primer (HrU-3) gave PCR products that were not readable and its PCR conditions would need to be improved by increasing the T_a. Four microsatellites were monomorphic, while nine were polymorphic with 2–6 alleles (Table 1). The expected heterozygosity (H_e) in nest 1 was 0.440 ($H_o = 0.556$) with 2.22 alleles per locus. The genetic variability was slightly higher in the second nest with H_e of 0.494 ($H_o = 0.630$) and 2.56 alleles per locus. Given the small sample size, we did not run a Hardy–Weinberg equilibrium test nor tested for null alleles. The proportion of passerine primers that amplified and were polymorphic was exceptionally high given the evolutionary distance between passerines and rails, suggesting that additional passerine primers might be successfully amplifyed in this species. Indeed, previous studies of cross-amplification among bird species predicted 20% of amplification and less than 10% of polymorphism (Primmer et al. 1996), or 30% and 17.5%, respectively (Primmer et al. 2005). Cross-amplification tends to produce shorter alleles (Van Hooft et al. 1999). Two of the polymorphic loci (Ase-18 and FhU-2) had sizes of less than 50 bp and may therefore be prone to misgenotyping due to a low resolution of current sequencers in that size region and should be used with caution. However, the banding pattern had been clear and consistent between runs on the ABI PRISM3100 Genetic Analyzer used here.

The origin of our samples from two nests limits our cross-amplification results in regard to the number of alleles and heterozygosity reported. However, a marker that is already polymorphic in a small sample size is very likely to be highly polymorphic in a larger sample size. Our results are therefore good indicators of the polymorphism of the amplified markers in a population.

For the first time, we provide nine polymorphic microsatellite markers that are of potential use in future studies of genetic variability, population structure and reproductive strategy of wild populations of the European Coot *F. atra*.

Zusammenfassung

Polymorphe Mikrosatelliten, identifiziert durch zwischenartliche Amplifizierung beim Europäischen Blässhuhn (*Fulica atra*)

Wir prüften die zwischenartliche Amplifizierung voninsgesamt 29 Mikrosatelliten in 8 Europäischen Blässhühnern aus 2 Populationen. Davon waren 26 spezifisch für Sperlingsvögel und 3 für den Genus *Gallinula*. 16 der Mikrosatelliten amplifizierten erfolgreich und 9 waren mit 2–6 Allelen polymorph (Mittelwert = 3.7 Allele). Die erwartete Heterozygotie (H_e) pro Mikrosatellit reichte von 0.375–0.805 (Mittlere $H_e = 0.589$). Im Mittel fanden wir 2.22 Allele pro Locus und eine mittlere H_e von 0.440 im ersten Nest, und 2.56 Allele pro Locus und eine mittlere H_e von 0.494 im zweiten Nest. Die 9 polymorphen Mikrosatelliten sind von potentiellem Nutzen für Studien der genetischen Variabilität, der genetischen

Table 1 Initial species, GenBank accession numbers, primer sequences, annealing temperature (T_a), size range, number of alleles, observed heterozygosity (H_o) and expected heterozygosity under Hardy–Weinberg equilibrium (H_e) for 29 cross-amplified microsatellite loci tested

	Initial species	GenBank accession no.	Repeat motif	Primer sequence (5′-3′)	T_a (°C)	Amplification	N_{amp}	Size range (bp)	No. of alleles	H_o	H_e
Ase-18	*Acrocephallus sechellensis*	AJ276375	$(GT)_{12}$	F-ATC CAG TCT TCG CAA AAG CC R-TGC CCC AGAVGGG AAG AAG	48	Yes	6	33–41	3	0.333	0.486
Ase-19	*Acrocephallus sechellensis*	AJ276376	$(CA)_4GA(CA)_5$	F-TAG GGT CCC AGG GAG GAA G R-TCT GCC CAT TAG GGA AAA GTC	50	No					
Ase-50	*Acrocephallus sechellensis*	AJ276379	$(CA)_{12}$	F-CTG TGG AAT GCT GTC TGG C R-ATC GAC TCC CGT CTA ACT TGC	53	Yes	8	228–264	2	0.500	0.375
Ase-56	*Acrocephallus sechellensis*	AJ276385	$(GT)_{18}$	F-TTC ACT GAG AAG TGA GAA TGT G RGTC CTT GAT TGA TTA CAG GCT-	50	No					
Cuμ-28	*Catharus ustulatus*	AF122894	$(CA)_{12}$	F-GAG GCA CAG AAA TGT GAA TT R-TAA GTA GAA GGA CTT GAT GGC T	50	No					
Escμ-6	*Emberiza schoeniclus*	X77082	$(CA)_{15}CG(CA)_{10}$	F-CAT AGT GAT GCC CTG CTA GG R-GCA AGT GCT CCT TAA TAT TAT TTG G	50	No					
FhU-2	*Ficedula hypoleuca*	X84361	$(CT)_{12}$	F-GTG TTC TA AAA CAT GCC TGG AGG R-GCA CAG GTA AAT ATT TGC TGG GCC	50	Yes	8	41–45	4	1.000	0.734
FhU-3	*Ficedula hypoleuca*	X84362	(GT)8A(TG)12	F-ATA TTC CCC ATA AGA TAA TGG R-ATA GTG TTG TCT TAA GGT CTC T	50	No					
FhU-4	*Ficedula hypoleuca*	X84363	$(GT)_{19}(GTTT)_{11}(T)_7$	F-GGA TTC CTA GTA ATT TAA ACT C R-CCT TCC AAA CTG AAG AGT AAG	50	No					
HrU-3	*Hirundo rustica*	X84088	$(CA)_{13}((A)_n(T)_n)_5$	F-CAC TGG CC TAG GCT GCT ATC R-CTG TCC CAT GTC AGG CCA GTC	50, 53	Yes	8	Smear			
HrU-4	*Hirundo rustica*	X84089	$(TC)_2A(TC)_{13}TA(TC)_4$	F-GAT CTT GTG AGA GGT TTG AAC R-CTT TCT GGA GGC AAA CCT TCA	50	No					
HrU-5	*Hirundo rustica*	X84090	$(GT)_{17}$	F-TCA ACA AGT GTC ATT AGG TTC R-AAC TTA GAT AAG GAA GGT ATA T	50	No					
HrU-6	*Hirundo rustica*	X84091	$(AAAG)_{17}(AG)_2(AAAG)_2$	F-GCT GTG TCA TTT CTA CAT GAG R-ACA G CAG TGT TAC TCT GC	50	No					
HrU-10	*Hirundo rustica*	X97562	$(CTCTT)_{32}$	F-ATA TTA ATA TAA ATG TTA AAT TC R-ATC TGA AAT CAG AGT CAC TCA	50	No					
Mjg-1	*Aphelocoma ultramarina*	U82673	(AAAG)	F-CCC GG AAA C TTC GTC TC R-GGA GAT T ATA TCG GTG GC	50	Yes	5	166–176	5	0.200	0.740
Patmp2-43	*Poecile atricapillus*	Otter et al. (1998)	Not published	F-ACA GGT AGT CAG AAA TGG AAA G R-GTA TCC AGA GTC TTT GCT GAT G	50	Yes	8	121	1		

Table 1 continued

	Initial species	GenBank accession no.	Repeat motif	Primer sequence (5′-3′)	T_a (°C)	Amplification	N_{amp}	Size range (bp)	No. of alleles	H_o	H_e
Pca-2	*Parus caeruleus*	AJ279804	$(CCAT)_{12}$	F-GTT GGC CTT CTT GGC CCC R-TGT TGG AGG TTA GGC CTC T	48	Yes	7	73	1		
Pca-5	*Parus caeruleus*	AJ279807	$(CA)_{17}$	F-TTG GCT GGG AGC AGA GCT G R-CCA GCC TGT CCT CAG CAG C	50, 53	No					
Pca-7	*Parus caeruleus*	AJ279809	$(TG)_{24}$	F-TGA GCA TCG TAG CCC AGC AG R-GGT TCA GGA CAC CTG CAC AAT G	53	Yes		>1,000	–		
Pca-8	*Parus caeruleus*	AJ279810	$(GATA)_{16}(TG)_{25}$	F-ACT TCT GAA ACA AAG ATG AAA TCA R-TGC CAT CAG TGT CAA ACC TG	53	Yes	7	301–325	4	0.143	0.500
Pdoμ-3	*Passer domesticus*	X93506	(CCAT)	F-CTG TTC ATT AAG TCA CAG GT R-AGT GAA ACT TTA ATC AGT TG	50	No					
Pdoμ-4	*Passer domesticus*	X93505	(GAAAGAGA)	F-CGA TAA GCT TGG ATC AGG ACT AC R-CTT GGG AAG AGA ATG AGT CAG GA	50	No					
Pdoμ-5	*Passer domesticus*	Y15126	$(TG)_{21}$	F-GAT GTT GCA GTG ACC TCT CTT G R-GCT GTG TTA ATG CTA TGA AAA TGG	50	Yes	8	180	1		
Pdoμ-6	*Passer domesticus*	Y15125	$(GAAA)_{28}$	F-CTG ATC ATG TGT AGA TGT AAGACT GC R-CAG ATC CTT AAG CAG GAA GTT AGG	50	Yes		~60			
PK-12	*Parus caeruleus*	AF041466	Not published	F-CGC TTG GAG ATA AAG ACA TT R-TAG CCT GGC ACT AAG AAC G	50	Yes	5	252–345	3	0.540	0.600
Pocc-6	*Phylloscopus occipitalis*	U59117	$(CA)_{16}$	F-TCA CCC TCA AAA ACA CAC ACA R-ACT TCT CTC TGA AAA GGG GAG C	53	Yes	4	562	1		
Tm-27	*Gallinula mortierii*	Buchan (2000)	$(TG)_{13}$	Not published	55	Yes	8	134–144	3	1.000	0.625
Tm-31B	*Gallinula mortierii*	Buchan (2000)	$(GT)_9GC(GT)_{14}$	Not published	52	Yes	7	174–188	3	0.571	0.439
Tm-105	*Gallinula mortierii*	Buchan (2000)	$(CA)_{21}$	Not published	57	Yes	6	135–161	6	1.000	0.806

– Data not evaluated

Populationsstruktur und der Reproduktionsstrategien des Europäischen Blässhuhns.

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