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PRIMER NOTE

Cross-amplification of polymorphic microsatellites reveals extra-pair paternity and brood parasitism in *Sturnus vulgaris*

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Abstract

We tested the cross-amplification of 37 microsatellites in a population of starlings (*Sturnus vulgaris*). Twenty-three of them amplified and five exhibited a large number of alleles per locus and high heterozygosity (on average: 14.6 alleles/locus and H_E = 0.704). We assessed the occurrence of extra-pair paternity (EPP) and intraspecific brood parasitism (IBP) in this population. The EPP rate was 16% to 18% offspring from 43% to 45% of nests. IBP was very variable between two successive years (14% to 27% chicks from 25% to 64% of clutches). These five polymorphic markers will be of potential use in studies of genetic diversity, population structure and reproductive strategy of this species.

Keywords: brood parasitism, EPP, microsatellites, *Sturnus vulgaris*

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The European starling (*Sturnus vulgaris*) is a semicolonially breeding passerine living in large permanent social groups. Taken together facultative polygyny and breeding synchrony can favour the spread of alternative breeding strategies in this species, such as extrapair paternity (EPP) and/or intraspecific brood parasitism (IBP). To investigate the frequency and intensity of both strategies in starlings, high-resolution genetic markers are needed.

Blood samples (20 µL) were obtained by venipuncture of breeding adults and their nestlings and diluted in 180 µL of Queen's lysis buffer (Seutin *et al*. 1991). DNA was extracted following a salt/chloroform procedure (Ehinger *et al*. 2002).

We used 37 microsatellites isolated from passerines to test their cross-amplification in six starlings of the same population (Table 1, Appendices 1, 2).

Polymerase chain reactions (PCRs) were performed in a 10 µL volume/sample containing: 1X PCR reaction Buffer (10 mM TrisHCl, pH = 9, 50 mM KCl, 1.5 mM $MgCl_2$, 0.1% TritonX100, 0.2 mg/mL BSA, Incubation Buffer, Q-Biogene), 75 µM of each dNTP, 200 µM of each primer, 0.25 U of *Taq* DNA Polymerase (Q-Biogene) and 15–50 ng of DNA.

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Samples were amplified in a DNA thermal cycler GeneAmp PCR System 9700 (Applied Biosystem) according to: 12 min initial denaturation at 94 °C; 10 cycles of 94 °C for 15 s, corresponding primer pair annealing temperature (T_a) for 15 s, and 72 °C for 30 s; 20 cycles of 89 °C for 15 s, T_a for 15 s, and 72 °C for 30 s; and a final extension of 72 °C for 10 mn to end.

PCR products were controlled under UV light after electrophoresis on a 2% agarose gel stained with ethidium bromide. We successfully amplified 23/37 markers and 15 out of them were tested for their polymorphism on 15 adults.

We ran PCR with one primer of each pair labelled with fluorescent dyes at the 5′ end. The amplification products were separated on 5% polyacrilamide gels by using ABI PRISM 310 monocapillar sequencer and were analysed using GENESCAN 3.1 (Applied Biosystem). Five out of 15 were highly polymorphic with at least six alleles (Table 1, Appendix 1).

One population of 290 individuals (94 adults and their 196 offspring) was studied near the Lausanne University campus (46°31′00″ N, 6°34′50″ E, Switzerland) during two successive breeding seasons. Blood samples were obtained from 28 complete families in 2001 and 20 families in 2002. The PCR were performed with Ase-18, FhU-3, Mjg-1, Patmp2–43 and Pca-7 primers under the conditions described above.

Table 1 Initial species, GenBank Accession nos, primer sequences, primer-dye, annealing temperatures (T_a), number of individuals, size range, number of alleles, observed heterozygosity (H_O), expected heterozygosity (H_E), exact *P*-values and SE of the Hardy–Weinberg equilibrium test for five cross-amplified microsatellite loci in *Sturnus vulgaris*

	Initial species	GenBank Accession no.	Primer sequence (5′–3′)	Primer-dye	T_a (°C)	No. of indiv.	Size range (bp)	No. of alleles	H_O	H_E	HW *P*-value	HW SE
Ase-18	*Acrocephalus sechellensis*	AJ276375	F-ATC CAG TCT TCG CAA AAG CC R-TGC CCC AGA GGG AAG AAG	NED – F	50	94	161–206	13	0.863	0.767	0.664	0.006
FhU-3	*Ficedula hypoleuca*	X84362	F-ATA TTC CCC ATA AGA TAA TGG R-ATA GTG TTG TCT TAA GGT CTC T	NED – F	49	91	137–181	14	0.620	0.656	0.015	0.001
Mjg-1	*Aphelocoma ultramarina*	U82673	F-CCC GGG AAA GGC TTC GTC TTC R-GGA GAT TTT ATA TCG GTG GC	FAM – F	60	94	152–190	17	0.853	0.917	0.066	0.003
Patmp2–43	*Poecile atricapillus*	Otter *et al*. (1998)	F-ACA GGT AGT CAG AAA TGG AAA G R-GTA TCC AGA GTC TTT GCT GAT G	FAM – F	63	94	119–129	6	0.653	0.537	0.993	0.001
Pca-7	*Parus caeruleus*	AJ279809	F-TGA GCA TCG TAG CCC AGC AG R-GGT TCA GGA CAC CTG CAC AAT G	HEX – F	56	94	104–160	20	0.789	0.723	0.984	0.002

We examined the variability of each locus among adult starlings (GENEPOP 1.2: Raymond & Rousset 1995). All the five microsatellites were polymorphic with an average allele number of 14/locus (range six to 20 alleles/locus). The average proportion of heterozygotes was 0.720. We then ran a Hardy–Weinberg and a genotypic equilibrium tests. One locus (FhU3) showed a slight heterozygote deficit that may just be a sampling effect with an under-representation of rare alleles (six alleles with $f < 0.01$). There was no other heterozygote deficit. The population showed no departure from Hardy–Weinberg equilibrium (multilocus test: $P = 0.207$, S.E. = 0.027). All tests for pair wise linkage disequilibrium between loci were not significant and the loci were therefore considered to be at linkage equilibrium. Furthermore, we assessed the total exclusionary power (CERVUS 2.0: Marshall *et al*. 1998). It was of 0.93 (with none parent known) and 0.99 (with one parent known) reflecting the high resolution of the system.

We investigated the frequency of EPP and IBP strategies in the population. The putative father was not the genetic father in 16% of offspring (18 /112) for 43% of nests (12/28) in 2001, and in 18% of offspring (15/84) for 45% of nests (9/20) in 2002. Overall we found that 17% of chicks (33/196) resulted from EPC and that 44% of broods (21/48) contained extra-pair young. Data from 2001 revealed that 27% of chicks (30/112) from 64% of clutches (18/28) were brood parasites whereas in 2002 only 14% of chicks (12/84) from 25% of clutches (5/20) were parasites.

Our results are consistent with previous finding reporting similar rates (10% to 20%) of EPC in the spotless starling (*Sturnus unicolor*) (Cordero *et al*. 2003). Using multiloci DNA fingerprinting, Pinxten *et al*. (1993) in a Belgian population of European starlings and Smith & Von Schantz (1993) in a Sweden one observed lower rates of EPP and IBP (EPP: 9% to 10% offspring from 29% to 32% of nests; IBP: 0 to 2% of chicks from 0 to 8% of clutches). Consequently, the frequency of EPP and IBP seem to vary considerably between different populations of European starlings. Moreover, in a given population, brood parasitism appears to be very variable from year to year and may constitute a female opportunistic strategy to changing environmental conditions (Pinxten *et al*. 1993).

To summarize, the five microsatellite loci selected exhibit a large number of allele per locus and high heterozygosity. They consequently will be of potential use in future studies of genetic diversity, population structure and reproductive strategy of this species in the wild.

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Appendix 1

Initial species, GenBank Accession nos, primer sequences, primer-dye, annealing temperatures (T_a), number of individuals, size range, number of alleles, for 18 other successfully cross-amplified microsatellite loci tested in *Sturnus vulgaris*

	Initial species	Accession no.	Primer sequence (5′– 3′)	T_a (°C)	No. of indiv.	Amplification	Size range (bp)	No. of alleles
Ase-19	*Acrocephalus sechellensis*	AJ276376	F-TAG GGT CCC AGG GAG GAA G R-TCT GCC CAT TAG GGA AAA GTC	57	15	yes	269–296	6
Ase-40	*Acrocephalus sechellensis*	AJ276642	F-CAC TGC TCC AGG CAC TCT G R-TCC AAG GCA CAC AAA GGT G	58	6	yes	—	—
Ase-50	*Acrocephalus sechellensis*	AJ276779	F-CTG TGG AAT GCT GTC TGG C R-ATG GAC TCC CGT CTA ACT TGC	63	15	yes	159–171	3
Ase-56	*Acrocephalus sechellensis*	AJ276785	F-TTC ACT GAG AAG TGA GAA TGT G R-GTC CTT GAT TGA TTA CAG GCT	60	15	yes	279	1
Cuμ-10	*Catharus ustulatus*	AF122893	F-AAA ATG AGG AGA ATA CTA GGC A R-ACT TAT TTC AGT CCT AAA TTC ACC	60	6	yes	—	—
Cuμ-28	*Catharus ustulatus*	AF122894	F-GAG GCA CAG AAA TGT GAA TT R-TAA GTA GAA GGA CTT GAT GGC T	60	15	yes	123–132	4
HrU-2	*Hirundo rustica*	X84087	F-CAT CAA GAG AGG GAT GGA AAG AGG R-GAA AAG ATT ATT TTT CTT TCT CCC	50	4	yes	132–134	2
HrU-3	*Hirundo rustica*	X84088	F-CAC TGG CTC TAG GCTGTC ATC R-CTG TCC CAT GTC AGG CCA GTC	50	4	yes	178–258	3
HrU-6	*Hirundo rustica*	X84091	F-GCT GTG TCA TTT CTA CAT GAG R-ACA GGG CAG TGT TAC TCT GC	50	4	yes	—	—
Mcyμ-4	*Malurus cyaneus*	U82388	F-ATA AGA TGA CTA AGG TCT CTG GTG R-TAG CAA TTG TCT ATC ATG GTT TG	58	6	yes	—	—
Pca-2	*Parus caeruleus*	AJ279804	F-GTT GGC CTT CTT GGC CCC R-TGT TGG AGG TTA GGC CTC T	51	6	yes	—	—
Pca-4	*Parus caeruleus*	AJ279806	F-AAT GTC TTA CAG GCA AAG TCC CCA R-AAC TTG AAG CTT CTG GCC TGA ATG	52	6	yes	—	—
Pca-8	*Parus caeruleus*	AJ279810	F-ACT TCT GAA ACA AAG ATG AAA TCA R-TGC CAT CAG TGT CAA ACC TG	50	15	yes	96	1
Pdoμ-4	*Passer domesticus*	X93505	F-CGA TAA GCT TGG ATC AGG ACT AC R-CTT GGG AAG AGA ATG AGT CAG GA	50	6	yes	—	—
Pdoμ-5	*Passer domesticus*	Y15126	F-GAT GTT GCA GTG ACC TCT CTT G R-GCT GTG TTA ATG CTA TGA AAA TGG	51	15	yes	202	1
Pdoμ-6	*Passer domesticus*	Y15125	F-CTG ATC ATG TGT AGA TGT AAG ACT GC R-CAG ATC CTT AAG CAG GAA GTT AGG	51	6	yes	—	—
Pocc-6	*Phylloscopus occipitalis*	U59117	F-TCA CCC TCA AAA ACA CAC ACA R-ACT TCT CTC TGA AAA GGG GAG C	50	4	yes	178–258	3
PK-12	*Parus caeruleus*	AF041466	F-CGC TTG GAG ATA AAG ACA TT R-TAG CCT GGC ACT AAG AAC G	49	15	yes	208	1

—, not tested for polymorphism.

Appendix 2

Initial species, GenBank Accession nos or references, primer sequences, primer-dye, annealing temperatures (T_a) tested for 14 unsuccessful cross-amplified microsatellite loci tested in *Sturnus vulgaris*

	Initial species	Accession no.	Primer sequence (5′– 3′)	T_a (°C)	No. of indiv.
BMC-3	*Manorina melanophrys*	AF005376	F-CCT GGC TGC CTG CAC AGA C R-TGA ATT GCA GCT TCT GGG TGC	48, 49, 50	6
Cuμ-05	*Catharus ustulatus*	AF122892	F-ACC TCT AAA TAC CTG TGA GTG C R-ACT GTG GTA TTC TTT ACC TAG CA	60	6
Escμ-6	*Emberiza schoeniclus*	X77082	F-CAT AGT GAT GCC CTG CTA GG R-GCA AGT GCT CCT TAA TAT TTG G	50	4
FhU-1	*Ficedula hypoleuca*	X84360	F-TGA TCG AAA GAC CTG TAA GAT R-ATC AGC GTT AGA CCA ATA CTC TTA	48, 49, 50, 51, 52, 53	6
FhU-2	*Ficedula hypoleuca*	X8461	F-GTG TTC TTA AAA CAT GCC TGG AGG R-GCA CAG GTA AAT ATT TGC TGG GCC	48, 50, 52, 54, 56, 58	6
FhU-4	*Ficedula hypoleuca*	X84363	F-GGA TTC CTA GTA ATT TAA ACT C R-CCT TCC AAA CTG AAG AGT AAG	46, 48, 50, 52	6
FhU-5	*Ficedula hypoleuca*	X84364	F-TGG GAA GTG CAG CAG TCC AG R-CAG CCC TCT CAC CTG TGT GCA	55, 56, 57	6
FhU-6	*Ficedula hypoleuca*	X84365	F-ATC TGC TCC TCT GGG CCC TG R-GAT CCC TGT TCC TGG GTT AC	66	6
HrU-4	*Hirundo rustica*	X84089	F-GAT CTT GTG AGA GGT TTG AAC R-CTT TCT GGA GGC AAA CCT TCA	50	4
HrU-5	*Hirundo rustica*	X84090	F-TCA ACA AGT GTC ATT AGG TTC R-AAC TTA GAT AAG GAA GGT ATA T	50	4
HrU-10	*Hirundo rustica*	X97562	F-ATA TTA ATA TAA ATG TTA AAT TC R-ATC TGA AAT CAG AGT CAC TCA	50	4
Pca-5	*Parus caeruleus*	AJ279807	F-TTG GCT GGG AGC AGA GCT G R-CCA GCC TGT CCT CAG CAG C	53, 54, 55	6
Pdoμ-3	*Passer domesticus*	X93506	F-CTG TTC ATT AAG TCA CAG GT R-AGT GAA ACT TTA ATC AGT TG	48, 49, 50	6
Phtr-2	*Phylloscopus trochilus*	Fridolfsson *et al*. (1997)	F-CGC AGG CTC AGA AAT ACT TGA R-GCC CAC AGC TCA ATA GTC TT	48, 49	6