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Transferability of microsatellite markers among economically and ecologically important galliform birds

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ABSTRACT. We used the partially sequenced genomes of the turkey and chicken to find a large number of microsatellite markers. We then characterized 10 polymorphic microsatellite markers developed by cross-species amplification from economically and ecologically important birds to various European subspecies of the grey partridge. Even though we used cross-species amplification, a high degree of polymorphism was conserved in all microsatellite markers. Cross-species amplification from

birds of economic and ecological interest, such as chicken and turkey, could be an attractive approach to develop microsatellite markers and to use these to manage wild and captive populations of other galliforms, such as the grey partridge.

Key words: Grey partridge; Turkey; Chicken; Microsatellite markers; Cross-species amplification

INTRODUCTION

Among galliforms, the chicken (*Gallus gallus*) and turkey (*Meleagris gallopavo*) are of particular economic interest, and their genomes are respectively entirely and partially sequenced. Several hundred primer pairs have already been defined to amplify microsatellite markers in these two species. In this study, we proposed to test the transferability of these markers to the grey partridge (*Perdix perdix*), another galliform species of ecological and economic interest. Considering that the galliform phylogenetic tree shows that the chicken is basal and that the turkey is a sister taxon of the grey partridge (Dimcheff et al., 2002; Kimball et al., 1999), we hypothesized that primer pairs identified in chicken and conserved in turkey (or conversely) should also be conserved in grey partridge. The grey partridge is represented by 8 allopatric sub-species occurring in Eurasia. Like other galliforms, such as Capercaillie (Segelbacher and Storch, 2002) or rock ptarmigan (Bech et al., 2009), global warming would cause the loss of suitable habitat and would thus increase population fragmentation of the grey partridge. In France, this habitat fragmentation would lead to the geographical isolation of two sub-species, *Perdix perdix hispaniensis* (included in the red data book of threatened species) in the Pyrenees and *Perdix perdix armoricana* in the north of France. Moreover, *P. p. hispaniensis* and *P. p. armoricana* represent important small game species, and because they are depleted by hunting, they are regularly restocked. Because restocking involves individuals originating from distinct European sub-species, wild populations display a risk of genetic introgression (Martin et al., 2003).

Microsatellite markers are a very useful tool for both ecological monitoring of wild populations and stock management and enhancement of captive-bred individuals. In this paper, we present the characterization of microsatellite markers usable for the European grey partridge.

MATERIAL AND METHODS

Cross-species amplification

In this study, we tested 138 microsatellite primer pairs (see Supporting Information Online; Supplementary Material) on the grey partridge, which have been successfully amplified in both chicken and turkey. Three other criteria were also considered: i) primer pairs would show high annealing temperature in source species; ii) the microsatellite loci would be polymorphic in their source species, and iii) the microsatellite loci would be located on different chicken chromosomes in order to avoid potential linkage disequilibrium. Sampling included liver and feather samples,

which were collected during the hunting season ($N = 82$). Main samples came from two distinct wild sub-species (*P. p. hispaniensis* and *P. p. armoricana*). Some other samples came from stockbreeding with an imprecise geographical origin. Genomic DNA was extracted using silica columns (e.Z.N.A from OMEGA BIO-TEK), according to the manufacturer protocol. Polymerase chain reaction was performed using the QIAGEN multiplex kit. Multiplexes were carried out according to the manufacturer standard microsatellite amplification protocol in a final volume of 10 μL and at 57°C for annealing temperature.

Statistical analysis

Departures from Hardy-Weinberg expectations and linkage disequilibria were assessed for each specific microsatellite markers using the exact tests (1000 permutations) implemented in GENEPOP version 3.4 (Raymond and Rousset, 1995). Polymorphism of microsatellite markers, which gave us a specific amplification, was estimated with the number of alleles (A), and expected heterozygosity (H_e) (Weir and Cockerham, 1984) using FSTAT version 2.9.3.2 (Goudet, 2001) with 800 permutations.

RESULTS

Among 138 loci tested, 76 gave amplification with only one specific product (see Supporting Information Online; Supplementary Material). Of these, 10 microsatellite markers were polymorphic (Table 1). Among these, no evidence of linkage disequilibrium was detected ($P < 0.001$, significance threshold adjusted with the Bonferroni's procedure, for 45 tests). After Bonferroni's correction for multiple comparison ($P < 0.005$), only two loci showed significant deviation from Hardy-Weinberg expectations: MNT412 and MNT45 ($F_{IS} > 0$). This heterozygosity deficit could be interpreted as the result of a Wahlund effect because our sampling included several samples from different geographical regions but not real field populations, which are not yet determined. Concerning polymorphism, the number of alleles per locus ranged from 3 to 19 and gene diversity ranged from 0.51 to 0.89 (Table 1).

DISCUSSION

This study shows that genomic sequence databases of economically important animals are useful tools to identify conserved genetic markers. Because there are not many microsatellites in avian species (Primmer et al., 1997), cross-species amplification from birds of economic interest could be an attractive approach to manage wild and captive populations of other birds such as the grey partridge. In birds, it has been shown that the polymorphism rate of microsatellite markers decreases with the increase in genetic distance from the species from which they have developed (Ellegren et al., 1995; Primmer et al., 1996). With the use of cross-species amplification, a high polymorphism was conserved in our microsatellite markers, which is very promising in terms of usefulness. Studies based on these markers are now underway.

Table 1. Characteristics of 10 polymorphic microsatellite loci developed by cross-species amplification for the grey partridge.

Species name	Marker name	Number of alleles	Origin species			Primer sequences (5' - 3')	<i>Perdix perdix</i> (N = 82)					
			GenBank accession No.	Fragment size	Position on chromosome		T _a (°C)	Fragment size	Multiplex (Dye number)	A	H _e	F _{is}
<i>M. gallopavo</i>	MNT12	1	AF482371	147	4	F: AGGTGTTTTGGGCAGTCTC R: TGCAAGCACCATCTGCTAAG	59.5	145-185	2 (D4)	19	0.822	+0.139
<i>M. gallopavo</i>	MNT412	3	AL592829	162-166	19	F: CCCATGTGAGCAGTGAATTG R: GTCATCACAGTGGAGGATCG	61.7	234-272	2 (D2)	14	0.889	+0.131*
<i>G. gallus</i>	ADL0292	2	G01710	126	5	F: CCAAATCAGGCAAACTTCT R: AAATGGCCTAAGGATGAGGA	61.7	98-112	3 (D4)	3	0.444	+0.049
<i>M. gallopavo</i>	MNT477	1	AL593755	230	5	F: TTCACCACGCTCATTCAAAG R: TCCAAAATGTGACTAGATGATAAAGTG	56.5	227-263	3 (D4)	17	0.811	+0.071
<i>M. gallopavo</i>	MNT404	3	AL593651	283-297	13	F: AACCAGCTCTGGAGATACCG R: GGACTGCAAGGACAACATCC	61.7	242-254	2 (D3)	4	0.510	-0.040
<i>M. gallopavo</i>	MNT45	?	AY235058	140	3	F: ACATGGAGGCAGAGAACCTC R: TGTCAGCCTGAATGTTTCCTC	59.5	95-112	2 (D3)	7	0.686	+0.292*
<i>G. gallus</i>	MCW230	?	G32001	281-298	11	F: TGCACAGAGCCAAGCTGCTTC R: GCAACTTTCTGCAGGCTCA †	61.7	227-243	1 (D2)	5	0.746	+0.263
<i>M. gallopavo</i>	MNT467	4	AL593567	232	7	F: AGTGGCAGTTTGTGGATCTTG R: TCGTACTGGCAGGCATGTAG	61.7	235-239	1 (D4)	3	0.510	-0.145
<i>G. gallus</i>	ADL0260	2	G01680	129	4	F: GGAGCTGTCATCCACTCTTG R: ATCAGCCCATCCCAGTATCG	56.5	94-114	1 (D3)	4	0.574	+0.001
<i>M. gallopavo</i>	MNT408	3	AY926543	230	1	F: GTGTCCTGCCACACTACAG R: GGAATTTGCTCCAACCTGAC	56.5	223-237	3 (D3)	6	0.727	-0.061

Origin species of the marker; Marker name; Numer of alleles in the origem species; GenBank accession number; Fragment size in the origin species; Position of the marker on the chicken chromosomes; Primer sequences (F: forward, R: reverse); Ta: annealing temperature in the grey partridge (°C); fragment size in grey partridge; multiplex and dye number according to Beckman Coulter codes; A: number of alleles; H_e: expected heterozygosity; F_{is}*: significant deviation from Hardy-Weinberg equilibrium after Bonferroni's correction for multiple comparison (P < 0.005). † Reverse primer sequence of MCW230 (G32001) has been redesigned in order to have a better specificity.

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Supplementary material.

Supporting Information Online. List of microsatellite markers tested but not selected.

Origin species	Marker name	GenBank accession No.	Reference
<i>G. gallus</i>	ADL0019	L23887	(Reed et al., 2000a)
<i>G. gallus</i>	ADL0039	L23917	(Reed et al., 2000a)
<i>G. gallus</i>	ADL0044	L23909	(Reed et al., 2000a)
<i>G. gallus</i>	ADL0102	G01547	(Reed et al., 2000a)
<i>G. gallus</i>	ADL0106	G01550	(Reed et al., 2000a)
<i>G. gallus</i>	ADL0114	G01726	(Reed et al., 2000a)
<i>G. gallus</i>	ADL0127	G01736	(Reed et al., 2000a)
<i>G. gallus</i>	ADL0132	G01740	(Reed et al., 2000a)
<i>G. gallus</i>	ADL0136	G01561	(Reed et al., 2000a)
<i>G. gallus</i>	ADL0138	G01563	(Reed et al., 2000a)
<i>G. gallus</i>	ADL0143	G01568	(Reed et al., 2000a)
<i>G. gallus</i>	ADL0146	G01571	(Reed et al., 2000a)
<i>G. gallus</i>	ADL0147	G01572	(Reed et al., 2000a)
<i>G. gallus</i>	ADL0149	G01574	(Reed et al., 2000a)
<i>G. gallus</i>	ADL0150	G01575	(Reed et al., 2000a)
<i>G. gallus</i>	ADL0155	G01742	(Reed et al., 2000a)
<i>G. gallus</i>	ADL0158	G01582	(Reed et al., 2000a)
<i>G. gallus</i>	ADL0160	G01584	(Reed et al., 2000a)
<i>G. gallus</i>	ADL0171	G01593	(Reed et al., 2000a)
<i>G. gallus</i>	ADL0172	G01594	(Reed et al., 2000a)
<i>G. gallus</i>	ADL0176	G01598	(Reed et al., 2000a)
<i>G. gallus</i>	ADL0179	G01601	(Reed et al., 2000a)
<i>G. gallus</i>	ADL0181	G01603	(Reed et al., 2000a)
<i>G. gallus</i>	ADL0230	G01650	(Reed et al., 2000a)
<i>G. gallus</i>	ADL0234	G01654	(Reed et al., 2000a)
<i>G. gallus</i>	ADL0254	G01674	(Reed et al., 2000a)
<i>G. gallus</i>	ADL0262	G01682	(Reed et al., 2000a)
<i>G. gallus</i>	ADL0266	G01686	(Reed et al., 2000a)
<i>G. gallus</i>	ADL0267	G01687	(Reed et al., 2000a)
<i>G. gallus</i>	ADL0268	G01688	(Reed et al., 2000a)
<i>G. gallus</i>	ADL0272	G01692	(Reed et al., 2000a)
<i>G. gallus</i>	ADL0279	G01699	(Reed et al., 2000a)
<i>G. gallus</i>	ADL0306	G01721	(Reed et al., 2000a)
<i>G. gallus</i>	ADL0315	G16117	(Pang et al., 1999)
<i>G. gallus</i>	ADL210	G01630	(Reed et al., 2000a)
<i>G. gallus</i>	HUJ0005A	L10231	(Reed et al., 2000a)
<i>G. gallus</i>	HUJ0006A	L10294	(Reed et al., 2000a)
<i>G. gallus</i>	LEI0089	X83239	(Gibbs et al., 1997)
<i>G. gallus</i>	LEI0098	X82860	(Gibbs et al., 1997)
<i>G. gallus</i>	LEI0100	X82859	(Gibbs et al., 1997)
<i>G. gallus</i>	LEI0101	X82805	(Gibbs et al., 1997)
<i>G. gallus</i>	LEI0103	X82796	(Gibbs et al., 1997)
<i>G. gallus</i>	LEI0106	X82854	(Gibbs et al., 1997)
<i>G. gallus</i>	LEI0107	X83253	(Gibbs et al., 1997)
<i>G. gallus</i>	LEI0108	X85517	(Gibbs et al., 1997)
<i>G. gallus</i>	LEI0112	X82789	(Gibbs et al., 1997)

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Origin species	Marker name	GenBank accession No.	Reference
<i>G. gallus</i>	LEI0147	X83256	(Reed et al., 2000a)
<i>G. gallus</i>	LEI126	X82799	(Reed et al., 2000a)
<i>G. gallus</i>	LEI132	X82856	(Reed et al., 2000a)
<i>G. gallus</i>	LEI161	X85524	(Reed et al., 2000a)
<i>G. gallus</i>	LEI169	X85535	(Gibbs et al., 1997)
<i>G. gallus</i>	MCW0005	-	(Reed et al., 2000a)
<i>G. gallus</i>	MCW0023	-	(Reed et al., 2000a)
<i>G. gallus</i>	MCW0080	-	(Reed et al., 2000a)
<i>G. gallus</i>	MCW0169	-	(Reed et al., 2000a)
<i>G. gallus</i>	MCW0228	-	(Reed et al., 2000a)
<i>G. gallus</i>	MCW0246	-	(Reed et al., 2000a)
<i>G. gallus</i>	MCW0300	-	(Reed et al., 2000a)
<i>G. gallus</i>	MCW0331	-	(Reed et al., 2000a)
<i>G. gallus</i>	MCW211	G31988	(Reed et al., 2000a)
<i>G. gallus</i>	UMA1.019	-	(Reed et al., 2000a)
<i>M. gallopavo</i>	MNT001	AF176506	(Reed et al., 2000b)
<i>M. gallopavo</i>	MNT003	AF176508	(Reed et al., 2000b)
<i>M. gallopavo</i>	MNT005	AF176510	(Reed et al., 2000b)
<i>M. gallopavo</i>	MNT006	AF176511	(Reed et al., 2000b)
<i>M. gallopavo</i>	MNT007	AF176512	(Reed et al., 2000b)
<i>M. gallopavo</i>	MNT011	AF482370	(Reed et al., 2002)
<i>M. gallopavo</i>	MNT019	AF482378	(Reed et al., 2002)
<i>M. gallopavo</i>	MNT020	AF482379	(Reed et al., 2002)
<i>M. gallopavo</i>	MNT021	AY235034	(Reed et al., 2003)
<i>M. gallopavo</i>	MNT041	AY235054	(Reed et al., 2003)
<i>M. gallopavo</i>	MNT043	AY235056	(Reed et al., 2003)
<i>M. gallopavo</i>	MNT044	AY235057	(Reed et al., 2003)
<i>M. gallopavo</i>	MNT049	AY235062	(Reed et al., 2003)
<i>M. gallopavo</i>	MNT058	AY235071	(Reed et al., 2003)
<i>M. gallopavo</i>	MNT061	AY235074	(Reed et al., 2003)
<i>M. gallopavo</i>	MNT118	AF540421	(Dranchak et al., 2003)
<i>M. gallopavo</i>	MNT127	AY235106	(Reed et al., 2003)
<i>M. gallopavo</i>	MNT129	AF540431	(Dranchak et al., 2003)
<i>M. gallopavo</i>	MNT130	AF540432	(Dranchak et al., 2003)
<i>M. gallopavo</i>	MNT131	AY235107	(Reed et al., 2003)
<i>M. gallopavo</i>	MNT139	AY235115	(Reed et al., 2003)
<i>M. gallopavo</i>	MNT141	AF540433	(Dranchak et al., 2003)
<i>M. gallopavo</i>	MNT160	AY235129	(Reed et al., 2003)
<i>M. gallopavo</i>	MNT171	AY235139	(Reed et al., 2003)
<i>M. gallopavo</i>	MNT181	AY235149	(Reed et al., 2003)
<i>M. gallopavo</i>	MNT188	AY235156	(Reed et al., 2003)
<i>M. gallopavo</i>	MNT201	AY235168	(Reed et al., 2003)
<i>M. gallopavo</i>	MNT204	AY235171	(Reed et al., 2003)
<i>M. gallopavo</i>	MNT211	AY235176	(Reed et al., 2003)
<i>M. gallopavo</i>	MNT215	AY235180	(Reed et al., 2003)
<i>M. gallopavo</i>	MNT219	AY235184	(Reed et al., 2003)
<i>M. gallopavo</i>	MNT220	AY235185	(Reed et al., 2003)
<i>M. gallopavo</i>	MNT221	AY235186	(Reed et al., 2003)
<i>M. gallopavo</i>	MNT222	AY235187	(Reed et al., 2003)
<i>M. gallopavo</i>	MNT224	AY235189	(Reed et al., 2003)

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Origin species	Marker name	GenBank accession No.	Reference
<i>M. gallopavo</i>	MNT226	AY235191	(Reed et al., 2003)
<i>M. gallopavo</i>	MNT249	AY552821	(Knutson et al., 2004)
<i>M. gallopavo</i>	MNT250	AY552822	(Knutson et al., 2004)
<i>M. gallopavo</i>	MNT275	AY552847	(Knutson et al., 2004)
<i>M. gallopavo</i>	MNT276	AY552848	(Knutson et al., 2004)
<i>M. gallopavo</i>	MNT291	AY552863	(Knutson et al., 2004)
<i>M. gallopavo</i>	MNT399	AY926537	(Chaves et al., 2006)
<i>M. gallopavo</i>	MNT400	AY926538	(Chaves et al., 2006)
<i>M. gallopavo</i>	MNT401	AY926539	(Chaves et al., 2006)
<i>M. gallopavo</i>	MNT402	AL592658	(Chaves et al., 2006)
<i>M. gallopavo</i>	MNT405	AY926540	(Chaves et al., 2006)
<i>M. gallopavo</i>	MNT407	AY926542	(Chaves et al., 2006)
<i>M. gallopavo</i>	MNT409	AL593356	(Chaves et al., 2006)
<i>M. gallopavo</i>	MNT410	AL592718	(Chaves et al., 2006)
<i>M. gallopavo</i>	MNT411	AY926544	(Chaves et al., 2006)
<i>M. gallopavo</i>	MNT414	AY926546	(Chaves et al., 2006)
<i>M. gallopavo</i>	MNT416	AY926548	(Chaves et al., 2006)
<i>M. gallopavo</i>	MNT427	AL592640	(Chaves et al., 2006)
<i>M. gallopavo</i>	MNT429	AL592644	(Chaves et al., 2006)
<i>M. gallopavo</i>	MNT433	AL592677	(Chaves et al., 2006)
<i>M. gallopavo</i>	MNT436	AL592829	(Chaves et al., 2006)
<i>M. gallopavo</i>	MNT437	AL593814	(Chaves et al., 2006)
<i>M. gallopavo</i>	MNT442	AL593052	(Chaves et al., 2006)
<i>M. gallopavo</i>	MNT443	AL593060	(Chaves et al., 2006)
<i>M. gallopavo</i>	MNT448	AL593178	(Chaves et al., 2006)
<i>M. gallopavo</i>	MNT450	AL593308	(Chaves et al., 2006)
<i>M. gallopavo</i>	MNT453	AL593364	(Chaves et al., 2006)
<i>M. gallopavo</i>	MNT454	AL593401	(Chaves et al., 2006)
<i>M. gallopavo</i>	MNT456	AL593466	(Chaves et al., 2006)
<i>M. gallopavo</i>	MNT458	AL593487	(Chaves et al., 2006)
<i>M. gallopavo</i>	MNT466	AL593563	(Chaves et al., 2006)
<i>M. gallopavo</i>	MNT406	AY926541	(Chaves et al., 2006)

Origin species of the marker; Marker name; GenBank accession number; Article reference. In bold, specific but not polymorphic markers in grey partridge.

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