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N. Bech, C. Novoa, Jean-François Allienne, Jérôme Boissier. Transferability of microsatellite markers among economically and ecologically important galliform birds.. *Genetics and molecular research : GMR*, 2010, 9 (2), pp.1121-9. 10.4238/vol9-2gmr760 . halsde-00729015

**HAL Id: halsde-00729015**

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Submitted on 7 Sep 2012

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# Transferability of microsatellite markers among economically and ecologically important galliform birds

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Genet. Mol. Res. 9 (2): 1121-1129 (2010)

Received January 8, 2010

Accepted March 20, 2010

Published June 15, 2010

DOI 10.4238/vol9-2gmr760

**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  $\mu\text{L}$  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 <sub>a</sub> (°C)	Fragment size	Multiplex (Dye number)	A	H <sub>e</sub>	F <sub>is</sub>
<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: GCAACTTCTGCAGGCTCA †	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: ATCAGCCATCCCAGTATCG	56.5	94-114	1 (D3)	4	0.574	+0.001
<i>M. gallopavo</i>	MNT408	3	AY926543	230	1	F: GTGTCCCTGCCACACTACAG 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<sub>e</sub>: expected heterozygosity; F<sub>is</sub>\*: 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.

## ACKNOWLEDGMENTS

Research supported by Bureau des Ressources Génétiques, Office National de la Chasse et de la Faune Sauvage, Ministère de l'Enseignement Supérieur et de la Recherche Scientifique and Centre National de la Recherche Scientifique (all in France). Equally, we would like to thank especially David Ripoll and Mathieu Blanchard who contributed equally to this project.

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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>	<b>ADL0019</b>	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>	<b>ADL0143</b>	G01568	(Reed et al., 2000a)
<i>G. gallus</i>	<b>ADL0146</b>	G01571	(Reed et al., 2000a)
<i>G. gallus</i>	<b>ADL0147</b>	G01572	(Reed et al., 2000a)
<i>G. gallus</i>	<b>ADL0149</b>	G01574	(Reed et al., 2000a)
<i>G. gallus</i>	ADL0150	G01575	(Reed et al., 2000a)
<i>G. gallus</i>	<b>ADL0155</b>	G01742	(Reed et al., 2000a)
<i>G. gallus</i>	ADL0158	G01582	(Reed et al., 2000a)
<i>G. gallus</i>	<b>ADL0160</b>	G01584	(Reed et al., 2000a)
<i>G. gallus</i>	ADL0171	G01593	(Reed et al., 2000a)
<i>G. gallus</i>	<b>ADL0172</b>	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>	<b>ADL0230</b>	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>	<b>ADL0279</b>	G01699	(Reed et al., 2000a)
<i>G. gallus</i>	<b>ADL0306</b>	G01721	(Reed et al., 2000a)
<i>G. gallus</i>	<b>ADL0315</b>	G16117	(Pang et al., 1999)
<i>G. gallus</i>	<b>ADL210</b>	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>	<b>LEI0089</b>	X83239	(Gibbs et al., 1997)
<i>G. gallus</i>	<b>LEI0098</b>	X82860	(Gibbs et al., 1997)
<i>G. gallus</i>	LEI0100	X82859	(Gibbs et al., 1997)
<i>G. gallus</i>	<b>LEI0101</b>	X82805	(Gibbs et al., 1997)
<i>G. gallus</i>	<b>LEI0103</b>	X82796	(Gibbs et al., 1997)
<i>G. gallus</i>	LEI0106	X82854	(Gibbs et al., 1997)
<i>G. gallus</i>	<b>LEI0107</b>	X83253	(Gibbs et al., 1997)
<i>G. gallus</i>	<b>LEI0108</b>	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>	<b>LEI0147</b>	X83256	(Reed et al., 2000a)
<i>G. gallus</i>	LEI126	X82799	(Reed et al., 2000a)
<i>G. gallus</i>	<b>LEI132</b>	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>	<b>MCW0005</b>	-	(Reed et al., 2000a)
<i>G. gallus</i>	<b>MCW0023</b>	-	(Reed et al., 2000a)
<i>G. gallus</i>	<b>MCW0080</b>	-	(Reed et al., 2000a)
<i>G. gallus</i>	<b>MCW0169</b>	-	(Reed et al., 2000a)
<i>G. gallus</i>	<b>MCW0228</b>	-	(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>	<b>MCW0331</b>	-	(Reed et al., 2000a)
<i>G. gallus</i>	<b>MCW211</b>	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>	<b>MNT003</b>	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>	<b>MNT007</b>	AF176512	(Reed et al., 2000b)
<i>M. gallopavo</i>	<b>MNT011</b>	AF482370	(Reed et al., 2002)
<i>M. gallopavo</i>	<b>MNT019</b>	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>	<b>MNT043</b>	AY235056	(Reed et al., 2003)
<i>M. gallopavo</i>	MNT044	AY235057	(Reed et al., 2003)
<i>M. gallopavo</i>	<b>MNT049</b>	AY235062	(Reed et al., 2003)
<i>M. gallopavo</i>	<b>MNT058</b>	AY235071	(Reed et al., 2003)
<i>M. gallopavo</i>	MNT061	AY235074	(Reed et al., 2003)
<i>M. gallopavo</i>	<b>MNT118</b>	AF540421	(Dranchak et al., 2003)
<i>M. gallopavo</i>	<b>MNT127</b>	AY235106	(Reed et al., 2003)
<i>M. gallopavo</i>	MNT129	AF540431	(Dranchak et al., 2003)
<i>M. gallopavo</i>	<b>MNT130</b>	AF540432	(Dranchak et al., 2003)
<i>M. gallopavo</i>	<b>MNT131</b>	AY235107	(Reed et al., 2003)
<i>M. gallopavo</i>	<b>MNT139</b>	AY235115	(Reed et al., 2003)
<i>M. gallopavo</i>	<b>MNT141</b>	AF540433	(Dranchak et al., 2003)
<i>M. gallopavo</i>	<b>MNT160</b>	AY235129	(Reed et al., 2003)
<i>M. gallopavo</i>	<b>MNT171</b>	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>	<b>MNT204</b>	AY235171	(Reed et al., 2003)
<i>M. gallopavo</i>	<b>MNT211</b>	AY235176	(Reed et al., 2003)
<i>M. gallopavo</i>	MNT215	AY235180	(Reed et al., 2003)
<i>M. gallopavo</i>	<b>MNT219</b>	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>	<b>MNT222</b>	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>	<b>MNT226</b>	AY235191	(Reed et al., 2003)
<i>M. gallopavo</i>	<b>MNT249</b>	AY552821	(Knutson et al., 2004)
<i>M. gallopavo</i>	<b>MNT250</b>	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>	<b>MNT291</b>	AY552863	(Knutson et al., 2004)
<i>M. gallopavo</i>	<b>MNT399</b>	AY926537	(Chaves et al., 2006)
<i>M. gallopavo</i>	MNT400	AY926538	(Chaves et al., 2006)
<i>M. gallopavo</i>	<b>MNT401</b>	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>	<b>MNT414</b>	AY926546	(Chaves et al., 2006)
<i>M. gallopavo</i>	<b>MNT416</b>	AY926548	(Chaves et al., 2006)
<i>M. gallopavo</i>	MNT427	AL592640	(Chaves et al., 2006)
<i>M. gallopavo</i>	<b>MNT429</b>	AL592644	(Chaves et al., 2006)
<i>M. gallopavo</i>	<b>MNT433</b>	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>	<b>MNT442</b>	AL593052	(Chaves et al., 2006)
<i>M. gallopavo</i>	<b>MNT443</b>	AL593060	(Chaves et al., 2006)
<i>M. gallopavo</i>	<b>MNT448</b>	AL593178	(Chaves et al., 2006)
<i>M. gallopavo</i>	<b>MNT450</b>	AL593308	(Chaves et al., 2006)
<i>M. gallopavo</i>	<b>MNT453</b>	AL593364	(Chaves et al., 2006)
<i>M. gallopavo</i>	MNT454	AL593401	(Chaves et al., 2006)
<i>M. gallopavo</i>	<b>MNT456</b>	AL593466	(Chaves et al., 2006)
<i>M. gallopavo</i>	<b>MNT458</b>	AL593487	(Chaves et al., 2006)
<i>M. gallopavo</i>	<b>MNT466</b>	AL593563	(Chaves et al., 2006)
<i>M. gallopavo</i>	<b>MNT406</b>	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.

## REFERENCES (Supplementary material)

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