Novel polymorphisms to detect *Alectoris* introgression: a multiplex-primer extension approach

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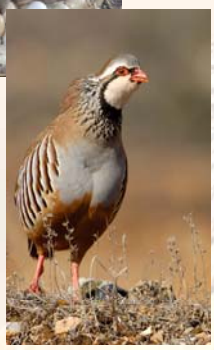
Alectoris chukar



Alectoris rufa

Introduction

Alectoris genus includes species with important cynegetic characteristics in European countries. As wild partridge populations decrease, numerous hunting areas across the countries are periodically reinforced with millions of captive-bred individuals. Several studies have recorded cases of artificial genetic pollution of *A. rufa* and *A. graeca* by *A. chukar*, due to a better growth and adaptation performance of the latter to captivity as a result of artificial selection. Hybrids detection is crucial to avoid uncontrolled restocking which may lead to a widespread introgression of foreign species in locally adapted partridge species.

Aim

The aim was to develop a medium-throughput genotyping method to allow easy introgression detection of *A. chukar* into wild *A. rufa* populations through a fast and low cost analysis of a large number of individuals.

Material and methods

For SNP detection and validation we used 98 *A. rufa* partridges from wild Spanish areas historically never restocked, and 63 *A. chukar* from Spanish and Greek farms.

Using the chicken genome we designed primers for SNP detection in the *Alectoris* genus by PCR-SSCP. Polymorphic bands were extracted from gels, purified, and sequenced to identify the polymorphism.

Twenty-four target sequences (23 genomic and 1 mitochondrial DNA polymorphisms) were amplified in two multiplex reactions, and resolved in two capillary runs previously hybridized by Primer Extension (Figure 1).

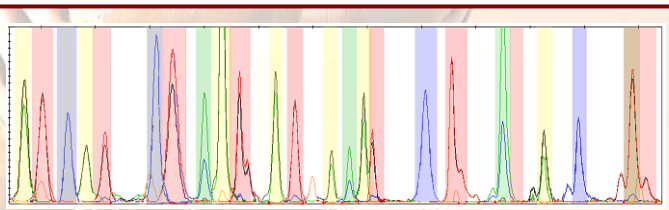


Figure 1. Electropherogram in an ABI3130 sequencer analysed using GeneMapper v4.0 (Applied Biosystems)

SNP locus	dbSNPs accession	Mutation	Allele frequencies			
			A.rufa		*A. chukar*	
			Allele 1	Allele 2	Allele 1	Allele 2
AGC1	ss105106807	G/C	0.990	0.010	0.071	0.929
ALDOB	ss105106809	A/G	0.552	0.448	0.000	1.000
ARSA1	ss105106811	A/G	0.845	0.155	0.040	0.960
CFRT	ss105106812	T/C	0.958	0.042	0.058	0.942
CG3869_1	ss105106813	A/G	0.918	0.082	0.085	0.915
CLU	ss105106814	C/T	0.371	0.629	0.000	1.000
GMCSF	ss105106815	C/T	0.773	0.227	0.008	0.992
GSN	ss105106817	A/T	0.907	0.093	0.025	0.975
HBB	ss105106824	T/C	1.000	0.000	0.210	0.790
LAMC1	ss105106832	T/C	0.903	0.097	0.038	0.962
MNK	ss105106833	T/A	0.990	0.010	0.524	0.476
MPO	ss105106834	A/T	0.947	0.053	0.000	1.000
NID	ss105106835	T/C	1.000	0.000	0.302	0.698
OTC	ss105106842	A/T	0.500	0.500	0.355	0.645
OXT	ss105106843	T/G	0.825	0.175	0.024	0.976
PDE6B	ss105106845	C/G	0.929	0.071	0.009	0.991
PKM2	ss105106847	C/A	0.897	0.103	0.000	1.000
PTHLH	ss105106851	G/C	1.000	0.000	0.016	0.984
RET	ss105106862	T/C	1.000	0.000	0.740	0.260
SPTBN1	ss105106870	A/G	0.976	0.024	0.009	0.991
PCBD2	ss105106872	T/C	0.974	0.026	0.024	0.976
THBS1	ss105106874	G/A	0.964	0.036	0.008	0.992
TNFAIP6	ss105106875	C/T	0.933	0.067	0.025	0.975
Dloop SNP1-R	ss107795934	A/G	1.000	0.000	0.000	1.000

Table 1. Allele frequencies

Results

Starting from 112 sequences belonging to 110 different *G. gallus* nuclear genes, a total of 109 SNPs and 5 INDELs located in 35 different genes were identified in the partridge genome.

Using a panel of 23 nuclear SNPs and 1 mitochondrial polymorphism, allele frequencies were calculated (Table 1) to perform analytical Type-I error probability and power calculations. An optimum subset of 15 markers (genes in blue in Table 1) was chosen to maximize power of hybrid detection for an F1 *A. rufa* x *A. chukar*, and three consecutive backcrosses with *A. rufa* B1, B2, and B3 with a significance level lower than 0.1 (Table 2).

Type-I error prob.	0.098
Power for F1	1
Power for B1	0.996
Power for B2	0.843
Power for B3	0.524

Table 2. Type-I error probability and detection powers

Conclusions

We show here that a domestic species, like *G. gallus*, can be used effectively to develop SNPs in a divergent genus, such as *Alectoris*.

The newly identified polymorphisms in the Alectoris genome and the simple and efficient SNP typing assay developed in the present study can be applied to the genetic control of reproductive-bred individuals in hunting areas and on farms before restocking, thus limiting any harm to wild populations.