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RAPD analysis for detection and eradication of hybrid partridges (*Alectoris rufa* × *A. graeca*) in Spain

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Abstract

The red-legged partridge (*Alectoris rufa*) is the only indigenous *Alectoris* species in the Iberian Peninsula. Local populations are often reinforced with captive-bred individuals, sometimes including hybrids between the red-legged partridge and the exotic rock partridge (*A. graeca*). Hunters and wildlife managers oppose releases of hybrids, but their identification by visual inspection is difficult beyond the first hybrid generation. Here we report the development of a set of RAPD markers to identify hybrid partridges using blood samples. We initially screened 46 RAPD primers on a subsample of pure red-legged and rock partridges, and finally selected six primers that produced 11 markers specific of the rock partridge. The selected primers were tested on hybrids of different generations bred in captivity. This set of loci permitted the detection of 100% ($n=31$) F1 hybrids, 100% ($n=14$) backcrosses of F1 to red-legged partridge, all but one (95%, $n=18$) hybrid of the second backcross, and 18 out of 27 (67%, $n=27$) hybrids of the third backcross. Efficient detection of backcross 1 and 2 individuals is essential, as these are the ones released for re-stocking purposes in hunting states. Although we have only used blood samples, other sources of high-quality DNA, such as muscle, should provide the same results. Therefore, it would be feasible to monitor the genetic purity of partridges in farms and hunting states at different stages of production, including embryos, chicks or hunted specimens.

Keywords: Hybridisation; Reintroduction; Genetic introgression; Red-legged partridge; Rock partridge

1. Introduction

The red-legged partridge (*Alectoris rufa*) is a medium-sized galliform whose natural breeding range is in the Iberian Peninsula, France and Italy including the Mediterranean islands of Corsica, Elba and the Balearics. There is also a large introduced population in the UK (Johnsgard, 1988). It is the most important game species in Spain, where about four million birds are shot every hunting season (Delibes, 1992). These animals are in such a high demand by hunters that natural populations in numerous hunting states across the country are periodically re-inforced with captive-bred individuals, of which about two million are released every year.

There is a perception among partridge hunters (see e.g. Nadal, 1992) that captive-bred individuals are of inferior quality to wild ones, in terms of general appearance (e.g. duller hues in the colour of bill and legs), vigour and anti-predatory or mating behaviour. These problems are attributed to conditions during captive rearing, and to genetic deterioration of the captive stock. It is known (Nadal, 1992) that some partridge farms cross red-legged partridges with the non-native rock partridge (*A. graeca*), even though the trade of hybrids for releasing in the wild is illegal. The rock partridge occurs naturally in Italy and the Balkans (Johnsgard, 1988). It is also present in parts of France, where it hybridises naturally with red-legged partridges in the southern edge of the Alps (Bernard-Laurent, 1984; Randi and Bernard-Laurent, 1999).

The reason why rock partridges are deliberately hybridised with the native red-legged partridges in Spanish game farms is that rock partridges are better adapted to captivity, and are more productive (Nadal, 1992). In fact, rock partridges are bred industrially for human consumption in Mediterranean countries, including Spain. First generation hybrids present a

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characteristic intermediate plumage in relation to pure parentals, such as the double black band in flank feathers. However, individuals resulting from the first backcross of the first generation to red-legged partridge or more advanced crosses are phenotypically very similar to pure red-legged partridges. In our experience, and given the large variation in plumage characteristics of true red-legged partridges, most hybrids beyond the first generation would pass unnoticed. Partridge farmers take advantage of this situation and sell backcrosses as if they were pure red-legged partridges for re-stocking purposes in hunting states, where they may interbreed with individuals from the native populations.

The preservation of the genetic integrity of native species, or safeguard of local biodiversity, is now an international preoccupation (IUCN, 1998; De Greef and Triest, 1999). Specifically referring to bird recovery programmes, Black (1991) suggested that the introduced stock must be of the same taxon as that occurring in the area. If captive stocks are to be used, they must be the closest available subspecies or population to the original stock, something that is definitely possible using pure red-legged partridges (although at a higher price). In the case of Spanish partridges, human intervention has brought about hybridisation that would hardly happen naturally due to the distance that separates the natural breeding ranges of the species involved. Environmental authorities are willing to stop the release of hybrid partridges. The main problem is that identification of hybrids by plumage characteristics is difficult beyond the first generation. Genetic markers that would unambiguously identify the hybrids would be the best solution.

Recently, allozymes have been used to characterise the natural hybrid zone between red-legged and rock partridges in France (Randi and Bernard-Laurent, 1999). Although allele frequencies differed significantly between species, only one allele seemed to be exclusive to the rock partridge. Therefore, the utility of the reported set of markers would be limited for detection of hybrids beyond the first generation.

In this study, our aim was to find a set of random amplified polymorphic DNA (RAPD) markers (Williams et al., 1990) specific to the exotic rock partridge, to assess genetic purity of red-legged partridges in Spain with high confidence ($>$ 95% in the first backcross of F1 hybrids between *A. rufa* $\times$ *A. graeca* to pure red-legged partridges).

2. Materials and methods

2.1. Sample collection

In 1998 and 1999, blood samples were collected from 13 red-legged partridges, 13 rock partridges and a total of 92 hybrids of different generations and backcrosses to red-legged partridge (Table 1). Known hybrids were used to verify the main assumption of the RAPD technique: that the amplified DNA fragments behave as genetic markers inherited in a Mendelian fashion as dominant characters (Williams et al., 1990).

The pure partridges from the same locality were chosen among a large pool of birds, and the chances of choosing close relatives were deemed to be minimal. The hybrids originated from 10 different broods that had been bred in 1998 at a game farm in the province of Jaen (southern Spain). The parents of the hybrid birds (hybrids themselves of previous generations) were not available for analysis. Approximately 300 μl of blood was drawn from the brachial vein using 30-gauge heparinized needles and syringes. Blood was transferred to 2 ml plastic vials containing lysis buffer (Seutin et al., 1991), transported to the laboratory in a cooler and stored at 4°C until analysis.

2.2. DNA extraction and PCR conditions

Total DNA was isolated according to Gemmell and Akiyama (1996). Each sample (c. 200 μl) was first digested with proteinase K for 2 h at 50°C, and 37°C overnight. After digestion, an equal volume of 5 M LiCl was added. The samples were thoroughly mixed for 1 min by inverting the vials, and 600 μl of chloroform were added prior to a high-speed centrifugation for 15 min. DNA was precipitated with twice the volume of absolute ethanol at room temperature, and recovered by centrifugation. DNA was finally resuspended in TE buffer. The DNA concentration and purity was determined spectrophotometrically.

DNA was amplified using the RAPD technique (Williams et al., 1990). Six primers of the "RAPD Analysis Primer Set" of Amersham-Pharmacia-Biotech (PH), and 40 primers obtained from Operon Technologies Inc. (OP) were initially used. The reaction mixture contained c. 10 ng of template DNA, 25 pmol (PH primers) or 18.75 pmol (OP primers) of a single decanucleotide, 0.2 mM dNTPs, 0.5 U of Taq polymerase in the reaction buffer provided by the manufacturer, and distilled H_2O to a final volume of 25 μl. Amplification was performed in an MJ Research PTC-100 thermocycler programmed as follows: 1 cycle of 3 min at 94°C; 45 cycles of 30 s at 94°C, 30 s at 36°C and 1 min at 72°C; with a final extension of 5 min at 72 C. Amplification products were run on 1.4% agarose gels at 100 V (constant voltage) for 45–120 min, along with a 100 base-pair ladder that was used as a molecular weight marker, stained with ethidium bromide, and photographed over UV light.

2.3. Statistical analysis

A linkage analysis was performed on the siblings of brood 530 ($n = 14$, backcross category 1) to determine

whether markers segregated independently of each other (Weising et al., 1995). Fisher's exact tests were calculated for all pairwise combinations of markers, and absence of linkage was inferred if $P > 0.05$.

3. Results

3.1. Marker search strategy

RAPD primers were first tested (step 1) on two red-legged partridges and two rock partridges to identify primers that (a) amplified partridge DNA, and (b) produced bands specific to the rock partridge. Of the 46 decamer primers surveyed, 44 (96%) produced scorable PCR products. We discarded 35 primers that yielded either the same band patterns for the two species, or different patterns for the two rock partridges that were tested simultaneously. The remaining nine primers were tested (step 2) with all available rock and red-legged partridges. Three of the primers proved difficult to reproduce and a final set of six primers was used (step 3) with all the hybrids (Table 2). When conducting PCR amplification of the hybrid samples, we always included four red-legged partridges and four rock partridges. PCR products of the hybrids were run along with those of the pure species to allow identification of the diagnostic bands. Pure individuals were tested at least twice for each primer, independently by two persons in steps 2 and 3, and the same individual pattern was always observed.

3.2. RAPD banding patterns

Electrophoresis of PCR products yielded patterns consisting of 5–12 well-defined bands plus a variable number of fainter bands in the 200–2000 base-pair range (Fig. 1). The set of six primers that were finally selected produced a total of 11 bands specific to the rock partridge (considered as loci in this study, see Table 2) that segregated independently according to the linkage analysis. When these 11 loci were examined in the two broods of first-generation hybrids, five loci produced bands in all individuals ($n = 31$, Table 3). This is as expected if the parental rock partridges were homozygous for the dominant "band" allele and the parental red-legged partridges were homozygous for the recessive

Table 1
Provenance and number (N) of partridge samples used in this study[a]

Population	Location (province)	Generation	N
Red-legged partridge (*Alectoris rufa*)	Finca Lugar Nuevo (Jaén)	Pure	5
	Finca Pesqueril (Sevilla)	Pure	6
	City of Sevilla (Sevilla)	Pure	2
Rock partridge (*A. graeca*)	Finca Lugar Nuevo (Jaén)	Pure	3
	Nanta, Reus, (Tarragona)	Pure	10
Hybrids (*A. rufa* × *A. graeca*)	Finca Lugar Nuevo	F1 (brood 535)	15
		F1 (brood 536)	16
		F3 (brood 531)	2
		F4 (brood 528)	2
		BC-1 (brood 530)	14
		BC-2 (brood 529)	7
		BC-2 (brood 532)	9
		BC-3 (brood 526)	15
		BC-3 (brood 533)	6
		BC-3 (brood 534)	6

[a] Ten hybrid broods, generation F1 to F4 or unidirectional backcross category to red-legged partridge (BC-1 to BC-3) have been included.

Table 2
Selected RAPD primers together with sequence and marker fragment length and code

Primer code	Sequence (5′–3′)	Fragment length (kb)	Marker code
Ph-03 (Pharmacia)	GTAGACCCGT	1600	Ph-03-1600
		1100	Ph-03-1100
		700	Ph-03-700
		550	Ph-03-550
		275	Ph-03-275
Ph-04 (Pharmacia)	AAGAGCCCGT	1400	Ph-04-1400
Ph-05 (Pharmacia)	AACGCGCAAC	230	Ph-05-230
OP-08 (Operon)	TGGACCGGTG	1000	OP-08-1000
OP-09 (Operon)	CTCACCGTCC	900	OP-09-900
		425	OP-09-425
OP-15 (Operon)	GACGGATCAG	1200	OP-15-1200

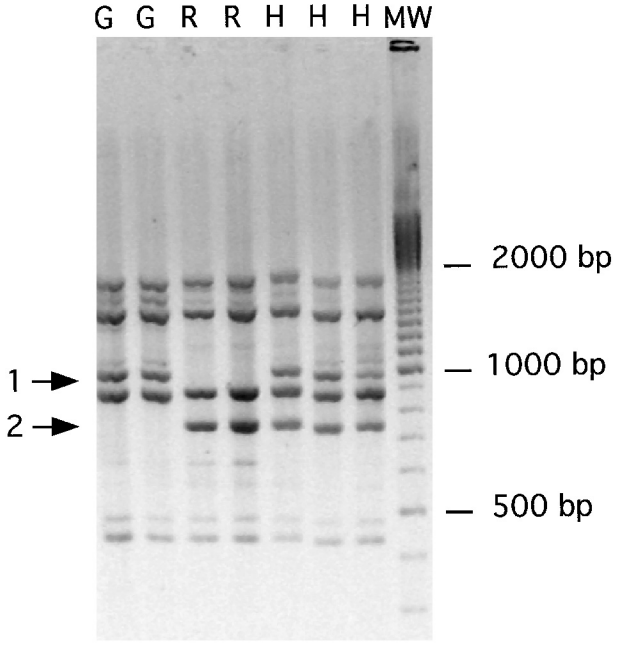

Fig. 1. RAPD banding profile of marker OP-08-1000. From left to right, two rock partridges *Alectoris graeca* (G), two red-legged partridges *A. rufa* (R), three hybrids *A. graeca* × *A. rufa* (H), and a molecular weight marker (MW). The arrows point to bands specific of (1) the rock partridge and (2) the red-legged partridge, both present in the hybrids.

Table 3
Presence/absence of rock- partridge marker bands in first-generation hybrid broods 535 and 536

Individual	OP-09-900	OP-09-425	OP-08-1000	OP-15-1200	PH-04-1400	PH-05-230	PH-03-1600	PH-03-1100	PH-03-700	PH-03-550	PH-03-275
535-1	1	1	1	0	1	1	1	1	0	1	0
535-2	1	1	0	0	1	1	1	1	0	1	1
535-3	1	1	0	0	1	1	0	1	0	1	1
535-4	1	1	1	0	1	1	1	1	0	1	1
535-5	1	1	1	0	1	1	0	1	1	1	1
535-6	1	1	1	1	1	1	0	1	1	1	1
535-7	1	1	1	0	1	1	0	1	1	1	1
535-8	1	1	0	1	1	1	1	1	0	1	1
535-9	1	1	1	0	1	1	1	1	0	1	1
535-10	1	1	1	1	1	1	0	1	0	1	1
535-11	1	1	1	1	1	1	1	1	1	1	1
535-12	1	1	0	1	1	1	1	1	1	1	1
535-13	1	1	1	1	1	1	1	1	1	1	1
535-14	1	1	1	0	1	1	1	1	0	1	0
535-15	1	1	0	0	1	1	0	1	0	1	1
536-1	1	1	1	1	1	1	0	1	1	1	1
536-2	1	1	1	1	1	1	0	1	1	1	1
536-4	1	0	1	1	1	1	0	1	1	1	1
536-5	1	0	1	1	1	1	0	1	1	1	1
536-6	1	0	1	1	1	1	0	1	1	1	1
536-7	1	0	1	1	1	1	0	1	1	1	1
536-8	1	1	1	1	1	1	0	1	1	1	1
536-9	1	0	0	1	1	1	0	1	1	1	1
536-10	1	0	0	1	1	1	0	1	1	1	1
536-11	1	0	1	1	1	1	0	1	1	1	1
536-12	1	1	1	1	1	1	0	1	1	1	1
536-13	1	0	0	1	1	1	0	1	1	1	1
536-14	1	1	1	1	1	1	0	1	1	1	1
536-15	1	0	1	1	1	1	0	1	1	1	1
536-16	1	1	1	1	1	1	0	1	1	1	1

"null" allele. The remaining six loci produced bands in some but not all F1 hybrids (Table 3), possibly because the parental rock partridge was either heterozygous or homozygous (in one instance) for the concerned loci. The parent rock partridge in family 535 seemed to be heterozygous for loci OP-08-1000, OP-15-1200, PH-03-1600, PH-03-700 y PH-03-275. The parent rock partridge in family 536 seemed to be heterozygous at loci OP-09-425, OP-08-1000, whereas it was possibly homozygous at locus PH-03-1600 (i.e. none of its progeny of 15 showed the marker band). We can discard mitochondrial inheritance in the latter case (and for the remaining markers as well), as the female parent in family 536 was a rock partridge whereas it was a red-legged partridge in family 535.

3.3. Hybrid detection power

Overall, the set of 11 loci permitted the detection of 100% ($n=31$) F1 hybrids, 100% ($n=14$) hybrids resulting of the first backcross of F1 to red-legged partridge, all but one (95%, $n=18$) hybrids of the second backcross, and 18 out of 27 hybrids of the third backcross (67%, $n=27$). It is thus clear that the combined analysis of the 11 loci for a given individual up to the second backcross will tell us with a high degree of confidence whether it has mixed ancestry. As for the two siblings of F3 (brood 531), one was identified as a hybrid (4 positive markers out of 11) and the other was not. The two siblings of F4 (brood 528) were both identified as hybrids (by 4 and 5 markers out of 11, respectively).

Given that our sample sizes of pure partridges of both species do not permit an unbiased estimate of the frequency of the dominant allele, we have not attempted a calculation for the probability of detecting hybrids of the different backcrosses considering the set of 11 loci. However, we have calculated the probability of having false negatives (i.e. true hybrids considered as red-legged partridges) using five of our best markers — the ones that were present in all F1 hybrids. When using a single marker, the probability of not detecting a true hybrid in the first backcross is 0.5 (50%), 0.75 (75%) in the second backcross, and 0.875 (87.5%) in the third backcross. When several independent markers are combined, the probability is reduced in a multiplicative way. When combining five markers, the estimated probability is $(0.5)^5=0.03$ for the first backcross; $(0.75)^5=0.24$ for the second backcross, and $(0.875)^5=0.51$ for the third backcross. The actual proportions of hybrids that went undetected using the subset of five markers were 0 ($n=14$), 0.19 ($n=16$), and 0.37 ($n=29$), for the first,

second and third backcrosses, respectively. Observed and expected proportions did not differ significantly (Fisher's exact tests, $P > 0.05$), and this again supports the hypothesis that the rock partridges in the lineage of the hybrids (not available for analysis) were dominant homozygous.

To further test that the rock partridges from which the hybrids originated presented fixed dominant alleles for the subset of five markers, we used a model by Boecklen and Howard (1997). This model permits the calculation of expected frequencies of dominant RAPD markers under a scenario of unidirectional backcrossing, and thus fits very well with the data for our hybrids. For the first to the third backcross categories, the expected and observed distributions of relative frequencies of individuals that have up to five dominant markers did not differ significantly (Table 4), and we can conclude that the parent rock partridges were dominant homozygous for all those markers.

4. Discussion

RAPD analysis (Williams et al., 1990) is a relatively new molecular technique that has already proven very effective for the development of species-specific markers in hybridisation studies. Most examples so far concern plants (e.g. Arnold et al., 1991; Dawson et al., 1996; De Greef and Triest, 1999), with fewer examples from vertebrates (Fritsch and Rieseberg, 1996). We have demonstrated that RAPD analysis is a useful method for detecting hybrids between rock partridge and red-legged partridges. Our set of 11 markers met the condition that they were present in all pure rock partridges that we tested, but were absent from all red-legged partridges. These markers met the classical assumptions of RAPD markers, i.e. that they are dominant and inherited in a Mendelian fashion, and can be scored reproducibly (Williams et al., 1990). Markers specific to the red-legged partridge were also detected in our study, but they are not reported here because they have no interest for hybrid control in Spain.

The set of 11 markers was very powerful to detect hybrids of the first and second backcross of first-generation to red-legged partridge. Individuals belonging to those types of backcrosses were the targets of our study, because they are the ones supposedly sold for restocking purposes in hunting states. Our set of markers was not designed to tell whether a hybrid belongs to a particular backcross category. If this information was sought, and if advanced backcrosses were also involved, theoretical models by Boecklen and Howard (1997) predict the need to use 70 markers or more. Even if such a high number of markers was found, its application to screen a large population would be prohibitive.

When we applied our primers to first-generation hybrids, six markers failed to appear in some first generation hybrids or were missing altogether (locus PH-03-1600 for family 535). The latter is consistent with the parent rock partridge being heterozygous for those loci (or homozygous for the null allele in one case). The parents of the hybrids were unfortunately not available for analysis and our sample of pure individuals was relatively small. Numerous pure birds of the two species from different provenances would be needed to achieve unbiased estimates of dominant allele frequency in the case of each marker (Fristch and Rieseberg, 1996), and to determine whether our current marker set is useful all across the distribution range of the red-legged partridge. Ideally, and for the sake of hybrid detection, the frequency of a good marker should be close to 1 in the rock partridge, and close to 0 for the red-legged partridge.

RAPD markers could be applied to any biological material that contains high-quality partridge DNA, including blood and muscle. Therefore, it would be possible to monitor the genetic purity of partridges in farms at different stages of production, including newly hatched chicks, without sacrificing any animals for sample collection. In comparison, other marker types, such as allozymes, are more invasive and require the

Table 4
Comparison of observed and expected relative frequencies of individuals in backcross categories 1–3 that have Z dominant markers characteristics of the rock partridge out of five markers examined. Expected relative frequencies were obtained from Boecklen and Howard (1997). N × M exact tests were calculated according to Wells and King (1980)

Backcross category	Z	Individuals	Observed frequency	Expected frequency
BC-1	0	0	0.000	0.031
	1	3	0.214	0.156
	2	6	0.428	0.312
	3	3	0.214	0.312
	4	2	0.143	0.156
	5	0	0.000	0.031
		N × M exact test, $P = 0.999$		
BC-2	0	3	0.190	0.237
	1	4	0.250	0.395
	2	6	0.380	0.263
	3	3	0.190	0.087
	4	0	0.000	0.014
	5	0	0.000	0.001
		N × M exact test, $P = 0.99$		
BC-3	0	10	0.370	0.512
	1	10	0.370	0.366
	2	3	0.111	0.105
	3	4	0.148	0.015
	4	0	0.000	0.001
	5	0	0.000	0.000
		N × M exact test, $P = 0.99$		

sampling of internal organs such as the heart or the liver (Randi and Bernard-Laurent, 1999). Environmental authorities thus have a method for conducting farm inspections or to check the genetic status of birds destined to be released. If well publicised, these measures would quickly discourage game farmers that use mixed genetic stock, as they would not find a market for their birds.

Genetic monitoring of wild populations, to determine whether genetic introgression has already taken place, could also be undertaken with the co-operation of hunters and gamekeepers. Muscle samples could be collected during the regular hunting season from shot individuals and submitted for analysis to designated laboratories. Farmed partridges are released at a very large scale in Spain, and if a significant proportion of them contained foreign genes this would pose a serious threat to the original genetic make-up of native populations. Nothing is known about partitioning of genetic variability of red-legged partridge populations in Spain, but there may be local adaptations with a genetic basis, as red-legged partridges are found in a wide variety of habitats, including deserts, steppes, agricultural areas and high-mountain scrubland. Two subspecies are recognized in the Iberian Peninsula, *A.r. hispanica* in the north and *A.r. intercedens* in the south, that differ in the colour of their plumage (Villafuerte and Negro, 1998). Genetically distinct populations could be disrupted by introducing farmed partridges of different provenances, and the consequences would be even more serious if they contained rock partridge genes.

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References

Arnold, M.L., Buckner, C.M., Robinson, J.J., 1991. Pollen-mediated introgression and hybrid speciation in Louisiana irises. Proceedings of the National Academy of Sciences of the USA 88, 1398–1402.

Bernard-Laurent, A., 1984. Hybridation naturelle entre Perdrix bartavelle (*Alectoris graeca saxatilis*) et Perdrix rouge (*Alectoris rufa rufa*) dans les Alpes-Maritimes. Gibier Faune Sauvage 2, 79–96.

Black, J.M., 1991. Reintroduction and restocking: guidelines for bird recovery programmes. Bird Conservation International 1, 329–334.

Boecklen, W.J., Howard, D.J., 1997. Genetic analysis of hybrid zones: numbers of markers and power of resolution. Ecology 78, 2611–2616.

Dawson, I.K., Simons, A.J., Waugh, R., Powell, W., 1996. Detection and pattern of interspecific hybridization between *Gliricidia sepium* and *G. maculata* in Meso-America revealed by PCR-based assays. Molecular Ecology 5, 89–98.

De Greef, B., Triest, L., 1999. The use of random amplified polymorphic DNA (RAPD) for hybrid detection in *Scirpus* from the river Schelde (Belgium). Molecular Ecology 8, 379–386.

Delibes, J., 1992. Gestión de los cotos de Perdiz Roja. In: La perdiz roja. Gestión del hábitat. Fundación la Caixa, editorial Aedos, Barcelona, pp. 141–146.

Fristch, P., Rieseberg, L.H., 1996. The use of random amplified polymorphic DNA (RAPD) in conservation genetics. In: Smith, T., Wayne, R. (Eds.), Molecular Genetic Approaches in Conservation. Oxford University Press, Oxford, pp. 54–73.

Gemmell, N.J., Akiyama, S., 1996. An efficient method for the extraction of DNA from vertebrate tissue. Trends in Genetics 12, 338–339.

IUCN, 1998. Guidelines for Re-introductions. Prepared by the IUCN/SSC Re-introduction Specialist Group. IUCN, Gland, Switzerland and Cambridge, UK.

Johnsgard, P.A., 1988. The Quails, Partridges, and Francolins of the World. Oxford University Press, New York.

Nadal, J., 1992. Problemática de las poblaciones de perdiz roja, bases ecoetológicas para tener éxito con las repoblaciones. In: La perdiz roja. Gestión del hábitat. Fundación la Caixa, editorial Aedos, Barcelona, pp. 87–100.

Randi, E., Bernard-Laurent, A., 1999. Population genetics of a hybrid zone between the Red-legged partridge and rock partridge. The Auk 116, 324–337.

Seutin, G., White, B.N., Boag, P., 1991. Preservation of avian blood and tissue samples for DNA analyses. Canadian Journal of Zoology 69, 82–90.

Villafuerte, R., Negro, J.J., 1998. Digital imaging for colour measurement in ecological research. Ecology Letters 1, 151–154.

Weising, K., Nybom, H., Wolff, K., Meyer, W., 1995. DNA Fingerprinting in Plant and Fungi. CRC Press, Boca Raton, FL.

Wells, H., King, J.L., 1980. A general exact test for N x M contingency tables. Bull. S. Calif. Acad. Sci. 79, 65–77.

Williams, J.G.W., Kubelik, A.R., Livak, K.F., Rafalski, J.A., Tingei, S.V., 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research 18, 6531–6535.