

Fitness consequences of anthropogenic hybridization in wild red-legged partridge (*Alectoris rufa*, Phasianidae) populations

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Abstract Hybridization is a widespread phenomenon, which plays crucial roles in the speciation of living beings. However, unnatural mixing of historically isolated taxa due to human-related activities has increased in recent decades, favouring levels of hybridization and introgression that can have important implications for conservation. The wild red-legged partridge (*Alectoris rufa*, Phasianidae) populations have recently declined and the releases of farm-reared partridges have become a widespread management strategy. The native range of the red-legged is limited to the south-west of Europe (from Italy to Portugal). This species does not breed in sympatry with the chukar partridge (*A. chukar*), whose range is Eurasian (from Turkey to China). However, red-legged partridges have often been hybridized with chukar partridges to increase the

productivity of farmed birds, and game releases may have spread hybrid birds into the wild. In this study, we investigated the fitness (survival and breeding) differences between hybrid and “pure” red-legged partridges in a wild population located in central Spain. Incubation probability was similar in hybrids and “pure” partridges. Hybrid females laid larger clutches than “pure” ones, but hatching success did not differ between hybrid and “pure” partridges. Hybrid birds had lower survival rate than “pure” ones, mainly because of higher predation rates. Our results show that, despite lower survival, hybrid partridges breed in natural populations, so this could increase extinction risk of wild pure partridge populations, through releases of farmed hybrid birds. The consequences of continued releases could be of vital importance for the long term conservation of wild red-legged partridges.

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Introduction

Understanding the genetic basis of fitness in natural populations has long been a central aim in ecology and evolution (Arnold 1997; Grant and Grant 2000; Kingsolver and Huey 2003; Grant et al. 2003). Hybridization is a widespread phenomenon (Rhymer

and Simberloff 1996; Avise 2004; Mallet 2005), which plays crucial roles in the speciation and evolution of animals (Rhymer and Simberloff 1996; Allendorf et al. 2001; Barton 2001; Avise 2004; Grant et al. 2004). Hybridization may cause either decreased fitness (“outbreeding depression”) or increased fitness (“hybrid vigour”; Rhymer and Simberloff 1996; Grant and Grant 1992; Grant et al. 2003, 2004). Outbreeding depression is a fitness reduction seen in hybrid individuals suffering from underdominance (heterozygosity disadvantage), or the disruption of beneficial interactions (e.g. between genes and the environment) or intrinsically coadapted gene complexes (Templeton 1986; Frankham 1999; Laikre et al. 2010). However, hybridization can also create beneficial genetic interactions, including overdominance (heterozygote advantage) and dominance (the masking of deleterious effects), leading to hybrid vigour (Allendorf et al. 2001).

Because of its potential effects on individual fitness (Allendorf et al. 2001; Keller and Waller 2002), hybridization is also important to conservation biology. Unnatural mixing of historically isolated taxa due to human-related activities has increased in recent decades, favouring levels of hybridization (interbreeding of individuals from genetically distinct populations, regardless of the taxonomic status of the populations; Rhymer and Simberloff 1996) and introgression (gene flow between populations whose individuals hybridize; Allendorf et al. 2001) that can have important ecological consequences or implications for conservation (Olden et al. 2005; Randi 2008; Ryan et al. 2009; Laikre et al. 2010). Hybridization could thus be a threat to natural populations (loss of genetic pool or genetic diversity due to homogenization), when it occurs as a consequence of human activities such as the introduction of new species or fragmentation or modification of habitats (Rhymer and Simberloff 1996). Thus, it is important to separate the evolutionary role of natural hybridization from problems associated with increasing anthropogenic hybridization (Allendorf et al. 2001; Laikre et al. 2010).

Human-mediated genetic introgression with allochthonous species may currently be a broad-scale phenomenon in different Galliformes species (Deregnacourt et al. 2002, 2005; Barbanera et al. 2005, 2007, 2009a; Puigcerver et al. 2007; Tejedor et al. 2007; Blanco-Aguilar et al. 2008), mainly due to

releases of farm-reared gamebirds for hunting purposes (Randi 2008; Barbanera et al. 2010), but genetic risks associated with releases are often largely neglected in management and policy (Laikre et al. 2010). In *Alectoris* partridges, hybridization occurs naturally in several locations where the natural ranges of two species overlap (McCarthy 2006), for example between red-legged partridge and rock partridge (*Alectoris graeca*) in the southern French Alps (Bernard-Laurent 1984). Nevertheless, natural hybrids between red-legged partridges and chukar partridges (*A. chukar*) should not occur, because their distributions do not overlap (McCarthy 2006). The natural distribution of the red-legged partridge is restricted to the Mediterranean area (Spain, Portugal, France, north-east Italy, and the Italian islands of Elba and Corsica), but it has been introduced in the United Kingdom, some Atlantic islands (Azores, Canaries and Madeira), and with less success in United States, New Zealand and central Europe (Del Hoyo et al. 1994). Chukar partridges are naturally distributed from Turkey to China, with introduced populations in Canada, USA, Hawaii, New Zealand, and Australia (Del Hoyo et al. 1994). Yet, hybrids between these two species have been found in an introduced population in Italy (Baratti et al. 2004; Barbanera et al. 2005), on the Island of Majorca (Tejedor et al. 2007), in the UK (Potts 1989) and are now widespread in the Iberian Peninsula, especially in areas where farm-bred partridges are released for hunting purposes (Blanco-Aguilar et al. 2008). Therefore, genetic introgression in continental Spain seems to be a general pattern of human origin. Chukar partridges are more productive in farms, as well as their hybrids with red-legged partridges, and anthropogenic hybridization in captivity aims at increasing the productivity of farmed birds (Potts 1989; Nadal 1992). The lack of control of the genetic quality of farm-bred partridges and game releases have potentially extended the problem into the wild (Blanco-Aguilar 2007, 2008), which may have contributed to further depress the viability of wild populations (Potts 1989).

The red-legged partridge is currently the most important gamebird in Spain, especially in farmland areas, and has a high socioeconomic value in rural environments (Bernabeu 2000; Martínez et al. 2002). However, this small gamebird is currently considered as SPEC category 2 (Species of European

Conservation Concern), due to widespread declines in wild populations (Birdlife International 2004). Given the problems experienced by wild partridge populations (habitat alteration, mainly changes in agrarian management systems, predation, overhunting, e.g. Blanco-Aguilar et al. 2004; Keane et al. 2005; Vargas et al. 2006; Blanco-Aguilar 2007; Casas and Viñuela 2010), many game managers have been releasing large numbers of birds over the last 30 years in Spain (at least 3–4 millions every year; Garrido 2002). This contributes to a profitable business of captive breeding and rearing of partridges in farms, which has increased exponentially since the 80s (Blanco-Aguilar et al. 2008), and has made of restocking one of the main management tools currently used in Spain. However, this management system does not appear to help the recovery of wild partridge populations (Gortázar et al. 2000; Pérez et al. 2004). Released partridges are known to breed in the wild (Duarte and Vargas 2004), but virtually nothing is known about fitness of hybrids in wild populations of the original distribution area. Thus, the potential negative effects of wild breeding of these hybrid birds on native populations (Potts 1989; Tejedor et al. 2007; Blanco-Aguilar et al. 2008; Barbanera et al. 2009a, 2010) have been so far largely neglected in research and policy.

Here, we report information about the fitness (survival and breeding performance) differences between hybrid and “pure” red-legged partridges in a wild population in central Spain over 3 years. We could expect hybrid birds to survive less well than “pure” wild partridges (Gortázar et al. 2000). On the other hand, farm-reared birds are selected to maximize breeding outputs, and chukar or hybrid partridges are more productive in farms (Potts 1989; Nadal 1992), thus hybrid red-legged partridges breeding in natural populations were predicted to produce larger clutches and potentially to be more productive than “pure” ones.

Materials and methods

Study area

We conducted the study in central Spain, in a 125 km² area located in Campo of Calatrava, Castilla-La-Mancha (38° 80'N, 3° 80'W, 610 m a.s.l.; Fig. 1). The study area is mainly agricultural,

dominated by a mosaic of crops (mainly cereal), and interspersed with olive groves, vineyards, a few patches of dry annual legume crops (mainly vetch *Vicia sativa*) and sugar beet *Beta rubra*. The study area includes four game estates managed for small game hunting purposes with three different hunting management policies (mainly predator control, provision of drinking and food stations, and release of captive farm-bred birds: Casas and Viñuela 2010).

Captures and measurements

Fieldwork was carried out in February–October 2003–2005. Over the 3 years, 115 adult partridges were captured in late winter/early spring (2003: n = 39, 2004: n = 44, 2005: n = 32) using cage traps with live adult partridges as decoys to attract wild birds, which were baited with wheat daily (Casas and Viñuela 2010). Birds were individually ringed and sexed from plumage, biometry and ornaments (Sáenz de Buruaga et al. 2001). We took a blood sample from the brachial vein (0.5–1 ml) as a source of DNA. The sex of each bird was confirmed genetically (J. T. García & M. Calero-Riestra, unpublished data). Each individual was fitted with a necklace radio-transmitter equipped with a mortality sensor (10 g; Biotrack, Wareham, Dorset, UK), and released at the capture site shortly (ca. 20 min) after capture.

Survival and breeding performance

We located and monitored partridges by radio-tracking (using AOR-AR8200 multiband receivers and three element YAGI antennas; Biotrack) at least 2 times every week from capture date to the beginning of hunting season (early October), because we were mainly interested in natural mortality causes (not losses due to hunting). Birds that did not survive at least 7 days after tagging and those which transmitter failed (unknown fate) were excluded from the analyses. Thus, we finally used data collected over 3 years on 89 adult red-legged partridges (51 females and 38 males), which were monitored until death, signal loss (i.e. until batteries run out), or the beginning of hunting season. The cause of natural mortality was identified for 34 birds, and included predation, poaching (hunted outside the hunting season), agricultural practices and other unknown mortality causes. Because we were interested in

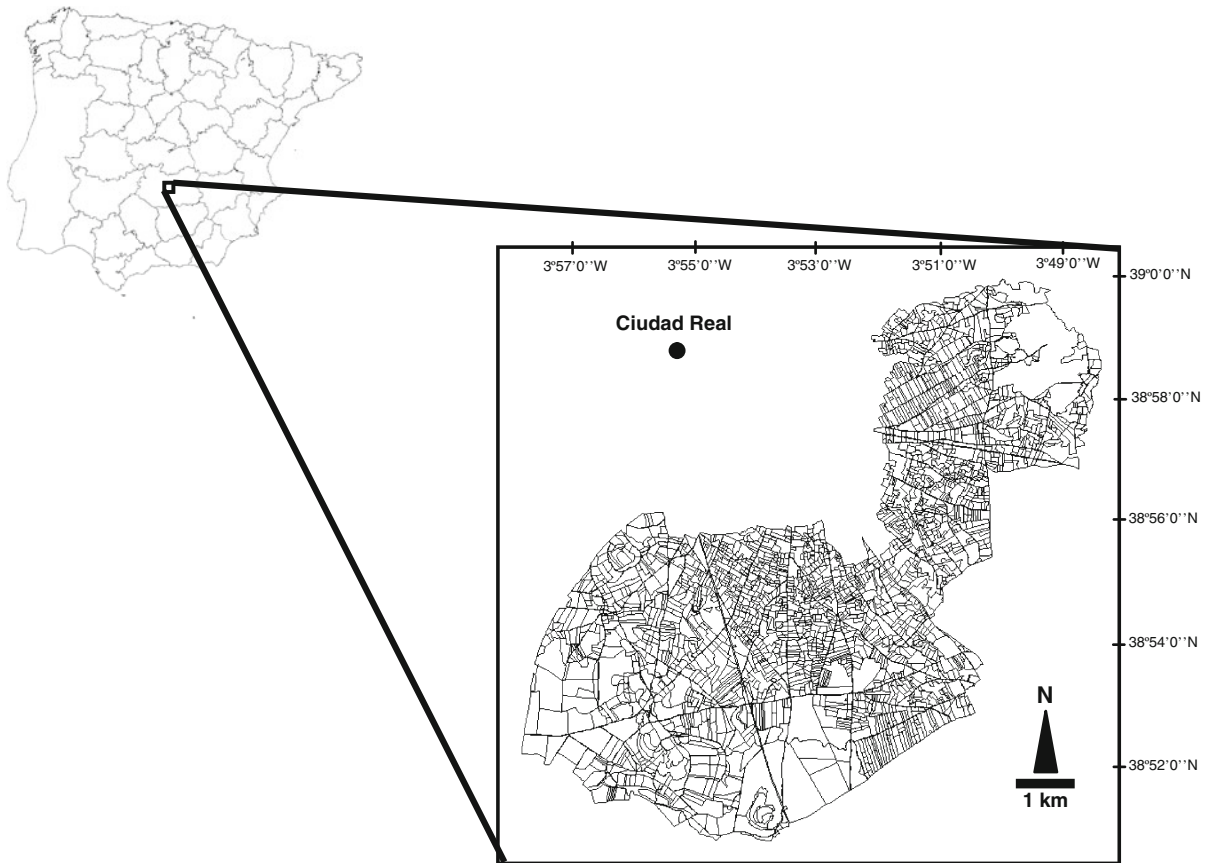


Fig. 1 Map of the study area (campo de Calatrava) located in the Ciudad Real province in central Spain ($38^{\circ} 80'N$, $3^{\circ} 80'W$). Subdivision lines show the distribution of agrarian fields

(mainly cereal, ploughed fields, fallows, annual legume crops, vineyards and olive groves). Nearest city to the study area (black dots)

natural mortality, we excluded losses due to agricultural practices (one male killed by a combine harvester at night), poaching ($n = 5$) or unknown cases ($n = 3$) from our analyses of mortality causes.

For each female, we recorded whether it laid and incubated a clutch (1 = incubation, 0 = no incubation), the day incubation began (julian date; 1 = 1st of March), clutch size (number of eggs per clutch), nesting success (1 = clutch successfully hatched at least 1 chick; 0 = failed to hatch any chick), and hatching success (hatched nestlings/clutch size in successful clutches). Double-brooding occurs frequently in red-legged partridges (Green 1984; Casas et al. 2009): females often lay eggs in two nests, one incubated by the female, the other one by the male, so we analyzed female and male breeding performances separately. For females, we tested for differences between hybrids and “pures” in incubation

probability, laying date, clutch size, nesting success and hatching success. For males, we tested for differences between hybrids and “pures” in incubation probability and nesting success, but not laying date, clutch size or hatching success, because we did not know the genetic identity of the female that laid the clutch. Some individuals died before breeding ($n = 18$ females, $n = 15$ males) so sample size was reduced for the analyses of breeding performance.

Genetic analyses

Molecular analysis

All birds captured had a typical red-legged partridge phenotype (small white bib framed by a broad black-streaked neck, brown hindneck and tricoloured flack feathers; Cramp and Simmons 1980), and were thus

not F1 hybrids between *A. rufa* and *A. chukar*, which can be identified using morphological characters (Potts 1989; Negro et al. 2001; Barbanera et al. 2005). All hybrid partridges studied here were introgressed individuals (F2, F3 or backcrossed), which can only be identified using molecular markers.

All genetic analyses were performed after field-work. Total genomic DNA was isolated from whole blood using a standard proteinase K/phenol method (Sambrook et al. 1989). DNA was isolated from museum specimens using the DNeasy Tissue Kit (Qiagen, Valencia, CA, USA) in a dedicated ancient DNA laboratory, and extraction blanks were included to assure the absence of contamination. To detect introgression, we used 8 diagnostic microsatellite loci (loci 1e45, 1e78, 1f32, 1f138, 1g47, 1h15, 1i68, 1v16; Online Resource 3) from a previously developed *Alectoris rufa* microsatellite enriched library. This library was built following Kandpal et al. (1994), and allowed identifying allele of different, non-overlapping, sizes in each taxa (Fig. 2; Online Resource 1 and 2; Blanco-Aguiar 2007; Dávila 2009). “Pure” reference samples to assess the categorical diagnostic character of the marker set were taken from more than 800 *A. rufa* partridges naturalized in museums before the 1980s and from the Iberian red-legged partridge population (Dávila 2009; Online Resource 4) and from 164 chukar partridges from across the distribution range, including populations in China (Qing yang, Gansu province, $n = 8$), the Aegean Islands (Ikaria, Kos, Karpathos, Makronisos, Crete and Andros, $n = 20, 5, 8, 2, 10, 2$, respectively), Cyprus ($n = 10$), Lebanon ($n = 24$), Armenia (Ehegnadzor, $n = 3$), Kirghizstan ($n = 2$), and five Spanish

game-farms ($n = 10, 20, 10, 10, 20$, respectively). Because allele sizes at the eight selected microsatellite loci differed between *A. rufa* and *A. chukar* and did not overlap (Fig. 2; Online Resource 1 and 2), they could be used as diagnostic genetic markers of nuclear introgression by hybridization between both species (Blanco-Aguiar 2007; Dávila 2009).

In addition to the 8 nuclear microsatellite loci, we used a RFLP-PCR on a cytochrome b sequence (mtDNA Cyt-b), using primers Aru-Cyt-b F and R, as in Blanco-Aguiar et al. (2008). Introgression of the maternally inherited *A. chukar* mtDNA into *A. rufa* was detected by means of diagnostic mutations located in the cytochrome b sequence (see Blanco-Aguiar et al. 2008). Digested fragments of cytochrome b were resolved by 2% agarose gel electrophoresis and ethidium bromide post-staining.

The use of diagnostic markers allowed us to categorically detect chukar introgression by a simple count of diagnostic alleles at the study loci (Fig. 2). We categorised a bird as “hybrid” when at least one of the genetic markers showed introgression from chukar, or as “pure” when none of the markers screened indicated introgression. However, there is some error probability in assigning a bird as “pure” when none of the markers screened indicated introgression because of advanced backcrosses. Assuming that nuclear markers are autosomal and that backcrossing is unidirectional (i.e backcross \times backcross or F1 \times backcross mating do not occur), we expect that about 10% of second backcrosses to be homozygous for *A. rufa* alleles at the eight nuclear markers. In fact, the probability that a BC-5 will be classified as pure *A. rufa* does not drop below 10% until about 70 nuclear diagnostic markers are examined (Boecklen

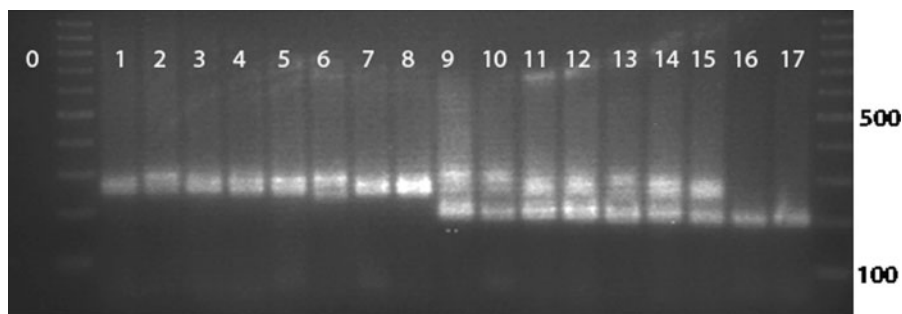


Fig. 2 Diagnostic PCR banding profiles obtained using loci Aru 1h15 microsatellite marker through a 2% agarose gel in TAE. The molecular weight ladder is shown in *base pairs*.

Lane 0 is a negative control. *Lanes 1–8* correspond to *A. rufa*; *lanes 9–15*, heterozygous hybrids; *lanes 16 and 17*, *A. chukar*

and Howard 1997). The additional use of the maternally inherited mitochondrial marker does not improve much this power of resolution (i.e. only ¼ of a first backcross is expected to show foreign mtDNA). Nonetheless, although the number of markers employed gives a coarse assignment of hybridization, the power of resolution is enough for the question at hand. In addition, the number of markers required to tell advanced backcrosses from pure species with low error is simply too large to be practical.

Statistical analyses

We used SAS 8.01 (SAS 2001) and Statistica 6.0 (StatSoft Inc 2002) for statistical analyses. Dependent variables were checked for normality (Shapiro–Wilk test), and were fitted to models using the following error distributions: (1) laying date: normal error distribution and identity link function; (2) clutch size: Poisson error distribution and log function; (3) probability of incubation, nesting success and hatching success (hatched nestlings/clutch size), probability of mortality or predation: binomial error distributions and logit link functions.

We estimated the survival rate of radio-tagged red-legged partridge during the study period (from late winter/early spring to early autumn) using the Kaplan–Meier product-limit estimator. This estimator allows determining the likelihood that a bird survives until a period t of time from the beginning of the study, and it takes into account that animals could not be captured simultaneously (Kaplan and Meier 1958; Pollock et al. 1989). The Kaplan–Meier estimator was used to examine differences between sexes, years and hybridization statuses. We also applied a proportional hazard model that uses Cox’s partial likelihood method (Cox 1972) to evaluate factors potentially associated with mortality (sex, year and genotype), considering hunting management practices as a grouping variable. We analysed the probability of mortality due to predation including year, sex and hybridization as independent variables. Initial models included sex, year, hybridization and their two-way interactions as explanatory variables, with model selection being done using a backward stepwise procedure (Crawley 1993). No significant differences among hunting estates with slightly different management policies were found for any

breeding parameter (Casas et al. 2009; Casas and Viñuela 2010), so we it was not necessary to include this variable in the analyses of the breeding performance data. All tests were two-tailed.

Results

Differences in breeding performance between hybrid and “pure” partridges

Thirty-three of 115 genotyped birds (28.7%) were categorised as hybrids indicating a high prevalence of hybrids in the spring breeding population. In females, incubation probability did not differ between hybrid and “pure” red-legged partridges ($\chi^2 = 0.33$, $df = 1$; $P = 0.57$; Table 1). In males, incubation probability varied between years ($\chi^2 = 10.57$, $df = 2$; $P = 0.005$), but not between hybrids and “pure” ($\chi^2 = 2.15$, $df = 1$; $P = 0.14$; Table 1). Laying dates did not differ significantly between hybrid and “pure” females ($F_{1,30} = 2.2$; $P = 0.15$). However, hybrid females laid larger clutches than “pure” females ($F_{1,30} = 6.96$; $P = 0.013$; Table 1), with no significant differences between years (year effect: $F_{2,30} = 1.48$; $P = 0.25$; year \times hybridization interaction: $F_{1,30} = 0.9$; $P = 0.35$).

Variation in nesting success was not explained by hybridization ($\chi^2 = 0.81$, $df = 1$; $P = 0.35$) or by the interaction hybridization \times sex ($\chi^2 = 1.8$, $df = 2$; $P = 0.17$), but was explained by sex ($\chi^2 = 6.51$, $df = 1$; $P = 0.01$; Table 1). Nesting success was higher in males than in females (see also Casas et al. 2009). Similarly, hatching success did not differ significantly between hybrid and “pure” birds (84.16 ± 5.89 and 96.96 ± 4.66 , respectively; $\chi^2 = 0.82$, $df = 1$; $P = 0.36$).

Differences in survival between hybrid and “pure” partridges

Survival rate (late winter/early spring to early autumn) differed significantly between hybrid and “pure” birds ($Z = 3.37$; $P < 0.001$; Fig. 3), tended to differ between years ($\chi^2 = 5.92$, $df = 3$; $P = 0.052$), but did not differ between sexes ($Z = -0.45$; $P = 0.65$).

Proportional hazard models revealed that hybridization significantly reduced survival (Wald = 3.89;

Table 1 Incubation probability, clutch size, incubation start date (1 = 1st March) and nesting success differences between hybrid and “pure” Red-legged partridges (only birds alive during the breeding season)

| Hybridization | Sex (sample size) | Incubation rate ^a (%) | Clutch size | Laying date ^b | Nesting success ^c (%) |
|---------------|-------------------|----------------------------------|--------------|--------------------------|----------------------------------|
| Hybrids | Female (n = 11) | 90.9 ± 9.1 | 11.78 ± 0.72 | 61.95 ± 2.0 | 33.3 ± 9.8 |
| | Male (n = 6) | 66.6 ± 21.0 | – | – | 66.7 ± 16.7 |
| “Pure” | Female (n = 25) | 96.0 ± 4.0 | 9.24 ± 0.55 | 56.67 ± 2.79 | 40.0 ± 16.3 |
| | Male (n = 25) | 36.0 ± 9.8 | – | – | 100.0 ± 0.0 |

All data are expressed as means ± S.E

^a Percentage of radio-tagged males or females that incubated a clutch

^b Day 1 = 1st of March

^c Percentage of nests that hatched at least 1 young

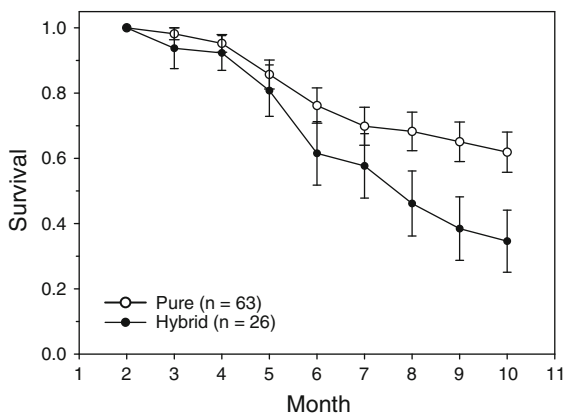


Fig. 3 Cumulated mean (±SE) monthly survival probability of hybrid and “pure” red-legged partridges (data from all years and sexes combined). Sample size refers to number of radio-tracked birds

$P = 0.048$), with no significant differences between sexes (Wald = 0.29; $P = 0.58$) or among years (Wald = 0.03; $P = 0.87$).

Mortality causes

Predation probability differed between hybrid and “pure” birds, depending on sex (hybridization: $\chi^2 = 6.46$, $df = 1$; $P = 0.011$; sex: $\chi^2 = 3.62$, $df = 1$; $P = 0.057$; sex × hybridization: $\chi^2 = 7.63$, $df = 1$; $P = 0.0057$). Predation occurred in 42.3% of hybrids birds (n = 26), but only in 23.7% of “pures” (n = 59). Hybrids males suffered significantly higher predation rate than “pure” ones, but were equally likely to die from other causes. The commonest predators were identified as carnivores (red foxes,

feral cats and feral dogs, n = 18) and raptors (n = 4). Hybrids birds (n = 11) were only found predated by carnivores (100%), while “pure” birds (n = 14) were found predated by carnivores (50%), raptors (28.57%) or other, unknown predators (21.43%, cases in which predator identity could not be ascertained because carcasses were highly consumed).

Discussion

Our results compare some major fitness components between “pure” and hybrid partridges (*A. rufa* × *chukar*) in a native wild population in Spain. Given that hybrid females had the same probability of laying a clutch than “pure” ones, had a similar hatching success, but laid larger clutch sizes, hybrid females might spread their genotypes more efficiently than “pure” ones in wild populations. Thus, genetic introgression may be extending through red-legged partridges range by wild breeding, not only by releases of hybrid birds (Blanco-Aguiar et al. 2008). In fact, although the number of partridges known to be released in our study area is relatively low (about 2000 birds/year), and restricted to two game estates of relatively small size and high hunting pressure (see details in Casas and Viñuela 2010), we found a relatively high percentage of hybrids in the whole population (29% of breeding birds). Hence, some of the hybrid birds we have monitored might have originated from backcrosses in the wild, although initial introgression must have been generated by human releases of farm-bred birds, most likely via releases in two of the game estates in the study area, because chukar and

red-legged partridges ranges do not overlap (McCarthy 2006). Our results contrast with those reported by Potts (1989) for British populations, who did not find any remarkable difference in clutch size, fertility or chick survival between chukar, hybrid or red-legged partridges, but, for reasons unknown, chukar and hybrid partridges had lower productivity than red-legged partridges. However, in that study there was not genetic identification of birds, so information could be incomplete or skewed. Alternatively, the effects of releases and hybridization might differ depending on prevailing environmental conditions experienced by wild populations, but additional research comparing UK and Spain would be necessary to test this possibility.

Larger clutches of hybrid females might be a consequence of artificial selection in farms, or strictly due to greater genetic laying ability of hybrids (Potts 1989; Nadal 1992). Thus, larger clutch size of hybrid birds could simply reflect a farmed origin, independently of hybridization. Larger clutches could be associated with increased predation risk in birds (Skutch 1982), but we have not found this to be the case in our study population (Casas et al. 2009; Casas and Viñuela 2010). In this study, we did not find significant interannual variations in clutch size in nests incubated by females. The significant year-to-year variation in clutch size reported in this species (Casas et al. 2009) may be mainly explained by clutch size variation in nests incubated by males, and in the proportion of females that lay in two nests (one incubated by the male, and the other one by herself; Green 1984; Casas et al. 2009). We found that hybrid males tended to incubate more often than “pure” ones, although the difference was not significant. The fact that hybrids may breed as well as, or even better than “pure” ones, in wild populations have important implications regarding the persistence and possible spread of hybrids in nature. It stresses out the widespread concern about the genetic integrity of red-legged partridge populations (Potts 1989; Aebischer and Potts 1994).

We found that hybrid partridges survived less well than “pure” ones in wild populations. Hybrid birds suffered higher mortality that was attributable mainly to predation, in particular by carnivores. “Pure” birds were predated mainly by carnivores too, but also by raptors. These results should be interpreted cautiously, given that foxes often scavenge on dead birds, so we could underestimate mortality due to other causes. Predation is typically the main cause of

mortality in farm-reared released gamebirds and partridges (Leif 1994; Gortázar et al. 2000; Putaala and Hissa 2003), most likely because of inappropriate antipredator behaviour in captive-bred birds (McPhee 2003). Partridges are particularly vulnerable to predators during the first weeks after release (Gortázar et al. 2000; Pérez et al. 2004), and our results suggest that this may be the case in the longer-term too.

Although survival rate was lower in hybrid birds, enough hybrids seem to survive, recruit and breed to be maintained in natural populations at a relatively high rate. This might be because despite surviving less well, those hybrids that recruit might be more productive than “pure” birds (greater laying capacity). An alternative explanation is that there is a continuous influx of hybrid birds into wild populations because of repeated (annual) and massive (3–4 millions/year in Spain) releases of captive-bred birds, and because of a high prevalence of hybrids in farms, with poor or no genetic control (hybrids have been detected in c. 63% of farms used for releasing partridges; Blanco-Aguilar et al. 2008). Thus, even although few released birds would survive in the wild, massive releases conducted over many years in the same area would allow hybrid partridges to be maintained in wild breeding populations (Barbanera et al. 2009b). Summarizing, the lower survival rate of hybrids could limit their spread into wild populations in the long term, but our data do not allow a complete demographic evaluation, because this lower adult survival could be compensated by a higher productivity. Data about chick survival of hybrid versus “pure” partridges are urgently needed to develop a complete demographic model, allowing a full evaluation of hybrid performance in the wild.

Hybridization is very important for the speciation and evolution of animals, but may also be an undesired consequence of human perturbations, and thus a general “hybrid policy” that applies to all situations and to different species would be very difficult (Allendorf et al. 2001). Providing appropriate and helpful recommendations is probably better on a case-by-case basis. Gamebird restockings are spreading introgression into wild populations (Puigcerver et al. 2007; Blanco-Aguilar et al. 2008; Barbanera et al. 2010). According the classification of Allendorf et al. (2001), our case of hybridization would be of “type 5”, i.e. widespread anthropogenic introgression, with the associated recommendations of maintaining

and expanding the remaining “pure” populations (Allendorf et al. 2001). We cannot rule out completely that fitness (breeding and survival) differences between hybrid and “pure” partridges might be due, in part, to domestication effects rather than effects associated with the introgression of chukar alleles. We have found that even in hunting estates where restocking with farm-reared partridges are performed (which populations could contain both “pure” and hybrid farm-bred partridges), the survival was lower in hybrids than in “pure” ones. Nevertheless, further investigation is needed (1) to disentangle the relative effects of domestication or hybridization on fitness, for which purpose additional studies in captivity or semi-captivity should be useful to study the fitness differences between “pure” and hybrids farm-bred birds, and (2) to study the long term hybridization effects.

Nonetheless, according to Spanish law (law 42/2007, Natural Heritage and biodiversity), the Spanish public administration ensure that releases of species for hunting purposes does not involve a threat to conservation of these or other species, in genetic or demographic terms, so management strategies should be implemented to control genetic introgression and to help recovering the original genetic structure of populations by (1) stopping releases of hybrid birds; (2) implementing an effective inspection protocol of partridge farms and game estates where partridge releases are performed, and (3) promoting management plans to avoid releases in those areas where restocking programs have not yet been performed, and where there is reliable evidence that populations consist of non-hybridized individuals. Gamebirds other than red-legged partridges can hybridize in the wild with introduced, non native birds that originates from hunting restocking programs (e.g. common quail *Coturnix coturnix* hybridizing with non native japanese quail *Coturnix japonica* or their hybrids; Puigcerver et al. 2007). Our work should stimulate more research not only on the occurrence of hybrids in the natural populations of other species, but also where possible on the fitness of hybrids in order to better understand the risks to native populations.

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