J Ornithol
DOI 10.1007/s10336-013-0947-2

ORIGINAL ARTICLE

Phenotypic differences in body size, body condition and circulating carotenoids between hybrid and "pure" red-legged partridges (*Alectoris rufa*) in the wild

F. Casas · F. Mougeot · M. E. Ferrero · I. Sánchez-Barbudo · J. A. Dávila · J. Viñuela

Received: 15 November 2012 / Revised: 8 February 2013 / Accepted: 7 March 2013
© Dt. Ornithologen-Gesellschaft e.V. 2013

Abstract In the last decades, the release of large numbers of farmed-reared birds became a widespread management practice for game species. The red-legged partridge (*Alectoris rufa*) is a quarry species with a high economic impact in rural areas of southwest Europe. In order to increase productivity and produce heavier birds, farmed red-legged partridges have often been hybridized with Chukar partridges (*A. chukar*), although these species have allopatric distribution ranges. During restocking, hybrid birds may be released into wild populations and may subsequently successfully breed, thus threatening the genetic integrity of native *A. rufa* populations. In this study, we compared several phenotypic indicators (body size, body condition and physiological state) between "pure" and hybrid partridges in order to evaluate the possible consequences of *A. chukar* genetic introgression into *A. rufa*. For this purpose, we captured 115 wild red-legged partridges during the breeding seasons 2003–2005 in four game estates of central Spain. We observed a greater occurrence of hybrid *A. rufa* × *A. chukar* partridges nearby the sites where the release of farmed-birds took place. We also found that hybrid males were smaller and hybrid females had better body condition and lower plasma carotenoid concentration than pure partridges of the same sex. Low carotenoid levels in blood plasma might be a limitation for female reproduction (fewer carotenoids available for ornamentation or to allocate to eggs). Overall, our results showed a greater occurrence of hybrids near restocking areas and phenotypic differences between hybrids and "pure" partridge in the wild. Genetic controls of farm-reared partridges should be a key step to prevent the releases of hybrids and ensure the maintenance of the genetic integrity of wild red-legged partridge populations.

Keywords *Alectoris rufa* · Farm-reared partridge · Hybridization · Introgression · Red-legged partridge · Spain

Zusammenfassung

Phänotypische Unterschiede in Körpergröße, Körperkondition und Carotinoidspiegel zwischen freilebenden „reinen" Rothühnern (*Alectoris rufa*) und solchen hybrider Abstammung

In den letzten Jahrzehnten hat sich die Freilassung großer Mengen von Vögeln aus Geflügelzuchten zu einer verbreiteten Managementpraxis für Jagdbestände entwickelt. Das Rothuhn (*Alectoris rufa*) ist als Federwildart in ländlichen Gegenden Südwesteuropas von hoher wirtschaftlicher Bedeutung. Um zur Produktivitätssteigerung schwerere Vögel zu züchten, wurden Rothühner von den Züchtern häufig mit Chukarhühnern (*A. chukar*) gekreuzt, obgleich diese beiden Arten allopatrisch verbreitet sind. Zur Bestandsaufstockung können diese Hybriden dann in Wildpopulationen freigesetzt werden und es kann daraufhin zu erfolgreichen Bruten kommen, welche die genetische Integrität der natürlichen *A. rufa*-Populationen gefährden. In dieser Studie vergleichen wir mehrere phänotypische Merkmale

Communicated by L. Fusani.

F. Casas · M. E. Ferrero · I. Sánchez-Barbudo · J. A. Dávila · J. Viñuela
Instituto de Investigación en Recursos Cinegéticos (IREC, CSIC-UCLM-JCCM), Ronda de Toledo s/n., 13071 Ciudad Real, Spain

F. Casas (✉) · F. Mougeot
Estación Experimental de Zonas Áridas (EEZA-CSIC), Carretera de Sacramento s/n, La Cañada de San Urbano, 04120 Almería, Spain
e-mail: fabian.casas@eeza.csic.es

Published online: 31 March 2013

(Körpergröße, Körperkondition und physiologischer Status) von „reinen“ und hybriden Hühnern, um mögliche Folgen der genetischen Introgression zwischen *A. chukar* und *A. rufa* abzuschätzen. Dazu fingen wir während der Brutperioden 2003–2005 115 wilde Rothühner in vier Jagdgebieten in Zentralspanien. Wir beobachteten ein verstärktes Vorkommen von *A. rufa* x *A. chukar*-Hybriden im Umkreis von Orten, an denen Zuchtvögel freigelassen worden waren. Außerdem stellten sich hybride Männchen als kleiner heraus, weibliche Hybriden waren in besserer körperlicher Verfassung und hatten einen niedrigen Carotinoid-Plasmaspiegel als „reine“ Hühner gleichen Geschlechts. Ein niedriger Carotinoidspiegel im Blutplasma könnte einen limitierenden Faktor für die weibliche Reproduktion darstellen (da weniger Carotinoide für die Ausbildung der Farbmerkmale oder die Ausstattung der Eier zur Verfügung stehen). Insgesamt belegen unsere Ergebnisse ein vermehrtes Auftreten von Hybriden in der Nähe von Freilassungsstellen und phänotypische Unterschiede zwischen Hybriden und „reinen“ Hühnern im Freiland. Die genetische Kontrolle von Hühnern aus Zuchtbetrieben hätte somit eine Schlüsselrolle bei der Vermeidung der Hybridfreisetzung inne und könnte die Aufrechterhaltung der genetischen Integrität wilder Rothuhn-Populationen gewährleisten.

Introduction

The release of captive farm-bred galliforms has become a common hunting management practice in recent years (Griffith et al. 1989; Sokos et al. 2008; Sánchez-García et al. 2009; Laikre et al. 2010). Although this management tool can be used to recover wild populations (i.e., reintroductions, for example threatened populations of Grey partridge *Perdix perdix* in UK, Buner et al. 2011), it is also routinely used to improve game harvest (i.e., for shooting mainly during the hunting season, “put and take”; Sokos et al. 2008), especially in intensive estates (Díaz-Fernández et al. 2012). Actually, it is estimated that at least 3–4 million farm-bred birds are released every year in Spain (Garrido 2002; Sánchez-García et al. 2009). The red-legged partridge (*Alectoris rufa*) is a medium sized galliform with a high socio-economic value for hunting in Europe (Martínez et al. 2002), which has suffered major population declines in its native range over the last decades (Aebischer and Potts 1994; Blanco-Aguiar et al. 2004). In Spain, red-legged partridge populations have decreased by >50 % between 1973 and 2002 (Blanco-Aguiar 2007; Delibes-Mateos et al. 2012), the population declines being mainly associated with land-use changes (Blanco-Aguiar 2007; Buenestado et al. 2009; Casas and Viñuela 2010; Delibes-Mateos et al. 2012). In farms, red-legged partridges have been artificially hybridized with Chukar partridges (*Alectoris chukar*) in order to increase laying period and to produce heavier and tamer birds (Potts, 1989). In the wild, both species (*A. rufa* and *A. chukar*) have clearly separate ranges, with no natural hybridization zones (Cramp and Simmons 1980, Del hoyo et al. 1994). However, human-mediated hybrids (*A. rufa* × *chukar*) have been found across most of both native and introduced ranges of *A. rufa* (Potts 1989; Baratti et al. 2004; Barbanera et al. 2005, 2010; Blanco-Aguiar et al. 2008). Hybrid red-legged partridges show lower survival than “pure” birds, but are able to breed successfully in the wild. Hence, the risk of genetic contamination of wild populations remains significant (Casas et al. 2012). Furthermore, the extent of differences in adaptation between “pure” and hybrid partridges could generate differences in morphological and behavioural characteristics, perhaps affecting their viability (Allendorf et al. 2001). In addition, together with genetic introgression, the relatively intensive husbandry methods employed in farms generate individuals with marked physiological, behavioural and parasites burden differences compared with wild ones (Millán et al. 2001, 2004; Villanúa et al. 2008; Gaudioso et al. 2011; Díaz-Sánchez et al. 2012).

Virtually nothing is known about the phenotypic differences between “pure” and hybrid birds in wild red-legged partridge populations (Blanco-Aguiar 2007). There are several phenotypic traits that could be particularly useful to examine, as they are often related to fitness (body size, body condition and plasma carotenoid levels). Body size might be important in a competitive context, in particular for intra-sexual competition (among males for access to territories and mates, and among females for access to mates; Alonso et al. 2008). Because males are larger than females, and because Chukar partridges are larger than red-legged partridges (Cramp and Simmons 1980), size might play a different role in hybrids and pure birds, depending on sex. Body condition should reflect the animal’s health, fitness and behaviour, so any index taking it into account may potentially work well as an indicator of animal quality. Considering that hybrid birds may have different vulnerability to diseases (Blanco-Aguiar 2007) or predators (Casas et al. 2012) as well as impaired health and condition with respect to pure red-legged partridges, we used the body mass corrected for size and plasma carotenoid level as an indicator of health and condition.

Carotenoid pigments are used by partridges either for ornamental coloration (Pérez-Rodríguez and Viñuela 2008), for self maintenance (parasite resistance and immune response; Blas et al. 2006; Mougeot et al. 2009) or for reproduction (females allocate carotenoid to eggs; Bortolotti et al. 2003). Vertebrates cannot synthesize carotenoids de novo, but must ingest them, so diet may limit ornament expression and good foragers, in better condition, are expected to acquire more carotenoids (Olson and Owens 1998; Hill and McGraw 2006). In addition, carotenoids act as immune-enhancers and are beneficial to health and self maintenance (Olson and Owens 1998; Moller et al. 2000). Differences in condition and carotenoid levels between hybrid and "pure" partridges would thus be indicative of differences in foraging ability, health and breeding prospect.

The occurrence of hybrid partridges during the breeding season should be greater in the local estates where releases are performed. Indeed, Blanco-Aguiar et al. (2008) found that hybrids were more present in localities where recent restocking had occurred. In order to determine the phenotypical effects of hybridization with *A. chukar* in wild *A. rufa*, first, we genotyped 115 wild red-legged partridges captured in four game estates during the breeding season, using both nuclear and mitochondrial DNA markers. We then investigated a few phenotypic traits (body size, body condition and physiological state) to test for the eventual differences between pure and hybrid birds. We discuss our results in the light of available information referring to restocking plans performed in the study area. We made genetic and phenotypic analyses on partridges in estates where either only hunting or hunting and restocking activity were carried out, in order to also evaluate the consequences of supplementations to wild populations.

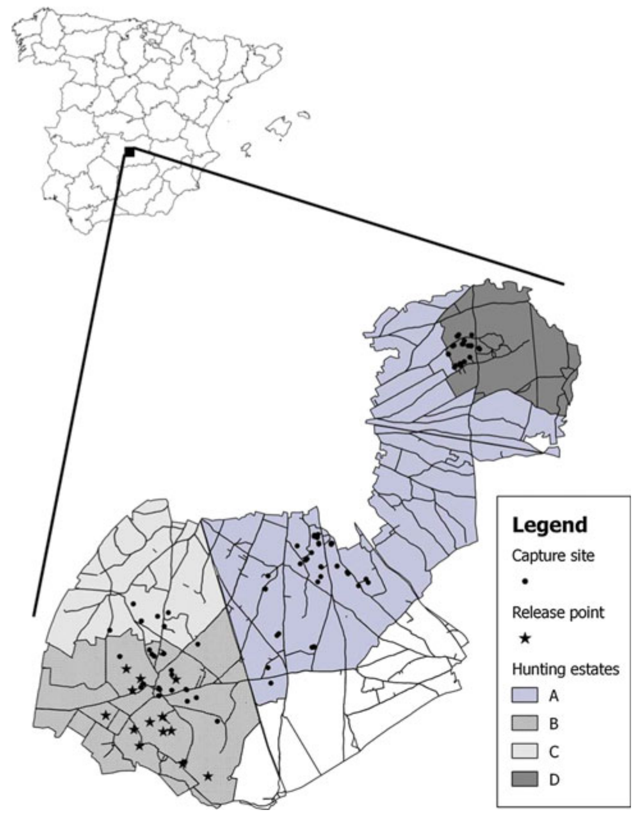

Fig. 1 Map of the study area showing the location of capture sites (*black dots*), releasing points (*black stars*) and hunting estates included in our study, 2003–2005. The inset map illustrates the location of our study area in Spain

Table 1 Genetic status (hybrid/"pure") of the individuals captured in the four game estates during the study period (2003–2005)

Years	Game estate				Total
	A	B	C	D	Hybrid/"pure"
2003	2/8	11/9	4/5	–	17/22
2004	2/14	8/7	–	0/13	10/34
2005	4/12	–	–	2/14	6/26
Total	8/34	19/16	4/5	2/27	33/82
Distance (m)	1,688	–	835	9,781	

The distance refers to nearest distance (m) between the limit of each game estate and the nearest partridge release point in game estate B (i.e., the main focus of local farm-reared red-legged partridge releases)

Materials and methods

Study area and sampling

Our study was carried out in February–May during 3 years (2003–2005), on a 125 km^2 farmland area located in the Campo of Calatrava region (Central Spain, 38°80′N, 3°80′W, 610 m a.s.l.). The study area included four game estates with different hunting management practices (hereafter "sites", Fig. 1). Because red-legged partridge were harvested on all game estates studied, some management tools were applied for hunting (mainly predator control, provision of water and food and release of captive farm-reared birds; see Casas and Viñuela 2010). In one game estate (estate B), farm-reared partridges were released each year (around 2,000 birds released annually before the hunting season started, in autumn). In another game estate (estate C), releases occurred irregularly (not all years, also before the hunting season started). In the other two game estates (A and D), no restocking of farm-reared partridges occurred at least during the 10 years prior to this study (Casas and Viñuela 2010).

Over the three study years, 115 adult partridges were captured in late winter/early spring (2003: $n = 39$, 2004: $n = 44$, 2005: $n = 32$; Table 1). We used cage traps with live adult partridges as a decoy. Traps were baited with wheat daily (Casas et al. 2009). Once partridges were captured, birds were individually ringed and sexed from plumage, biometry and ornaments (Sáenz de Buruaga et al. 2001). The sex of each bird was confirmed genetically (García and Calero-Riestra *unpublished data*). Upon each capture, we recorded the capture date (julian date; 1 = 1st of January) and measured the following: (1) body mass, with a 1,000 g Pesola® precision scale (nearest 5 g); (2) tarsus length, (3) beak length and (4) head width, with a digital calliper to the nearest 0.01 mm; (5) tail length, (6) total body length, and (7) wing length, with a ruler (nearest 0.5 mm). We also took a blood sample from the brachial vein (0.5–1 ml). All measurements were taken according to Svensson (1992) and by the same person (FC). Each bird was released at the capture site after c. 20 min.

Genetic analyses

We followed the same procedure as in Casas et al. (2012), using nine DNA diagnostic markers (eight nuclear microsatellite loci + one mitochondrial PCR–RFLP locus on a cytochrome b sequence; Blanco-Aguiar 2007; Blanco-Aguiar et al. 2008; Dávila 2009; Ferrero et al. 2011 and Casas et al. 2012 for the primer sequences) to categorically detect chukar introgression, using a simple count of diagnostic alleles at the studied loci. We considered 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.

Plasma carotenoid analysis

After collection, blood samples were kept refrigerated until centrifugation (Sigma 113, 4,000 rpm, 10 min), separating the plasma (used for carotenoid analysis) and cellular fraction (used for genetic analysis, see above). Both were stored separately, at −20 °C until analysed. Plasma carotenoid concentration was determined by diluting 60 μl of plasma in acetone (dilution 1:10). The mixture was vortexed and centrifuged at 10,000 rpm for 10 min to precipitate the flocculent proteins. The supernatant was examined in a Shimadzu UV-1603 spectrophotometer at 446 nm (see for more details, Pérez-Rodríguez et al. 2007). Finally, plasma carotenoid concentration (μg/ml) was calculated using a standard curve for lutein (Sigma Chemicals). Lutein was chosen as a reference pigment because previous works established that this was a main carotenoid circulated in the blood in red-legged partridge (Blas et al. 2006).

Statistical analyses

We tested for differences between years and sexes in the proportion of hybrid and “pure” partridges by performing a Chi square analysis on a contingency table. We tested if the proportion of introgressed individuals (hybrid = 1, “pure” = 0) was related to the distance to the nearest release point using a general linear model (GLM) with a binomial error distribution and logit link function. We used

Table 2 Results of principal component analysis on wild red-legged partridges biometrics (n = 107; Princomp procedure; SAS 2001)

	Principal components	
	First (PC1)	Second (PC2)
Total body length	+0.44	+0.31
Wing length	+0.43	−0.03
Tail length	+0.36	+0.68
Tarsus length	+0.42	−0.12
Head width	+0.42	−0.25
Beak length	+0.36	−0.59
Eigenvalue	3.81	0.86
Variance explained		
Cumulative	63.5 %	14.3 %
Proportion	63.5 %	77.8 %

the Princomp procedure (SAS 2001) for the Principal Component Analyses on biometrics (body length, wing length, tail length, tarsus length, head width and beak length; see Table 2). We tested for differences between hybrid and “pure” partridges in body mass, body size (PC1; see Table 2) and plasma carotenoid concentration using GLMs with a normal error distribution and identity link function. Initial models included sex (owing to the typical sexual size dimorphism in this species; Cramp and Simmons 1980), year, hybridization and their interactions as explanatory variables. When analysing variation in body condition, the dependent variable was body mass, with the first principal component of the PCA on size variable included as a covariate, as an index of partridge body size. We used SAS 8.01 (SAS 2001) and Statistica 6.0 (StatSoft 2002) for statistical analyses. All tests are two-tailed and data expressed as mean ± SD.

Results

Variation of introgression rate in the breeding populations

We found genetic introgression by *A. chukar* in 33 of 115 birds analysed (28.7 %). The proportion of hybrid partridges did not differ significantly between sexes ($\chi^2 = 1.34$, $df = 1$, $P = 0.25$; 23.6 % of males, $n = 55$, and 33.3 % of females, $n = 60$) or between years ($\chi^2 = 1.46$, $df = 2$, $P = 0.48$), but differed among game estates ($\chi^2 = 16.50$, $df = 3$, $P < 0.001$). The percentage of hybrid partridges was highest in the estate where partridge releases occurred yearly (estate B: 54.3 %), followed by the nearest study area where we had evidence that birds had been occasionally released (estate C: 44.4 %). The occurrence of hybrid partridges was lower in the estates where no releases were performed (19.1 % in estate A, and 7.4 % in estate D; Table 1). The proportion of hybrid partridges was related to the distance between the capture site and the nearest farm-reared partridge release point ($\chi^2 = 18.06$, $df = 1$, $P < 0.001$, Fig. 1). The average distance between capture site and release point was also lower for hybrid (2,125 ± 492 m) than for "pure" partridges (5,511 ± 439 m).

Differences in body size

We used a principal component analysis on all the body measurements to calculate an index of body size for males and females (Table 2). The first principal component (PC1) explained 64 % of variation, with all the measurements having positive loadings. The second principal component (PC2) explained a further 14 % of variation with tail length having the highest positive loading. The PC1 was indicative of overall size, and was used as an index of body size in subsequent analyses.

Variation in body size (PC1) was explained by sex ($F_{1,103} = 392.04$, $P < 0.001$; males were larger than females; Fig. 2a) and was also significantly explained by the interaction between sex and hybridization (hybridization: $F_{1,103} = 392.04$, $P < 0.001$; Hybridization × sex: $F_{1,103} = 4.08$, $P < 0.05$). Body size differed between "pure" and hybrid in males ($F_{1,50} = 4.79$, $P < 0.05$), but not in females ($F_{1,53} = 0.08$, $P = 0.77$). "Pure" males were larger than hybrid ones (Fig. 2a).

Differences in body condition index

In females, variation in body mass was significantly explained by body size (PC1: $F_{1,51} = 8.73$, $P < 0.05$) and by year ($F_{2,51} = 15.92$, $P < 0.001$), but not by sampling date (linear: $F_{1,50} = 1.62$, $P = 0.21$). Females were in better condition in 2003 (0.072 ± 0.052) than in 2004 (−0.025 ± 0.069) or 2005 (−0.029 ± 0.042). After controlling for PC1 and year, variation in female body mass was significantly explained by hybridization ($F_{1,50} = 4.32$, $P < 0.05$), but not by the interaction hybridization × year ($F_{2,49} = 0.07$, $P = 0.93$). Hybrid females were heavier relative to their size (better body condition index) than "pure" females (Fig. 2b).

In males, variation in body mass was significantly explained by body size (PC1: $F_{1,46} = 13.98$, $P < 0.001$), by year ($F_{2,46} = 3.94$, $P < 0.05$) and by sampling date (linear term: $F_{1,46} = 5.32$, $P < 0.05$; quadratic term: $F_{1,46} = 3.75$, $P < 0.05$). Male condition increased non-linearly with date and was higher in 2003 (0.015 ± 0.051) than in 2004 (−0.005 ± 0.048) or 2005 (−0.013 ± 0.055).

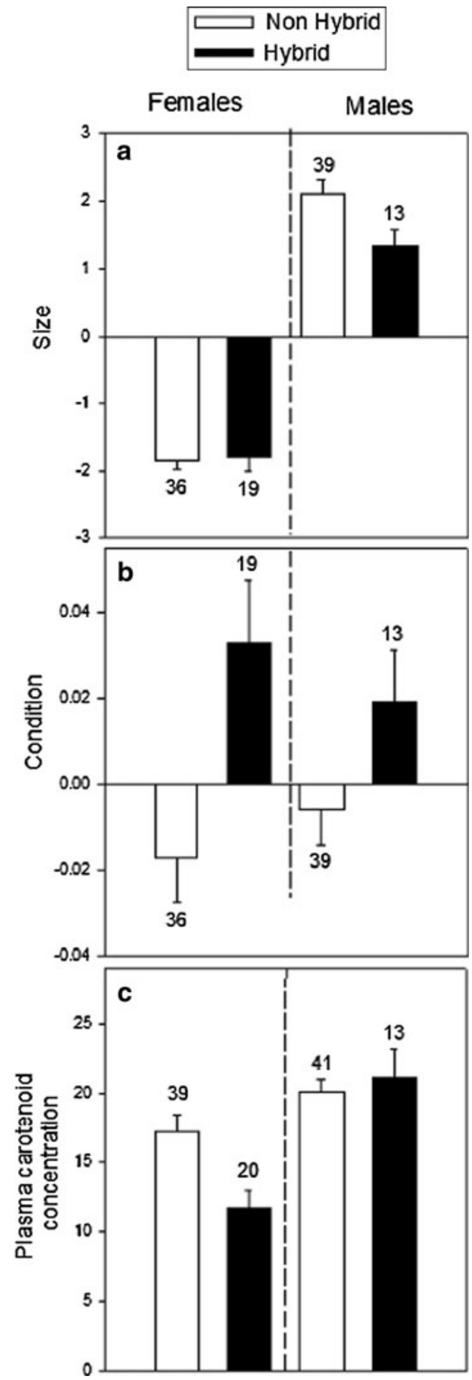

Fig. 2 Differences between hybrid (*black bars*) and non-hybrid (*white bars*) red-legged partridges according to sex in: **a** body size (mean ± SE score of the first principal component of the PCA on six body measurements; see Table 2); **b** body condition (mean ± SE body mass corrected for body size); and **c** plasma carotenoid concentration (mg × ml apex-1). Sample size below/above *error bars* refers to number of birds

After controlling for PC1, year and sampling date, variation in male body mass was not significantly explained by hybridization ($F_{1,43} = 0.59$, $P = 0.45$, Fig. 2b), nor by the interaction hybridization × year ($F_{1,43} = 0.34$, $P = 0.71$).

Differences in plasma carotenoid concentration

Males had higher plasma carotenoid concentration than females ($F_{1,108} = 7.43$, $P < 0.01$; Fig. 2c). Because of expected differences between sexes in carotenoid use prior to breeding, we analysed variation in plasma carotenoid concentration by sex.

In females, variation in plasma carotenoids was significantly explained by year ($F_{2,55} = 6.69$, $P < 0.01$) and sampling date (linear: $F_{1,55} = 5.33$, $P < 0.05$; quadratic: $F_{1,54} = 0.08$, $P = 0.77$). Carotenoid concentration was higher in 2004 (17.03 ± 6.73) and 2005 (19.58 ± 6.67) than in 2003 (8.57 ± 4.74) and increased linearly with sampling date. After controlling for year and sampling date, variation in plasma carotenoids was significantly explained by hybridization ($F_{1,54} = 4.47$, $P < 0.05$), but not by the interaction hybridization × year ($F_{2,52} = 1.32$, $P = 0.27$). Hybrid females had significantly lower plasma carotenoid concentrations than "pure" females (Fig. 2c).

In males, variation in plasma carotenoids was not significantly explained by year ($F_{2,49} = 0.26$, $P = 0.77$), sampling date (linear: $F_{1,49} = 1.41$, $P = 0.24$; quadratic: $F_{1,49} = 0.04$, $P = 0.85$) or hybridization ($F_{1,49} = 0.12$, $P = 0.73$; Fig. 2c).

Discussion

Our results showed a greater occurrence of hybrid birds near restocking areas as well as phenotypic differences between hybrids and "pure" partridge in the wild. The occurrence of hybrid birds in our study was within the range of that previously found in birds shot in autumn–winter (0–55 % depending on estates, Table 1; Blanco-Aguiar 2007), but it was higher in those game estates where restocking with farm-reared partridge took place (estates B and C). Moreover, the shorter the distance between the capture site and the farm-reared partridge release points, the greater the proportion of hybrid partridges (Fig. 1). This results support the hypotheses that the occurrence of hybrids is associated with farm-reared partridge releases (Blanco-Aguiar et al. 2008). It is noteworthy that as many as 55 % of the birds sampled during breeding season in a release site (estate B) were hybrid partridges, as this occurrence is similar to the maximum found in autumn–winter, when releases usually take place (Blanco-Aguiar 2007). Hybrids partridge can account for a high percentage of birds across different game farms (Blanco-Aguiar et al. 2008, Negri et al. 2013). Therefore, the high proportion of hybrids found in estate B could be related to a high proportion of hybrids in the farm that provided the birds for the releases there. However, we do not know the exact origin of the released birds, so we cannot exclude the possibility that the released partridges came from a farm that had a particularly high degree of hybridization.

In our study, we found phenotypic differences between hybrid and pure partridges, depending on sex. "Pure" males were larger than hybrid ones, while no difference in size was found according to genotype in females. These size differences between "pure" and hybrid partridges might be a consequence of artificial selection in farms favouring smaller males. A serious management problem in farms is related to males hurting females within the breeding cages (Padrós 1991) and smaller males could have been positively selected for being less harmful to females, However, in wild populations, body size often plays a role in intra-sexual competition, with larger males probably being favoured in territorial contests. Thus, the larger size of "pure" males may give them an advantage over hybrid males, in terms of competition for territories, territory size, competition for resources or access to mates.

When considering body condition, hybrid females were in better condition (relatively heavier) than "pure" ones. We did not find any difference in condition between hybrid and "pure" males. Our results are partly consistent with a previous study conducted during the hunting season on harvested partridges: hybrid females were found to be in relatively better condition than "pure" ones, whereas hybrid males were found to be in poorer condition than "pure" ones (Blanco-Aguiar 2007). Here, we did not find differences in body condition between hybrid and "pure" males in spring possibly because the hybrid males that survived over winter were those better able to recover a good condition. The differences in body condition observed in females could be related to hybridization and to different artificial selection pressures on farm-reared females aimed at increasing reproductive outputs (Padrós 1991; Gaudioso et al. 2002), which have been previously associated with body condition parameters (Williams 2005). In fact, hybrid females laid larger clutches in the wild than "pure" females (Casas et al. 2012).

Carotenoid levels decrease with increasing intestinal parasite infections as demonstrated in red-legged partridges (Mougeot et al. 2009) and other gamebirds (e.g., Martínez-Padilla et al. 2007; Mougeot et al. 2007). In addition, hybrid birds seem to be more susceptible to intestinal parasites (Blanco-Aguiar 2007). Thus, lower carotenoid levels might be indicative of greater parasite infections. In this study, no differences in blood carotenoid levels were found between hybrid and "pure" males. Nevertheless, hybrid females had significantly lower plasma carotenoid concentrations than "pure" birds, and a possible explanation might be that they were more parasitized. However, this does not fit with the finding that hybrid females were in better condition than "pure" females, given that parasites typically negatively impact on host condition (e.g., Blanco-

Aguiar 2007). An alternative explanation would be that the lower carotenoid levels in blood plasma of hybrid females reflected a reduced capacity to ingest and/or absorb carotenoids. This could have fitness consequences, for health related function (immune function; Mougeot et al. 2009) and cause a limitation in females for pairing (less carotenoid available for sexual ornamentation). However, incubation probability did not differ between hybrid and "pure" females (Casas et al. 2012), suggesting that carotenoid limitation may not affect pairing succes. Otherwise, given that hybrid females laid more eggs than "pure" females (Casas et al. 2012), but had less plasma carotenoids prior to laying, it might be also possible that hybrid females allocated fewer carotenoids to eggs, which could reduce hatchability (Cucco et al. 2007) or have negative consequences for offspring fitness (e.g., Surai et al. 2001; Blount 2004). However, more investigation on the mechanisms of ingestion, assimilation and allocation of carotenoids by hybrid and "pure" females is needed to detect possible fitness consequences.

Overall, "pure" and hybrid partridges differed in morphological and physiological characteristics, potentially affecting the adaptation and viability of hybrids in the wild. This seems particularly clear in hybrids males due to their lower body size (poorer competitive capacity), which may limit access to mate and territory. In contrast, hybrids females had a better body condition, as well as a greater laying capacity (Casas et al. 2012) than wild ones. The artificial selection and domestication in game-farms, where directional selection aims at reducing agonistic behaviour and improve productivity, could have promoted these phenotypic differences (Lynch and O'Hely 2001).

The occurrence of hybrids associated to restocking areas and phenotypic indicators (body size, body condition and physiological state) differences between "pure" and hybrid partridges confirm a serious threat to the genetic integrity and viability of wild red-legged partridge populations. The results of this study raise concerns about: (1) the risks of loss of genetic integrity at a local scale, where farmed partridges are released into the wild; (2) how hybrid and "pure" birds may interact in the wild; and (3) how differences in morphological and behavioural characteristics may affect fitness and population viability. Therefore, there is an urgent need to implement methods allowing the detection of hybrid birds prior to releases in order to avoid this important threat to wild red-legged partridge populations. Moreover, differences in parasites burdens and prevalence have been reported between farm-reared and wild partridges (Millán et al. 2004; Millán 2009; Díaz-Sánchez et al. 2012). Many releases are carried out without disease and parasites controls (Millán 2009), or using ineffective control methods (Villanúa et al. 2007b), posing an additional risk of new parasite introductions into wild red-legged populations (Villanúa et al. 2008) that may even affect other endangered species (Villanúa et al. 2007a). Therefore, stricter genetic and sanitary controls should be put in place for farm-reared partridges (see Casas et al. 2012), and non-invasive methods may be very useful to monitor the genetic quality of partridges, both in farms and in wild populations (Guerrini and Barbanera 2009).

Acknowledgments We thank all game managers and hunting societies' presidents for allowing studying partridges on their hunting estates. Particular thanks are due to all people have helped with the data collection. Fabián Casas was supported by a post-doctoral grant of the Junta de Comunidades de Castilla la Mancha (JCCM), and a JAE-Doc contract of the program «Junta para la Ampliación de Estudios» financed by the European Social Fund (ESF). This work alsoreceived a grant by the research project "Bases científicas preliminares para un plan de conservación de la perdiz roja en Castilla-La Mancha" of the Consejería de Agricultura y Medio Ambiente de la JCCM (Junta de Comunidades de Castilla-La Mancha), and CYCIT projects MCYT-REN200307851/GLO and CGL2004-02568/BOS. FM was supported by a Grant from the Ministerio de Educación y Ciencia, Spain (CGL 2006-11823) and from the JCCM, Spain (PAI06-0112).

References

Aebischer NJ, Potts GR (1994) Red-legged partridge. In: Tucker GM, Heath MF (eds) Birds in Europe. Their conservation status. Birdlife conservation, vol 3. Birdlife International, Cambridge, p 214

Allendorf FW, Leary RF, Spruell P, Wenburg JK (2001) The problems with hybrids: setting conservation guidelines. Trends Ecol Evol 16:613–622

Alonso ME, Prieto R, Gaudioso VR, Pérez JA, Bartolomé DJ, Díez C (2008) Influence of the pairing system on the behaviour of farmed relegged partridge couples (*Alectoris rufa*). Appl Anim Behav Sci 115:55–66

Baratti M, Ammannati M, Magnelli C, Dessi-Fulgheri F (2004) Introgression of chukar genes into a reintroduced red-legged partridge (*Alectoris rufa*) population in central Italy. Anim Genet 36:29–35

Barbanera F, Negro JJ, Di Giuseppe G, Bertoncini F, Cappelli F, Dini F (2005) Analysis of the genetic Structure of red-legged partridge (*Alectoris rufa*, *Galliforms*) populations by means of mitochondrial DNA and RAPD markers: a study from central Italy. Biol Conserv 122:275–287

Barbanera F, Pergams ORW, Guerrini M, Forcina G, Panayides P, Dini F (2010) Genetic consequences of intensive management in game birds. Biol Conserv 143:1259–1268

Blanco-Aguiar JA, (2007) Variación espacial en la biología de la perdiz roja (*Alectoris rufa*): una aproximación multidisciplinar. PhD Diss. Universidad Complutense de Madrid, Spain

Blanco-Aguiar JA, Virgós E, Villafuerte R (2004) Perdiz Roja (*Alectoris rufa*). In: Madroño A, González C, Atienza JC (eds) Libro Rojo de las Aves de España. Dirección General para la Biodiversidad-SEO/BirdLife, Madrid, pp 182–185

Blanco-Aguiar JA, González-Jara P, Ferrero ME, Sánchez-Barbudo I, Virgós E, Villafuerte R, Dávila JA (2008) Assessment of game restocking contributions to anthropogenic hybridization: the case of the Iberian red-legged partridge. Anim Conserv 11:535–545

Blas J, Pérez-Rodríguez L, Bortolotti GR, Viñuela J, Marchant TA (2006) Testosterone increases bioavailability of carotenoids:

insights into the honesty of sexual signalling. Proc Natl Acad Sci USA 103:18633–18637
Blount JD (2004) Carotenoids and life-history evolution in animals. Arch Biochem Biophys 430:10–15
Bortolotti GR, Negro JJ, Surai PF, Priet P (2003) Carotenoids in eggs and plasma of red-legged partridges: effects of diet and reproductive output. Physiol Biochem Zool 76:367–374
Buenestado FJ, Ferreras P, Blanco-Aguiar JA, Tortosa FS, Villafuerte R (2009) Survival and causes of mortality among wild Red-legged Partridges *Alectoris rufa* in southern Spain: implications for conservation. Ibis 151:720–730
Buner FD, Browne SJ, Aebischer NJ (2011) Experimental assessment of release methods for the re-establishment of a red-listed galliforms, the grey partridge (*Perdix perdix*). Biol Conserv 144:593–601
Casas F, Viñuela J (2010) Agricultural practices or game management: which is the key to improve red-legged partridge nesting success in agricultural landscapes? Environ Conserv 37:177–186
Casas F, Mougeot F, Viñuela J (2009) Double nesting behaviour and differences between sexes in breeding success in wild red-legged partridges *Alectoris rufa*. Ibis 151:743–751
Casas F, Mougeot F, Sanchéz-Barbudo I, Dávila JA, Viñuela J (2012) Fitness consequences of anthropogenic hybridization in wild red-legged partridge (*Alectoris rufa*, Phasianidae) populations. Biol Invasions 14:295–305
Cramp S, Simmons KEL (1980) The birds of the Western Palearctic, vol II. Oxford University Press, Oxford
Cucco M, Guasco B, Malacarne G, Ottonelli R (2007) Effects of β-carotene on adult immune condition and antibacterial activity in the eggs of the grey partridge, *Perdix perdix*. Comp Biochem Physiol A 147:1038–1046
Dávila JA (2009) Marcadores genéticos para detectar introgresión en aves del género Alectoris. Patent no 2 323 027, Oficina Española de Patentes y Marcas
Del Hoyo J, Elliott A, Sargatal J (1994) Handbook of the birds of the world, vol II. Lynx Editions, Barcelona
Delibes-Mateos M, Farfán MA, Olivero J, Vargas JM (2012) Impact of land-use changes on red-legged partridge conservation in the Iberian Peninsula. Environ Conserv 39:337–346
Díaz-Fernández S, Viñuela J, Arroyo B (2012) Harvest of red-legged partridge in central Spain. J Wildl Manag 76:1354–1363
Díaz-Sánchez S, Mateo-Moriones A, Casas F, Höfle U (2012) Prevalence of *Escherichia coli*, *Salmonella* sp. and *Campylobacter* sp. in the intestinal flora of farm-reared, restocked and wild red-legged partridges (*Alectoris rufa*): is restocking using farm-reared birds a risk? Eur J Wildl Res 58:99–105
Ferrero ME, Blanco-Aguiar JA, Lougheed SC, Sánchez-Barbudo I, de Nova PJG, Villafuerte R, Dávila JA (2011) Phylogeography and genetic structure of the red-legged partridge (*Alectoris rufa*): more evidence for refugia within the Iberian glacial refugium. Mol Ecol 20:2628–2642
Garrido JL (2002) Capturas de perdiz roja (Economía inducida por la caza de perdiz). In: FEDENCA (ed) Aportaciones a la gestión sostenible de la caza. FEDENCA, Madrid, pp 141–147
Gaudioso VR, Alonso ME, Robles R, Garrido JA, Olmedo JA (2002) Effects of housing type and breeding system on the reproductive capacity of the red-legged partridge (*Alectoris rufa*). Poult Sci 81:169–172
Gaudioso VR, Sánchez-García C, Pérez JA, Rodríguez PL, Armenteros JA, Alonso ME (2011) Does early antipredator training increase the suitability of captive red-legged partridges (*Alectoris rufa*) for releasing? Poult Sci 90:1900–1908
Griffith B, Scott JM, Carpenter JW, Reed C (1989) Translocation as a species conservation tool: status and strategy. Science 245:477
Guerrini M, Barbanera F (2009) Noninvasive genotyping of the red-legged partridge (*Alectoris rufa*, *Phasianidae*): semi-Nested PCR of mitochondrial DNA from feces. Biochem Genet 47:873–883
Hill GE, McGraw KJ (2006) Bird coloration: function and evolution. Harvard University Press, Cambridge
Laikre L, Schwartz MK, Waples RS, Ryman N (2010) Compromising genetic diversity in the wild: unmonitored large-scale release of plants and animals. Trends Ecol Evol 25:520–529
Lynch M, O'Hely M (2001) Captive breeding and the genetic fitness of natural populations. Conserv Genet 2:363–378
Martínez J, Viñuela J, Villafuerte R (2002) Socioeconomic and cultural aspects of gamebird hunting. REGHAB project, European Commission, Brussels
Martínez-Padilla J, Mougeot F, Pérez-Rodríguez L, Bortolotti GR (2007) Nematode parasites reduce carotenoid-based signalling in male red grouse. Biol Lett 3:161–164
Millán J (2009) Diseases of the red-legged partridge (*Alectoris rufa* L.): a review. Wildl Biol Pract 5:70–88
Millán J, Gortazar C, Villafuerte R (2001) Marked differences in the splanchnometry of farm-bred and wild red-legged partridges (*Alectoris rufa* L.). Poult Sci 80:972–976
Millán J, Gortázar C, Villafuerte R (2004) A comparison of the helminth faunas of wild and farm-reared red-legged partridges. J Wildl Manag 68:701–707
Møller AP, Biard C, Blount JD, Houston DC, Ninni P, Saino N, Surai PF (2000) Carotenoid-dependent signals: indicators of foraging efficiency, immunocompetence or detoxification ability? Avian Poult Sci Rev 11:137–159
Mougeot F, Pérez-Rodríguez L, Martínez-Padilla J, Leckie F, Redpath SM (2007) Parasites, testosterone and honest carotenoid-based signalling of health. Funct Ecol 21:886–898
Mougeot F, Pérez-Rodríguez L, Sumozas N, Terraube J (2009) Parasites, condition, cellular immunity and carotenoid-based ornamentation in male red-legged partridge *Alectoris rufa*. J Avian Biol 40:67–74
Negri A, Pellegrino I, Mucci N, Randi E, Tizzani P, Meneguz PG, Malacarne G (2013) Mitochondrial DNA and microsatellite markers evidence a different pattern of hybridization in red-legged partridge (*Alectoris rufa*) populations from NW Italy. Eur J Wildl Res. doi:10.1007/s10344-012-0686-3
Olson VA, Owens IPF (1998) Costly sexual signals: are carotenoids rare, risky or required? Trends Ecol Evol 13:510–514
Padrós J (1991) Situación actual del sector. Presente y futuro. In: La perdiz roja. Fundación la Caixa, AEDOS, Barcelona, pp 7–10
Pérez-Rodríguez L, Viñuela J (2008) Carotenoid-based bill and eye ring coloration as honest signals of condition: an experimental test in the red-legged partridge (*Alectoris rufa*). Naturwissenschaften 95:821–830
Pérez-Rodríguez L, Alonso-Alvarez C, Viñuela J (2007) Repeated sampling but not sampling hour affects plasma carotenoid levels. Physiol Biochem Zool 80:56–60
Potts GR (1989) The impact of releasing hybrid partridges on wild red-legged populations. Game Conserv Rev 20:81–85
Sáenz de Buruaga M, Lucio A, Purroy FJ (2001) Reconocimiento de sexo y edad en especies cinegéticas. EDILESA (ed) León, Spain
Sánchez-García C, Alonso ME, Prieto R, González V, Gaudioso VR (2009) Una visión sobre la avicultura para la producción de caza en España. ITEA-Anim 105:169–183
SAS (2001) SAS/STAT User's guide, version 8.01. SAS Insitute Inc., Cary
Sokos K, Periklis KB, Tsachalidis EP (2008) The aims of galliforms release and choice of techniques. Wildl Biol 14:412–422
StatSoft Inc (2002) STATISTICA data analysis software system, version 6. www.statsoft.com
Surai PF, Speake BK, Sparks NHC (2001) Carotenoids in avian nutrition and embryonic development. Absorption, availability and levels in plasma and egg yolk. Poult Sci 38:1–27
Svensson L (1992) Identification guide to European passerines. Mirstatryck, Stockholm

Villanúa D, Casas F, Viñuela J, Gortázar C, de la Morena ELG, Morales MB (2007a) First occurence of *Eucoleus contortus* in a little bustard *Tetrax tetrax*. A negative effect of red-legged partridge *Alectoris rufa* releases on steppe bird conservation? Ibis 149:405–406

Villanúa D, Pérez-Rodríguez L, Rodríguez O, Viñuela J, Gortázar C (2007b) How effective is pre-release nematode control in farm-reared red-legged partridges *Alectoris rufa*? J Helminthol 81:101–103

Villanúa D, Pérez-Rodríguez L, Casas F, Alzaga V, Acevedo P, Viñuela J, Gortázar C (2008) Sanitary risks of red-legged partridge releases: introduction of parasites. Eur J Wildl Res 54:199–204

Williams TD (2005) Mechanisms underlying the costs of egg production. Bioscience 55:39–48