



Decreased fitness of restocked hybrid quails prevents fast admixture with wild European quails



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ABSTRACT

Restocking with non-native species for hunting purposes is a widespread practice in some Galliformes species that may result in the introgression of maladaptive alleles into native populations. Quails farmed for restocking are produced by interbreeding domestic Japanese quails (*Coturnix japonica*) with European quails (*Coturnix coturnix*). Massive releases of these animals could represent a threat to native European quails. In this study we radio-track 16 female native European quails and 51 female farm-reared quails over four breeding seasons in a single locality. Our results show that farmed female quails attracted more wild common quail males than European quail females, probably because they produce more rally calls. Here for the first time we show empirical evidence that European quails and restocked farmed quails interbreed in the wild. Further, hybrid farmed females and European females had similar probabilities of mating, nesting success, clutch size, fertility, hatching probability and chicks' survival probability. However, female farmed quail had higher mortality than European quail females, and their nests had a higher rate of predation. These last observations could explain why the two lineages do not appear completely admixed after more than 20 years of massive restocking practices. However, our results clearly show a lack of reproductive barriers in the wild and that introgression may not be completely prevented. An immediate ban of the release of non-native quails is necessary to preserve their genetic integrity. Thus, banning restocking with Japanese quail or hybrids is necessary to prevent the progressive introduction of maladaptive alleles into the European quail populations.

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1. Introduction

Anthropogenic hybridization, either resulting from the introduction of species or from the invasion of alien species due to the modification of habitats, may create serious conservation problems (Levin et al., 1996; Rhymer and Simberloff, 1996). Introductions may be accidental (Kidd et al., 2009; McDowell, 2002) or part of a management plan, for example, to bolster endangered populations (Armstrong and Seddon, 2008; Massemin-Challet et al., 2006; Santos et al., 2009) or to increase hunting bags in the case of game species (Barbanera et al., 2010; Blanco-Aguar et al., 2008). Restocking with non-native species for hunting pur-

poses is a widespread management action for Galliformes (Barbanera et al., 2005, 2007, 2008; Blanco-Aguar et al., 2008; Casas et al., 2012; Derégnaucourt et al., 2002; Derégnaucourt et al., 2005a,b; Porkert et al., 1997; Puigcerver et al., 2007; Tejedor et al., 2007). These restocking practices are carried out mainly with farm-reared birds, in most cases of doubtful genetic origin (Perennou, 2009), and thus constitute a real danger for native populations (Chazara et al., 2010; Laikre et al., 2010; Olden et al., 2004).

The European quail (*Coturnix coturnix*), also called common quail in Europe, is a migratory Galliform with a distribution from the British Islands to Lake Baïkal and from the Arctic Circle to the tropics (Del Hoyo et al., 1994; Guyomarc'h et al., 1998; Moreau and Wayre, 1968). It is of conservation concern (SPEC 3) due to a large historical decline (Burfield, 2004). Even so, it is a very popular game bird with a high socio-economic value, especially in some Mediterranean countries. In Spain alone, which hosts the largest breeding population in Western Europe (Gallego et al., 1997; Rodríguez-Teijeiro et al., 2006), more than 1,300,000 individuals

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were hunted annually during the period 1973–2010 (Yearbooks of Agro-alimentary Statistics of the Spanish Ministry of Agriculture, Fisheries and Food). Because of hunting interest in this species, restocking with farm-reared quails is a very common practice in several European countries such as Italy, Greece, the Republic of Serbia, Montenegro, Romania and Spain (Puigcerver et al., 2007; Rodríguez-Teijeiro et al., 1993; Tsiompanoudis et al., 2011). For example, in Catalonia (Northeast Spain), more than 68,000 farm-reared quails have been restocked annually during the period 1990–2006 (personal communication from the Catalan Department of Agriculture, Farming, Fisheries, Food and Environment) in an area with a wild population of between about 5000 and 21,000 males (Rodríguez-Teijeiro et al., 2004).

Recent genetic studies show that these farm-reared quails are hybrids of European and domesticated Japanese (*Coturnix japonica*) quails (Amaral et al., 2007; Barilani et al., 2005; Chazara et al., 2010; Sanchez-Donoso et al., 2012) (in this manuscript we use the term “hybrid” to refer to all individuals whose genomes show extensive admixture of the two lineages, without limiting the definition to first generation hybrids). The Japanese quail is distributed across East Asia, including Japan. The natural ranges of the two species only overlap in the surroundings of Lake Baikal in Russia (Fefelov, 1998) and in the Kentei region in Mongolia (Kozlova, 1932). No natural hybridization has been reported (Del Hoyo et al., 1994; Guyomarc’h et al., 1998; Moreau and Wayre, 1968). However, hybrids of the two species generated in captivity are or have been used for releases in different European countries, and, as a result, the Japanese quail is considered an introduced species in Italy by the IUCN (<http://maps.iucnredlist.org/map.html?id=100600195>; accessed on November 5 2013) and in Spain by the Spanish Government (Royal Decree 630/2013). Thus, there is a real possibility that restocking could lead to the extensive introgression of domestic Japanese quail alleles into the European quail gene pool.

The European quail and the Japanese quail have almost identical habitat requirements (Taka-Tsukasa, 1941), and experiments in captivity show that they present only partial mate discrimination (Derégnaucourt and Guyomarc’h, 2003). Derégnaucourt et al. (2002) have also shown that hybridization between European and domestic Japanese quail, and backcrosses between hybrids and the parental species readily occurs in captivity and no differences have been found in average fertility, hatch and chick survival rates between hybrid pairs and European quails.

However, the arrival of Japanese quail alleles may represent a threat for European quails if they represent different adaptation to environmental conditions, co-adapted gene complexes or even sets of maladaptive alleles favorably selected in captivity. The European quail is a partially migratory species, whereas the domestic Japanese quail lost its migratory impulse during the domestication process (Derégnaucourt et al., 2005a,b; Guyomarc’h, 2003). As released hybrid quails have a lower frequency of migratory phenotypes (Derégnaucourt et al., 2005a,b), the introgression of their genes into wild populations could cause a decrease in the migratory tendency of European quail populations. This could lead to a decrease of the European quail population density, as non-migratory quails are more likely to suffer seasonal depletion of food resources in winter and a longer hunting season.

At least in theory, hybridization between European and farmed quails is likely to lead to a rapid increase in the proportion of hybrids and admixed individuals in wild populations. Once admixture has begun it is difficult to stop, especially if hybrids are fertile and mate both with other hybrids and with both parental species (Allendorf et al., 2001). As a result, in a few generations this process might result in a hybrid swarm in which almost all individuals are of hybrid origin (Allendorf et al., 2001; Huxel, 1999), leading to the collapse of the European quail population. But this may not be just a theoretical threat. Hybrid individuals have been detected across Europe

(Guyomarc’h, 2003; Rodríguez-Teijeiro et al., 1993), and genetic evidence for introgressive hybridization has been reported in Portugal (Amaral et al., 2007), Spain (Barilani et al., 2005; Sanchez-Donoso et al., 2012) and France (Chazara et al., 2010). However, these studies do not provide evidence of hybridization occurring in the wild. Consequently, Guyomarc’h (2003) called for changes in European Union law to stop the introgression of Japanese quail genes by banning the production and commercialization of Japanese quails as well as the breeding of hybrid quails for restocking purposes. Finally, the European Union Management Plan for the European quail (period 2009–2011) has stressed the need to ban Japanese/hybrid quail releases throughout Europe and across the rest of the European quail distribution (Perennou, 2009).

However, in spite of this persistent threat, Puigcerver et al. (2007, 2012) monitored the phenotype and song in wild quail populations in Catalonia over a period of 28 years (from 1984 to 2011) and failed to detect an increase in the proportion of hybrids. At the same time, genetic analyses over a 15 year period (1996–2010) showed that an increase over time in the proportion of admixed individuals cannot be detected (Sanchez-Donoso et al., submitted for publication). These data strongly suggest that despite the annual release of tens of thousands of hybrids, the introgression of Japanese quail alleles into the wild population is lower than initially expected.

The lack of an obvious increase in the proportion of admixed individuals can be explained by different factors:

- A very high mortality rate of restocked hybrids, which could be poorly adapted to life in the wild, because they lack the ability to protect themselves against cold weather, anti-predator behavior and the ability to find appropriate food (Guyomarc’h, 2003). High mortality is probably also due to hunting, since restocked individuals are usually released just before the opening of the hunting season, which starts before the birds are habituated to the new environment.
- In spite of the lack of reproductive barriers in captivity, ecological or behavioral barriers could prevent admixture in natural conditions. There is no direct evidence of mating between European quails and farmed birds in natural conditions.
- Mixed pairs (or pairs of farmed animals mating in the wild) could suffer high chick mortality (this could be due to causes such as lack of parental care resulting from adaptation to captivity, or susceptibility to parasites), as has been observed for other Galliformes. For example, Duarte and Vargas (2004) found a chick mortality rate of 91% in restocked farm-reared females of red-legged partridge (*Alectoris rufa*).

The aims of this study were to investigate whether massive restocking with farm quails of hybrid origin quails in one of the European countries where restocking with farm-reared quails is legal (Spain) may lead to extensive admixture with European quails in the wild, and to study mating and productivity differences between female wild European quails and females restocked from game farms. Finally, we investigate how these differences could contribute to explain why the proportion of admixed individuals is not clearly increasing through time in the wild population. Our results showed decreased survival and fitness for the released birds. The results also emphasized how the putative consequences of restocking practices cannot be directly inferred from just the number of individuals released.

2. Material and methods

In Spain, the country where the study has been carried out, the Law 42/2007 of Natural Heritage and Biodiversity allows restocking with native species but not with non-native ones or hybrids. In this case, restocking with European quail is allowed, but not

with Japanese quail or hybrids. In our study, we have used farm-reared quails intended for restocking that were certified as European quails by the regional Administration based on a veterinarian analysis. However, these farm-reared individuals turned out to be hybrids (Sanchez-Donoso et al., 2012). As indicated below (see “Field Experiment Design”) we obtained all necessary permits required to carry out this study, and the released farmed birds that survived to the end of the study as well as their chicks were recaptured.

2.1. Study area

The study was carried out in a 1 km² area in Northeast Spain (41°25'N, 1°23'E, 628 m above sea level), in a short and narrow valley with winter cereals (mainly barley and wheat) which constitutes a suitable habitat for European quail reproduction. The study area is surrounded by pine forest patches unsuitable for quails, but other cereal fields with quails are located less than 2 km away.

2.2. Field experiment design

The study was conducted during the breeding seasons of the period 2007–2010. The field work started around April 23rd, coinciding with the arrival of the first migrants, and finished around August 4th with the end of the breeding season due to the harvest of cereals, which implies the loss of the breeding habitat. On average, the field work lasted 102.5 days (*SD* = 7.2) per breeding season.

During the period 2007–2009, 20 walk-in funnel traps (144 cm × 67 cm × 87 cm) were randomly distributed along the edge of the cereal plots facing the cereal. Inside these traps we put a smaller cage containing a sexual decoy (Rodríguez-Teijeiro et al., 2003; Sardà-Palomera et al., 2011). In ten of these traps, the sexual decoy was a hybrid female from a game farm close to the study area, in Catalonia (Spain). These farm-reared hybrids showed small phenotypic differences when compared to their wild counterpart (as in Guyomarc'h, 2003): they had shorter wing and longer tarsus.

Groups of male European quail have been shown to attract both males and females (Sardà-Palomera et al., 2011). For this reason, the remaining ten traps were set in five pairs to replicate the aggregations of males observed in the field (normally involving 2 to 4 males, pers. obs.). Each pair was set in parallel separated by about 5 m. One trap of the pair contained one cage and the other one contained two cages, each cage containing one male decoy. Thus, each pair of traps represented an aggregation of 3 males (hereafter, male aggregation trap). Two of the male aggregation traps contained male European quail captured in neighboring areas and the remaining three contained male hybrid quail (Table 1).

In 2010, 19 walk-in funnel traps were placed in the study area. Six traps contained hybrid females as sexual decoy, while for comparative purposes three contained European quail females (captured in neighboring areas and kept in a pen for several months). No changes were made with regard to male aggregation traps (Table 1).

Traps were checked every 2–3 days to keep disturbance caused by researchers to a minimum. Water was supplied from an aseptic 8-l tank; food trays (with vitamin-supplemented wheat) had a capacity of 3 kg which allowed individuals to eat “ad libitum”. Cages had double wire to protect the quails against predators, and trap, which was protected against sunlight and rainfall, was sprayed at every visit with a mammal repellent. At every visit, food and water supplies were checked.

When a free-ranging male entered a funnel trap containing a female (either European quail or hybrid), we attached backpack radio transmitters (PIP-3 Biotrack button cell tags) to the females and we released them. The released female was replaced in the cage by another one of the same origin. Three days per week, the released individuals were monitored with a telemetry receiver (R1000, 148–174 MHz; Communications Specialists, Inc., Orange, CA) and a three-element Yagi antenna to locate them and to check if they had mated and nested, following Rodríguez-Teijeiro et al. (2003). We consider that a female mated successfully when she succeeded in establishing a pair bond with a male, no matter whether this female would succeed in nesting or not (she could be predated before that). For nesting success we understand the success in nesting by a female, no matter whether this nest was finally predated or not. At the end of the study, hybrid radio-tagged individuals were captured and returned to captivity to avoid the risk of genetic pollution. A total of 67 individuals were radio-tagged (51 hybrid females and 16 European quail females). Table 2 shows the number of radio-tagged females and the number of nests found in each year of study.

Once a nest was located, we determined the clutch size. After 10–12 days of incubation (more than 60% of the incubation period), we took the eggs from the nest to put them in a Masalles G-180 THP incubator (Sant Cugat del Vallès, Barcelona, Spain) and raised the chicks in captivity. This procedure allowed us to know the number of fertilized eggs, the number of hatched eggs and the number of chicks surviving in captivity during the critical period of the first three weeks of life (while flying abilities are not yet developed) for European quail and hybrid females. Also, it allowed us to know the number of nests preyed upon during the 10 days of the incubation period that were monitored. After the study was completed, these chicks remained in captivity in a large pen.

In addition, telemetry also allowed finding dead individuals. Since females tend to stay in the same area after their release, we defined the known survival time for each female as the time from the release until its death or until the study period reached its end due to harvest (at that time we proceeded to the recapture of the radio-tagged individuals).

Blood samples (100 µl) from the jugular vein were taken and used to genetically identify the origin of all individuals used in this study as European quails or hybrids following Sanchez-Donoso et al. (2012). All individuals were genotyped for 11 unlinked autosomal microsatellite loci (from Kayang et al., 2002, 2004) and genotypes were analyzed with STRUCTURE 2.3.2 (Pritchard et al., 2000) and NEWHYBRIDS 1.1 (Anderson and Thompson, 2002).

The study strictly followed the requirements of the Spanish Law 5/1995 of protection of animals used in experimentation and for

Table 1
Number and types of traps used each breeding season.

Sexual decoy in the trap	2007	2008	2009	2010	Total
One hybrid female	10	10	10	6	36
One European quail female	0	0	0	3	3
Hybrid male aggregation (3 males) ^a	3	3	3	3	12
European quail male aggregation (3 males) ^a	2	2	2	2	8
Total	15	15	15	14	59

^a Each one of these is composed by two traps and were intended to imitate groups of males found in European quail populations.

Table 2

Number of females radio-tagged in each year of study. In brackets: number of nests found.

Type of female	2007	2008	2009	2010	Total
European quail females	1 (1)	2 (2)	2 (1)	11 (7)	16 (11)
Hybrid females	11 (8)	15 (4)	17 (8)	8 (1)	51 (21)

other research goals, and the Decree 214/1997, which regulates the use of animals for experimentation and for other research goals. The animal experimentation procedure for this study (number B9900035) was approved by the Ethics Committee on Animal Experimentation of the University of Barcelona and by the Autonomous Government of Catalonia (“Direcció General del Medi Natural”, “Departament de Medi Ambient i Habitatge”), which has all the competences in this subject. Moreover, the University of Barcelona institutional code of good research practices was followed (Vicerectorat de Recerca, 2010).

2.3. Data analysis

2.3.1. Attraction ability

The attraction of wild quails to traps was analyzed with regard to the sex and origin (European quail or hybrid) of the individuals used as decoy. For each kind of trap (with a female decoy or a male aggregation decoy), the number of captures was modeled with Generalised Linear Models (GLM) as a function of the origin of the decoy in the trap. The number of captured wild quails was the response variable and the origin of the individuals acting as decoy was the independent variable of interest in both models. GLM models were constructed assuming a Poisson error distribution and log link function. They were tested for overdispersion (residual deviance/residual $df > 1$) and the heterogeneity of residuals was assessed by visual examination of the figures. The number of traps with each kind of decoy was also added as an offset term because they were not equally represented not only between years, but also within years (Table 1). As female decoys do not attract wild females, an additional independent variable was the sex of the captured quails only for traps simulating aggregations of males. In this model, “male aggregation trap” was also added as a random factor because males and females captured in the same male aggregation trap are not independent observations. Thus, this model was a Generalised Linear Mixed Model (GLMM). Since only in 2010 hybrid females and European quail females were simultaneously used as decoys, the comparison of their mate attraction was carried out only in this year. The interaction between explanatory variables was initially added to the model and removed if not significant. To establish the significance of the full model we used a likelihood ratio test, comparing its deviance with that of the null model including only the intercept. To test the significance of the interaction between explanatory variables we compared the deviance of the full model with that of a corresponding reduced model without interactions. These models allowed us to test whether there were differences in the number of quails captured per trap and breeding season considering the two different decoys. We could also investigate differences between sexes in the degree they are attracted by male aggregations.

2.3.2. Female mating and breeding success

A GLM was fitted for each variable related to female breeding success: (1) mating success (binary); (2) nesting success (binary); (3) clutch size (count); (4) fertilized eggs per laid egg (proportion); (5) nest predation (binary); (6) hatched eggs per fertilized egg (proportion); and (7) surviving chicks per hatched egg (proportion). Binary and proportion variables were fitted assuming a

binomial error distribution and logit link function and clutch size was fitted assuming a Poisson error distribution and log link function. If a model presented overdispersion (residual deviance/ $df > 1$), the corresponding variable was refitted using, depending of the case, a quasi-Poisson or a quasi-binomial error distribution.

A factor representing years and its interaction with origin (hybrid or European) were also initially added to the models to take into account changing conditions across years. These additional factors were removed from the final models if no significant effect was found.

Since female mating and nesting success are not only influenced by female ability to mate and nest but also by their survival probability, when modeling those variables, we added as covariate the female known survival time. Thus, this covariate allowed the models to test for differences in female mating and nesting success due to their origin independently of possible differences due to different mortality. These models also allowed us to estimate how long it takes for a female to achieve 95% probability of mating or nesting.

2.3.3. Survival

We used a Cox proportional hazards regression model (Cox, 1972) to assess the effect of female origin on its survival during the breeding season. This model assumed that the relative risk of death for both types of females (hybrid or European quail) remained constant over time. This risk was estimated by the model by considering the known survival periods. The model took into account if the end of the known survival period of each female was due to death or to the beginning of harvest. We also took into account the possible differences across years, and the interaction origin-year. If the interaction was not significant, it was removed from the models.

All analyses were conducted in R ver. 2.15.2 (R Core Team, 2012). GLM's models were fitted using the `glm()` function. Cox proportional hazards model was fitted using the function `coxph()` present in the package `survival` (Therneau, 2012). The significance of the factors added into a model were evaluated performing an analysis of deviance with the function `Anova()` available in the package `car` (Fox and Weisberg, 2011). In not overdispersed, the test for the analysis of deviance for GLM and Cox models was a likelihood-ratio (χ^2). In overdispersed GLM, an *F*-test was used. The differences between the levels of a significant factor were tested with the function `esticon()` from the package `doBy` (Højsgaard et al., 2013). The *p* values obtained from `esticon()` function were corrected using the `p.adjust()` function which controlled type I errors performing a false discovery rate correction (Benjamini and Hochberg, 1995). Confidence intervals (CI) of the estimated parameters were calculated for a 95% confidence level.

3. Results

Genetic origin of all individuals was unambiguously assigned and according to the expectations for wild (European quails) and farm (hybrid) quails (results not shown). Analyses with STRUC-TURE AND NEWHYBRIDS produced consistent results.

3.1. Attraction ability

In 2010, hybrid females acting as sexual decoys in walk-in funnel traps attracted 2.58 times more wild European quail males than did female European quails (confidence interval, CI: 1.16–6.87, $\chi^2_1 = 5.52$, $p = 0.019$, Fig. 1a).

Male aggregation traps attracted 5.75 times more wild males than females (CI: 2.87–13.16, $\chi^2_1 = 20.85$, $p < 0.001$, Fig. 1b). European quail male aggregation traps were 2.55 times more

visited, by both wild males and females (interaction not significant), than hybrid male aggregation traps (CI: 1.48–4.58, $\chi^2_1 = 11.03$, $p < 0.001$). The number of individuals entering the traps varied between years ($\chi^2_3 = 12.68$, $p = 0.005$); thus, in 2008 only 0.5 individuals were captured per trap, whereas in the rest of years 3.5 individuals were captured. These results strongly suggest that: (a) female hybrids are able to attract wild European quail males more effectively than female European quail; (b) male aggregations attract more males than females (although we do not have precise information about the sex ratio in the study area; males are known to be several times more numerous than females in the area); (c) hybrid male aggregations attract less wild quails (males and females) than European quail aggregations.

3.2. Female mating and breeding success

During the four years of the study, a total of 51 hybrid females and 16 European quail females were radio-tagged and monitored. The majority of the matings (94%) took place with a common quail male. For hybrid females, 61% (31) succeeded in mating, whereas 81% of European quail females (13) mated. Two hybrid females (4%) paired with freely roaming hybrid males. Mating probability was first modeled including “female origin” and “year” as factors, together with the “known survival period” of each female as covariate. Due to the low frequency of matings with free-roaming male hybrids, male origin was not included as a factor. This model showed a significant effect of year in female mating probability ($\chi^2_3 = 8.24$, $p = 0.041$). Mating probability in 2009 was higher than in 2007 ($\chi^2_1 = 4.16$, $p = 0.041$) and 2008 ($\chi^2_3 = 4.55$, $p = 0.033$). This mating probability also depended on the female known survival period ($\chi^2_1 = 37.68$, $p < 0.001$, Fig. 2a). But the model failed to show differences in the probability of mating between hybrid and European quail females ($\chi^2_1 = 0.93$, $p = 0.335$). On average, the model shows that mating probability reached 95% 40.8 days (CI: 25.3–72.8) after the release of the female (Fig. 2a).

Radio-tracking of the 51 hybrid females monitored showed that 21 of them (41%) nested, whereas of the 16 European quail females monitored, 11 of them (69%) succeeded in nesting. In the model for nesting success, neither female origin nor year had a significant effect. However, covariate “known survival period” had a significant effect. None of the interactions were significant. For these reasons, the final model was constructed using “female origin” as independent variable and “known survival period” as a covariate. Accord-

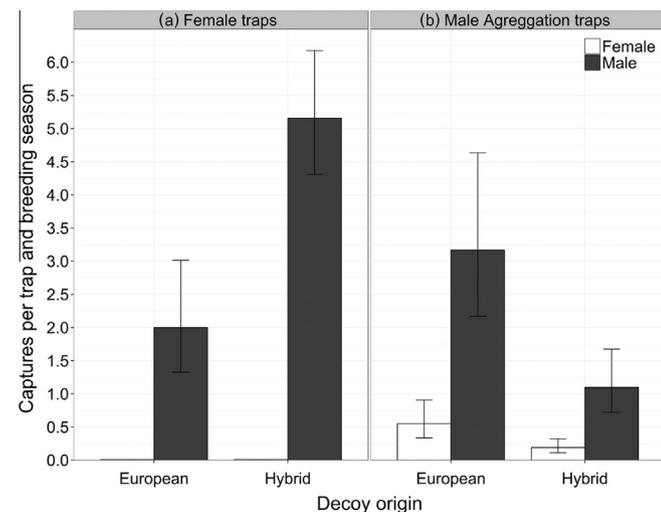


Fig. 1. Female and male wild quails captured per trap and breeding season (mean ± se) as a function of decoy origin (European quail or hybrid originating from game farms) for two types of trap: (a) one female used as decoy (only 2010 data) and (b) a group of males used as decoy (from 2007 to 2010 data).

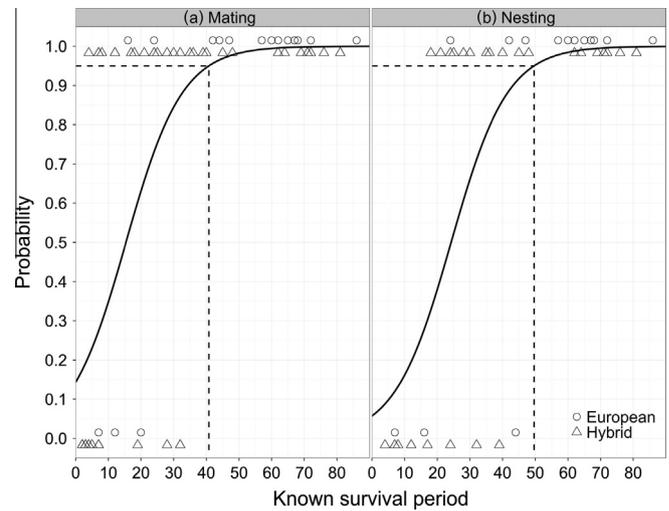


Fig. 2. Female mating (a) and nesting (b) probabilities as a function of the number of days in which females remained alive in the study area (known survival period). Adjusted functions: (a) $y = \text{inv.logit}(-1.79 + 0.12 \cdot x)$; (b) $y = \text{inv.logit}(-2.81 + 0.12 \cdot x)$. No differences were observed in these variables depending on female origin (European quail or hybrid). Dashed lines in both graphs show the time at which each Generalised Linear Model predicts a mating or nesting probability of 95%. Observations of both European and hybrid female quails, from which the models were built, are represented by circles and triangles respectively.

ing to this model, female origin did not have a significant effect on nesting success ($\chi^2_1 = 0.43$, $p = 0.510$), whereas known survival period showed a strong influence ($\chi^2_1 = 25.94$, $p < 0.001$, Fig. 2b). Females reached a 95% probability of nesting 49.6 days (CI: 27.7–99.2) after their release.

Factor “year” showed a significant effect only on fertility ($\chi^2_3 = 10.11$, $p = 0.0182$). Fertility in 2009 was lower than in 2007 ($\chi^2_1 = 6.61$, $p = 0.010$). Since factor “year” did not have a significant effect on clutch size, hatching success or chick survival, it was excluded from these models. The final models did not show differences between hybrid and European quail females regarding clutch size ($\chi^2_1 = 2.74$, $p = 0.098$, Table 3), fertility ($\chi^2_1 = 0.01$, $p = 0.915$, Table 3), hatching success ($F_{(1,20)} = 2.67$, $p = 0.102$, Table 3), or chicks survival ($F_{(1,19)} = 0.46$, $p = 0.496$, Table 3).

Finally, the probability of having the nest preyed upon was higher for female hybrids ($\chi^2_1 = 4.31$, $p = 0.038$, Table 3), and did not change from year to year (initial model: $\chi^2_3 = 7.45$, $p = 0.059$).

3.3. Survival

The mortality of the released hybrid females was 73% by the end of the annual study season; 23 of them were killed by predators (62% of the dead hybrid females) and 14 were found dead from unknown causes. This mortality rate is much higher than for European quail females (31%, 3 of them killed by predators and 2 found dead from unknown causes), clearly indicating that hybrid females

Table 3

Nests variables. Means and 95% confidence intervals (in parentheses) for clutch size, fertility, hatching success, chick survival during the first three weeks of life, and nest predation probability for both hybrid and European quail female nests.

Variables studied	Hybrid female	European quail female
Clutch size	9.14 (7.30–11.50)	11.09 (9.24–13.18)
Fertility	0.97 (0.88–0.99)	0.97 (0.88–0.99)
Hatching success	0.93 (0.80–0.98)	0.83 (0.72–0.92)
Chick survival	0.85 (0.73–0.93)	0.79 (0.69–0.87)
Nest predation ^a	0.22 (0.08–0.41)	0 ^b

^a Significant differences depending on female origin.

^b Confidence intervals cannot be calculated due to the lack of variability.

have lower survival than European quail females. The Cox proportional hazards regression model revealed that there is a significant difference in the relative risk of death for females of different origin ($\chi^2_1 = 17.55$, $p < 0.001$) and from different year ($\chi^2_3 = 12.47$, $p = 0.006$). More specifically, 2010 was the worse year. In 2010, the risk of death for a female was 5.47 (CI: 1.89–15.84) times higher than in 2007 ($\chi^2_1 = 9.84$, $p = 0.010$) and 3.54 (CI: 1.43–8.77) times higher than in 2009 ($\chi^2_1 = 7.5$, $p = 0.018$). The risk of death of a hybrid female, once released, was 7.22 (CI: 2.52–20.65) times higher than that of a European quail female. Fig. 3 shows the survival curves for European and hybrid quail females once released into the field. These curves show that 40.8 days after the release (time at which 95% of females have already mated, see Section 3.2) survival probability was higher for European quails (0.7, CI: 0.51–0.96) than for hybrids (0.29, CI: 0.19–0.44). Similarly, by the time 95% of females had nested (49.7 days after release, see Section 3.2), survival probability for female European quails was 0.68 (CI: 0.49–0.95), while for hybrids it was 0.27 (CI: 0.17–0.42).

4. Discussion

Previous studies have shown that, in captivity, there are not pre- or post-zygotic barriers preventing the interbreeding between European and Japanese quails or between European quails and hybrids (Derégnaucourt et al., 2002; Derégnaucourt and Guyomarc'h, 2003; Taka-Tsukasa, 1941). However, other studies suggest that the massive releases of farm bred quails have not resulted in a hybrid swarm (Puigcerver et al., 2007, 2012), indicating that perhaps ecological or behavioral barriers acting in the wild maintain separation (Pierotti and Annet, 1993). Nevertheless, this does not seem to be the case. Our results show, for the first time, that European quails and hybrids interbreed in the wild. Thus, the presence of admixed individuals in European quail populations in different countries during the breeding season may be explained not just as a result of restocking practices (as suggested by Puigcerver et al. (2007)), but also as the result of released hybrids reproducing in wild European quail populations.

Our results showed that female hybrids attract more wild common quail males than common quail females (Fig. 1). This could be due to the observation that they produced more rally calls from the trap, attracting more males (pers. obs.). This contrasts with the

results by Derégnaucourt and Guyomarc'h (2003) who have shown that, in captivity, European quail females are very selective, emitting the greatest number of rally calls in response to mating calls from conspecific males, a lower number in response to mating calls produced by Japanese quail and an intermediate number responding to mating calls from hybrid males. A possible explanation for this difference could be that hybrid females produced more vocalizations because they were less stressed in a cage. Although we tried to minimize this effect by keeping wild-caught females in captivity for some months before starting the experiments, we cannot assess if the differences in behavior are associated to the hybrid origin or are result of adaptation to life in captivity. In addition, female hybrids and European quails (Fig. 2a) had a similar probability of mating. These matings occurred mostly with European quail males (94%), which were much more abundant than hybrids in the area (Puigcerver et al., 2007). Therefore, mixed pairs formed in the wild are most likely formed by a hybrid female and a European quail male.

We did not know in advance whether our artificial hybrid male aggregations could attract wild European quails of both sexes, but we expected so because Sardà-Palomera et al. (2011) showed that European male aggregations attracted both males and females as a consequence of the mating system of the species. Our results show that wild European quail males, which have a complex polygamous mating system (Rodríguez-Teijeiro et al., 2003), are more attracted by artificial European quail male aggregations than to those of hybrid males, suggesting that natural European quail male aggregations found in the wild (Guyomarc'h et al., 1998) may be reasonably preserved despite the release of hybrids.

With regard to the breeding success of the females released from the traps together with a male, restocked hybrid females had similar mating and nesting success rates to European quail females. As in captivity (Derégnaucourt et al., 2002), fertility, hatching rate and chick survival rate (within the first three weeks of life) are also very similar between hybrid and European females (Table 3), although we evaluated chick survival after hatching the eggs in captivity, and this may not reflect survival rates in the wild. As farm-raised hybrid quails have been selected for life in captivity, their chicks should be expected to survive better in captivity than chicks coming from wild common quails. Therefore, the lack of differences in survival in captivity of chicks from hybrid and common quail does not imply similar survival in the wild. Since mating preferences do not show the existence of strong pre-zygotic barriers and the breeding success does not seem obviously lower, other mechanisms must explain why the proportion of hybrids has not been increasing in European quail populations (Puigcerver et al., 2007, 2012).

The reason for this may be found, at least in part, in the lower probability for female hybrids to survive long enough to mate and nest (Fig. 3). In addition, the nests of hybrid females are preyed upon more often (Table 3). These differences could contribute to explain why the massive releases of farmed quail have not resulted in a hybrid swarm. Mortality rates of restocked farm-reared birds are usually higher than for native populations because they are poorly adapted to the natural environment and lack the ability to defend themselves against cold and to forage and select food in those conditions (Guyomarc'h, 2003). After hunting, predation is probably one of the main causes of mortality for farm-reared hybrid quails, as happens for other farmed game bird species (Casas et al., 2012; Duarte and Vargas, 2004; Gortazar et al., 2000; Putaala and Hissa, 1998) due to the lack of an antipredatory behavior (Guyomarc'h, 2003; McPhee, 2003). The same poor knowledge about the local conditions might explain the higher nest predation rate for hybrid females.

It is unsurprising that hunting is the main cause of mortality for restocked farm quails since these tend to be released immediately before the hunting season or before special hunting events. We do

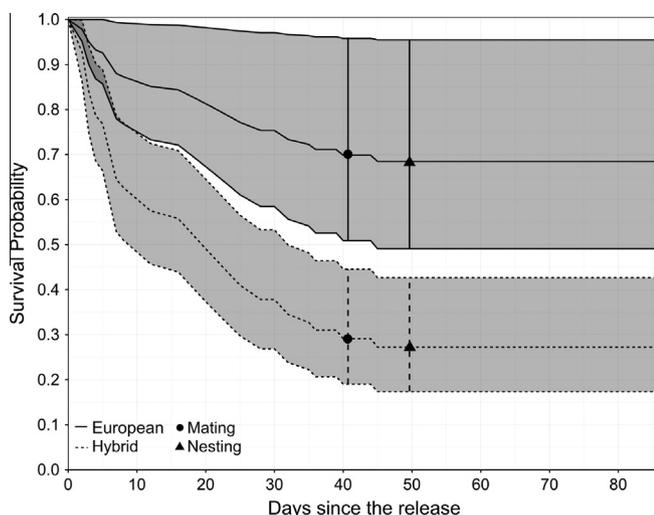


Fig. 3. Estimated survival probabilities (mean and 95% confidence intervals, based on a Cox proportional hazards regression model) for European (solid lines) and hybrid (dashed) female quails. Survival probabilities when mating (circles) and nesting (triangles) probabilities reach 95% (see Fig. 2) are represented to show that the probability of surviving long enough to mate and nest is higher for European quail females than for hybrid females.

not have data about the mortality during the hunting season of hybrid quails that were present during the breeding season compared to that of European quails. However, it seems reasonable to consider that the same lack of anti-predatory behavior would favor a higher hunting pressure on hybrids, thus increasing their mortality rates, as suggested by Guyomarc'h (2003), who reported 75% of restocked individuals from a total of 4959 hunted quails in a large hunting estate (64,000 ha) in France.

Previous field surveys spanning a long time period (Puigcerver et al., 2007, 2012) did not show an increase in the proportion of hybrids detected in nature. This could apparently suggest that restocking European quail populations with hybrid quails does not represent a conservation problem. However, our results show that the two groups are not genetically isolated and that interbreeding occurs in the wild. These observations seem incompatible, but can be explained by the lower chances of survival and nesting of hybrid females in the wild, as well as their higher rate of nest predation. This translates into a lower fitness for the quails of hybrid origin. This lower fitness could be due either to carrying maladaptive alleles of Japanese quail origin or to the adaptation to life in captivity. This result is in agreement with the results of a 15-year-long genetic study showing that the proportion of hybrid individuals in the population was not increasing over time, but also emphasizing that the results were compatible with a slow rate of introgression if hybrids had reduced fitness (Sanchez-Donoso et al., submitted for publication). In this case, a slow introgression of farm alleles could be expected, which would translate into extensive admixture in some generations. Our results confirm reproduction and also lower fitness for the hybrids, and so we expect a progressive build-up of alleles of farm origin into the wild population. This is likely to affect the genetic composition of European quail populations, which could result in a decrease in the adaptive and evolutionary potential of the species.

Our results show that in the case of small populations of European quails, the interbreeding with restocked hybrids could represent a loss of homospecific matings and a reduction of the potential for population growth. It is paradoxical that restocking practices for this species, instead of increasing the long-term chances for survival, could result in a reduction of the genetic effective population size and evolutionary potential (Ryman and Laikre, 1991) and could limit population growth. In addition, captive breeding of European quails for restocking could make sense if the species was under conservation concern; however, it is currently classified as “least concern” by the IUCN. In this case, restocking practices make no sense from the conservation point of view, especially considering that the introduction of farm-reared individuals in wild populations may lead to other threats, such as transmission of pathogens, that could cause substantial mortality in the native populations (Peeler et al., 2006).

The results obtained also emphasize how the putative consequences of restocking practices cannot be directly inferred from just the number of individuals released.

As reported in Casas et al. (2012) for the red-legged partridge (*A. rufa*), public Administrations should ensure that releases for hunting purposes do not represent a threat to the conservation of native species and populations. These authors suggest some management strategies to control genetic introgression and help the original population structure to recover: (1) stop releases of hybrid birds; (2) implement effective inspection procedures for farms providing individuals for restocking; (3) promote management plans avoiding releases in areas where restocking programmes have not yet been performed, and where there is reliable evidence that populations consist of non-admixed individuals.

It is important to highlight that the results of this study suggest that some restocked individuals could have been interbreeding with native European quails during the last decades. Until we bet-

ter understand the long-term consequences of the release of quails with – potentially – maladaptive genes, we believe all restocking programs involving Japanese quails or their hybrids within the natural distribution range of the European quail should be banned.

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