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Lack of introgression of Japanese quail in a captive population of common quail

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Abstract
Farm-reared quails are released to the wild in Europe in vast numbers every year to increase hunting bag quotas. Experimental studies indicate that rather than the native common quail (*Coturnix coturnix*), the restocking is often done with domestic Japanese quail (*Coturnix japonica*) or with hybrids of domestic Japanese quail and common quail. Such practices are thought to be a severe threat for the native species as it could lead to introgression of domestic Japanese quail alleles into the wild common quail genome and potentially alter the migratory and reproductive behaviour in wild populations. In this study, we assessed the genetic purity of a captive population of common quail that was established from wild-caught founders caught on the Southern Italian coast in Sicily (Italy). We evaluated the proportion of ancestry to common and Japanese quail in this captive population via genetic screening using nuclear microsatellite markers and mitochondrial DNA analyses. We showed that the captive farm quail in our study had no sign of admixture with domestic Japanese quail and had similar genotype frequencies relative to wild common quail, confirming the success of the breeding programme for the native species. We propose that raising common quails in captivity for restocking purposes rather than domestic Japanese quails or hybrids would be a feasible alternative that could minimise the risk of genetic pollution of wild common quail populations.

Keywords *Coturnix* · Game management · Hybridisation · Conservation management

Introduction

Captive breeding and restocking is one of the most common practices in wildlife conservation and management. Indeed, restocking for recreational activities is regularly carried out across various vertebrate species, including mammals (red

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deer, wild boar), fish (salmon, trout), and birds, especially in galliforms such as partridges, pheasants, and quail (see Champagnon et al. 2012 for a comprehensive review on restocking in vertebrates). The common or European quail (*Coturnix coturnix*) is a migratory galliform distributed across a large breeding range in Eurasia extending from Portugal and Ireland east to the Urals, south of the 60th parallel North (Guyomarc'h et al. 1998; Guyomarc'h 2003; Perennou 2009). Quail populations in many hunting areas across Europe, such as Italy, Spain, and Greece, are annually restocked to increase hunting bags with millions of quail bred and reared in farms (Rodríguez-Teijeiro et al. 1993; Galli 2003; Barilani et al. 2005; Tsiompanoudis et al. 2011).

Due to known captive breeding difficulties for wild-caught common quail compared to the domestic Japanese quail (*Coturnix japonica*)—e.g. low egg fertility (Sanchez-Donoso et al. 2012, VM *personal observation*)—restocking has often been performed with domestic lines of Japanese quail, or hybrids of different generations produced from crosses between wild common quail and domestic Japanese quail (e.g. Rodríguez-Teijeiro et al. 1993; Sanchez-Donoso et al. 2012). The crossing of the two species to obtain individuals for

hunting purposes most likely occurred by pairing domestic Japanese quail females with wild common quail males (Amaral et al. 2007; Sanchez-Donoso et al. 2012). This practice has raised serious concerns as common and domestic Japanese quail have very different life-history strategies, mainly due to centuries of artificial selection in the domestic Japanese quail for egg and meat production (Baumgartner 1994). Importantly, contrary to the common quail, domestic Japanese quail show only minimal, if any, level of migratory restlessness in captivity (Derégnaucourt et al. 2005). Moreover, the expression of the migratory phenotypes is significantly reduced among first generation hybrids generated by crosses between common and domestic Japanese quail (Derégnaucourt et al. 2005). As a consequence, the practice of restocking hybrids may lead to a reduction of the migratory phenotypes due to interbreeding events between restocked birds and the native species. Experimental evidence from studies in the laboratory showed that Japanese and common quails produce hybrids with no apparent reduced survival compared to purebred common quails (Derégnaucourt et al. 2002; Derégnaucourt and Guyomarc'h 2003). More recent work conducted by Sanchez-Donoso and collaborators experimentally demonstrated that hybrid males have better breeding performance (i.e. they showed higher fertilisation success rate and were also faster in fertilising eggs) compared to pure common quail males, a contributing factor potentially favouring the introgression of domestic Japanese quail alleles into the common quail population (Sanchez-Donoso et al. 2016). Despite limited long-term and large-scale data on the occurrence and frequency of interbreeding in the wild, simulation models predicted that admixture levels might be slowly increasing over time if release practices of hybrids are maintained (Sanchez-Donoso et al. 2014a).

As a result of the concerns outlined above, laws have been introduced in some European countries over the last 10 years (including Italy, Spain, Portugal, Spain, Greece) banning the release of Japanese quail or hybrids into the wild. The assessment of the specific origin of restocked/reared-farm quail populations is generally performed on the basis of phenotypic measurements by veterinarians. Such practices can however lead to misleading conclusions due to the morphological similarity of pure common quails and hybrids (Derégnaucourt 2000). Thus, it is highly desirable to develop reliable, high-throughput methods of species assignment such that farmed common quail stocks can be established free from Japanese quail introgression.

The main aim of our study was to assess the success of a captive breeding programme for common quail on the Southern Italian coast in Sicily (Italy) that was established from wild founders captured during spring migration and breeding season. We evaluated the proportion of ancestry to common and domestic Japanese quail in this population via genetic screening with established nuclear microsatellite markers and mitochondrial DNA analyses developed to accurately differentiate quail species (Kayang et al. 2002; Sanchez-Donoso et al. 2012).

Material and methods

General

The study was carried out in a captive colony of quail (herein referred as "farm quail") originated at the Istituto Sperimentale Zootecnico per la Sicilia (ISZS, Palermo, Italy) and maintained at ISZS and/or at the Konrad Lorenz Institute of Ethology (Vienna, Austria) as explained below. The birds were produced from wild founders captured in April–June (corresponding to spring migration/breeding season in the common quail) on the southern Italian coast near Palermo (38° 2′ 20″ 04 N, 13° 32′ 57″ 12 E—38.186523, 13.104779) in 2008, 2009, and 2010 (in total approximately 60 females and 120 males founders, Gianni Pola *personal communication*). No morphological or genetic screening was performed in the wild-caught quails.

Sampling

Twenty randomly selected farm birds from the captive colony at the ISZS (out of ~200 birds) were sampled for genetic analyses (November 2016); an additional 33 randomly selected farm quails from the stock captive population, which had been transferred from ISZS to the Konrad Lorenz Institute of Ethology (January 2017), were also sampled (July 2017) and included in the genetic analyses here (total $n = 53$). We genetically compared the farm quails with two distinct reference populations: (1) domestic Japanese quails ($n = 23$) obtained from a captive population maintained in the Zoology Department at the University of Glasgow (UK) originating from fertile eggs purchased from a commercial breeder (Moonridge farm, Scotland, UK) and (2) wild common quails ($n = 20$) captured between May–June across multiple years (2005, 2008, 2010, and 2011) and across different regions in Spain (full details in Table S1 in Supplementary Material)—these birds were previously identified as common quail on the basis of their song and phenotype.

For all samples, DNA was extracted from individually collected whole blood samples (stored at − 20 °C in 95% ethanol or at − 20 °C in Queen's buffer until extraction) or from red blood cells (stored at − 80 °C until extraction) using either DNeasy Blood/Tissue kit (Qiagen), or blood quickPure kit (Macherey-Nagel) following manufacturer's instructions. DNA extracts from all samples were screened using a Nanodrop spectrophotometer and had acceptable purity (ratios 260/280 ~ 1.8).

Animal procedures were performed in compliance with the Italian and Austrian legislation with approval of the Istituto

Sperimentale Zootecnico per la Sicilia (ISZS), the Ethics Committee of the University of Veterinary Medicine, Vienna, and the Federal Ministry of Science, Research and Economy (BMWFW-68.205/0037-WF/V/3b/2017). Sampling and handling of the wild quails fulfilled the ethics recommendations of the European Union and the Spanish legislation.

Microsatellite genotyping and mtDNA haplotype analyses

All individual birds were genotyped at 12 microsatellite loci designed for Japanese quail and shown to amplify also in chicken and guinea fowl (Kayang et al. 2002; Kayang et al. 2004). These microsatellites had previously been used in other studies to detect hybridization between common and domestic Japanese quails (Sanchez-Donoso et al. 2012; Sanchez-Donoso et al. 2014a, b). Loci were assigned for amplification in two multiplexed panels according to their primer annealing temperatures, fragment lengths, and fluorescent dye (see Table 1). Amplifications were carried out in 10 μl using 50–100 ng/μl of DNA with 5 μl of QIAGEN Multiplex PCR Master Mix, 0.1 μl of 0.001 mg/μl purified BSA (New England BioLabs; 10 mg/ml), and 0.2 μM of each of the forward and reverse primers. Loci were amplified in multiplex: 95 °C for 15 min, followed by 35 cycles of 0.5 min at 95 °C, 1.5 min at 60 °C (set 1) or 56 °C (set 2), and 1.0 min at 72 °C, followed by 72 °C for 10 min. Multiplexed PCR amplicons were analysed on an ABI 3130xl sequencer (Applied Biosystems) following standard conditions. Fragments were sized and scored using the software GeneMarker v2.6.7 (SoftGenetics, PA). Data were analysed for scoring errors, allelic dropout, and null alleles using the software Micro-checker (Van Oosterhout et al. 2004).

A 453-bp portion of the mitochondrial control region was amplified with the primers PHDL and PH-H521 (Barilani et al. 2005) for all samples. Full-length sequences were obtained for a subset of samples: 25 farm quail individuals (out of 53), 20 domestic Japanese quails (out of 23), and 16 wild common quail samples (out of 20). Reaction conditions and cycling parameters were as per Sanchez-Donoso et al. (2014a). Briefly, 30 ng of DNA was mixed with 1× Amplitaq Gold PCR buffer, 2.0 mM MgCl2, 0.2 mM dNTPs, 200 mM of each primer, and 0.5 units of Amplitaq Gold polymerase in a 25-μl reaction volume. Cycling was 10 min at 95 °C followed by 35 cycles of 95 °C for 30 s, 54 °C for 30 s, and 72 °C for 45 s, with a final extension of 72 °C for 7 min. PCR products were visualised on a 1.5% agarose gel to confirm product size and concentration. Cycle sequencing reactions were performed using Applied Biosystems Big Dye Terminator version 3.1 ready reaction cycle sequencing kit and were resolved via capillary electrophoretic separation on a 3130 xl Genetic Analyser (Applied Biosystems).

Data analyses

Standard genetic diversity estimates of observed heterozygosity (H_O), expected heterozygosity (H_E), number of alleles (A), and allelic richness corrected for population size (A_R) were calculated using the program FSTAT v.2.9.3.2 (Goudet 1995). The same program was used to generate pairwise F_{ST} statistics between the three sampled populations with associated significance values adjusted for multiple tests via Bonferroni correction. Population differentiation was further examined by constructing a PCA plot based on Nei's genetic distance (Nei et al. 1983) using the Excel add-on GenAlEx v.6.502 (Peakall and Smouse 2006).

Population cluster analysis was performed via the Bayesian clustering algorithm implemented in the program STRUCTURE v.2.3.4 (Pritchard et al. 2000). This program was used to determine the number of genetically distinct clusters (K) that maximise the likelihood of the data without prior population or sampling information (USEPOPINFO and LOCPRIOR deactivated). For each individual, an estimate of the fraction of the genome belonging to each one of the clusters (q) is represented as a coloured portion of a vertical bar within each population. Samples were analysed using the "admixture model" (allowing individuals to have ancestry in more than one parental population), with correlated allele frequencies. Simulations were run for 500,000 steps following a burn-in period of 50,000 steps. Values of K ranging from 1 to 10 were tested with 10 replications at each. The most likely value for K was chosen following the method proposed by Evanno et al. (2005), using the online program STRUCTURE HARVESTER (Earl and vonHoldt 2011), which takes into account the rate of change in the log likelihood between successive K values. For each individual, an estimate of the fraction of the genotype belonging to each of the K clusters (q) is given as a proportion. A graphical summation of the multiple independent runs for each K was generated using the program CLUMPAK (Kopelman et al. 2015) which illustrates the proportions as a coloured portion of a vertical bar.

To test the sensitivity of our marker set to detect hybrid individuals, we simulated hybrid offspring genotypes from the wild quail and domestic Japanese quail parental allele frequencies using the program HybridLab (Nielsen et al. 2006). Simulated genotypes were generated for 50 individuals of six different groups: pure wild common quails, pure domestic Japanese quails, F1 hybrids, F2 hybrids, backcross to wild parent, and backcross to Japanese parent. These genotypes were then analysed in STRUCTURE as above to assess degrees of hybridisation that could be detected by the markers used in this study.

Consensus control region sequences were constructed from forward and reverse reads and aligned against reference quail sequences (Sanchez-Donoso et al. 2014a) in CLC Workbench v 7.9.1 (Qiagen, Aarhus). Individual data was used for population

Table 1 Microsatellite loci used in the current study with basic genetic diversity estimates across all samples

Locus name	GenBank accession number	Repeat motif	Forward primer (5'-3')	Reverse primer (5'-3')	Multiplex set	Size range (bp)	T_A (°C)	A	A_R	H_O	H_E
GUJ0001	AB035652	(CA)7TG(CA)13	HEX-GAAAGCGAAAGCCGAGCCA	CAGCACTTCGGAGCACAGGA	1	226–240	60	8	5.9	0.73	0.71
GUJ0017	AB035827	(CA)14	TAM-AGAGAGATTAGAGGAGCTGC	GGCACTAAAACCATCGAGAG	1	154–178	60	15	10.9	0.39	0.83
GUJ0028	AB035838	(CA)9	FAM-TGAACAAAGCAGAAAGGAGC	CCTTACCTACATGAAACGTC	2	148–190	56	15	11.7	0.58	0.81
GUJ0039	AB035849	(CA)19	HEX-CAAAGAGCAGAGGGAATGGA	CCGAGAGATGGGTTTTTCC	1	163–199	60	15	10.6	0.59	0.69
GUJ0044	AB035854	(CA)16	TAM-GCCTTGAAACCTGAGTGATC	TGCATTTCAGCAGCTCTCAG	2	176–248	56	26	16.1	0.82	0.89
GUJ0057	AB063125	(CA)12	ATTO633-GCTCTGTTTCACAGCA	CAGGTGTTAAAGTCCAATGT	1	128–170	60	15	11.3	0.79	0.83
GUJ0065	AB063133	(CA)13	TAM-GCGTGCCATTTACTTCCCGG	AGCCAGGATGACCA GGAAGG	2	107–155	56	21	12.7	0.70	0.84
GUJ0070	AB063138	(CA)9	HEX-AAACCCCAAAGAAGCTGTCC	ACGTTGTCACCATCAGCTTG	2	196–214	56	8	6.2	0.68	0.68
GUJ0074	AB063142	(CA)10	HEX-GTTGTCCTGGCTGAGATGGC	GGGTTTGAGGGGTTGGGGTT	1	291–331	60	20	11.9	0.75	0.78
GUJ0085	AB063153	(GT)14	ATTO633-ACAACCACTTCTCC AGCTAC	GCTTGTCGCTGCTGTTGCTAA	2	242–285	56	29	17.1	0.69	0.82
GUJ0093	AB063161	(CA)16	FAM-CTCTTGTATTGTAACTGGGC	AGCCATAGAGGGCTATTAAG	1	188–242	60	20	12.4	0.53	0.70
GUJ0097	AB063165	(CA)14	HEX-GGATGCTCAGTGTGGAAAAG	GGAACCTGTCCTATCCTAGA	2	123–179	56	26	14.6	0.76	0.87

T_A annealing temperature, A number of alleles, A_R allelic richness, H_O observed heterozygosity, H_E expected heterozygosity

genetic parameters whereas unique sequences were used for phylogeny reconstruction. No double bands or mixed sequence traces were present in our samples which, together with the lack of unusually divergent outgroup sequences, suggest no problem with nuclear inserted mtDNA sequences (NUMTS, Thalmann et al. 2004). Population genetic parameters including segregating sites (S), haplotype diversity (Hd), nucleotide diversity (Pi), and genetic differentiation (F_{ST}) were calculated from all sequences in the three populations using the program DnaSP v.6 (Rozas et al. 2017). Unique haplotypes were used to construct a maximum likelihood tree in Mega (Kumar et al. 2016) based on the Tamura 3-parameter model (Kimura 1980) with gamma distributed evolutionary rates which was chosen as best fitting after testing the data against all available models. The analysis involved 72 nucleotide sequences and there were a total of 453 positions in the final dataset.

Data availability Data are available from Figshare (10.6084/m9.figshare.6840182).

Results

Observed heterozygosity ranged from 0.37 for locus GUJ0017 to 0.80 for locus GUJ0044 (see Table 1). Two loci (GUJ0017 and GUJ0028) showed significant departure from Hardy-Weinberg expectations (HWE) but only in the wild common quail and farm quail populations. Loci GUJ0039, GUJ0065, and GUJ0085 also showed a significant excess of homozygotes for the reference wild common quail samples. No loci departed from HWE for the domestic Japanese samples. No loci showed any evidence of linkage disequilibrium and only one locus (GUJ17) indicated a potential for null alleles but was retained as null alleles have been shown to have little effect on assignment analyses (Carlsson 2008). Diversity levels for the wild common quail and the farm quail were generally higher than those for the domestic Japanese population. The mean number of alleles was 11.3 and 14.2 for the farm quail and the wild common quail respectively while for the domestic Japanese population, it was 4.7. This result held also after rarefaction with allelic richness values of 9.3, 14.2, and 4.6 respectively (see Table S2 in Supplementary Material).

All three groups of quail populations showed signs of genetic divergence with significant pairwise microsatellite F_{ST} values across all groups after Bonferroni correction. However, divergence between the domestic Japanese quail population and the two other quail populations was greater than divergence between the farm quail individuals and the reference wild common quails (microsatellite F_{ST} values for domestic Japanese quail vs farm quail = 0.19; domestic Japanese quail vs wild common quail = 0.16; farm quail vs wild common quail = 0.03, see also Table 2). The PCA from Nei's genetic distance again markedly separates the domestic Japanese quail

Table 2 F_{ST} estimates between populations of quail from mtDNA control region sequences (above the diagonal) and microsatellite genotypes (below the diagonal)

	Farm quail	Wild common quail	Domestic Japanese quail
Farm quail	0	0.13	0.98
Wild common quail	0.03	0	0.94
Domestic Japanese quail	0.19	0.16	0

samples from the other populations (along the primary axis). There is some separation between the farm quail and the wild common quail populations along the secondary axis but many samples overlap among these two locations with no clear grouping (Fig. 1).

From the STRUCTURE analysis, the most likely value for the number of clusters (K) was 2 (Fig. 2) according to the Evanno et al. (2005) method. Likelihood values and its variability are reported in Table S3, Supplementary Material. The result was consistent across all runs with likelihood values converging in each case. The farm quail and the wild common quail populations grouped together and were clearly differentiated from the domestic Japanese quail individuals (Fig. 2). None of the farm quail q values for cluster membership fell under the lowest values observed among the wild common quails (lowest $q = 0.88$), suggesting no admixture with domestic Japanese quail (Fig. 2). Also, simulated data from the wild quail and domestic Japanese quail allele frequencies clearly separated F1 and F2 generation hybrids from the parental groups as well as differentiating the two parental backcrosses, conforming that the markers we used have sufficient statistical power for the individual assignment of parental and hybrid individuals (Figure S1, Supplementary Material).

The mtDNA control region sequencing produced two haplotypes from the domestic Japanese quails, three haplotypes from the farm quail individuals, and nine haplotypes from the wild common quail samples. As shown in Fig. 3 (tree with the

highest log likelihood (− 1068.217)), two haplotypes were shared between the farm quails and the wild common quails ("It H1", $n = 18$ with "Sp H1", $n = 5$, and "It H2", $n = 1$ with "Sp H2", $n = 2$—"It" and "Sp" prefix represent the farm quails from the ISZS (Palermo, Italy) and the wild common quails from Spain, respectively) and these haplotypes matched reference haplotypes "Cc W2" and "Cc W11", from Sanchez-Donoso et al. (2014a, b) (GenBank accession numbers: KF410838 and KF410847). Two wild common quail haplotypes (Sp H4, $n = 1$; Sp H5, $n = 1$) matched haplotypes Cc W4 and Cc W3 from Sanchez-Donoso et al. (2014a, b) (GenBank accession numbers: KF410840 and KF410839) and were not found in any other population. One domestic Japanese quail haplotype (Jp H1) matched reference haplotype Cj H1 from Barilani et al. (2005) (GenBank accession number: DQ087957) and the other haplotype (Jp H2) had not been previously reported. One farm quail (It H3, $n = 6$) and five wild common quail haplotypes (Sp H3, $n = 1$; Sp H6, $n = 1$; Sp H7, $n = 1$; Sp H8, $n = 1$; Sp H9, $n = 1$) were previously unknown (*pending GenBank accession numbers*). All haplotypes however, clustered together with wild common quail haplotypes in the maximum likelihood tree. Importantly, there was clear separation between the wild common quail clade and the Japanese quail/hybrid clade with 99% bootstrap support (Fig. 3). Diversity measures indicate highest nucleotide and haplotypic diversity for the wild population (Pi = 0.0041 and Hd = 0.89 respectively) whereas the domestic Japanese quail population was much lower (Pi = 0.0006 and Hd = 0.27) and the farm quails showed mid-range levels (Pi = 0.0012 and Hd = 0.44) (see Table S2, Supplementary Material).

Discussion

We evaluated for the first time the genetic origin of a captive population of farmed quail maintained since 2008 in the Istituto Sperimentale Zootecnico per la Sicilia (Palermo,

Fig. 1 Principal component analysis (PCA). Each dot represents an individual sample: blue diamonds = farm quail; green triangles = domestic Japanese quail; red squares = wild common quail
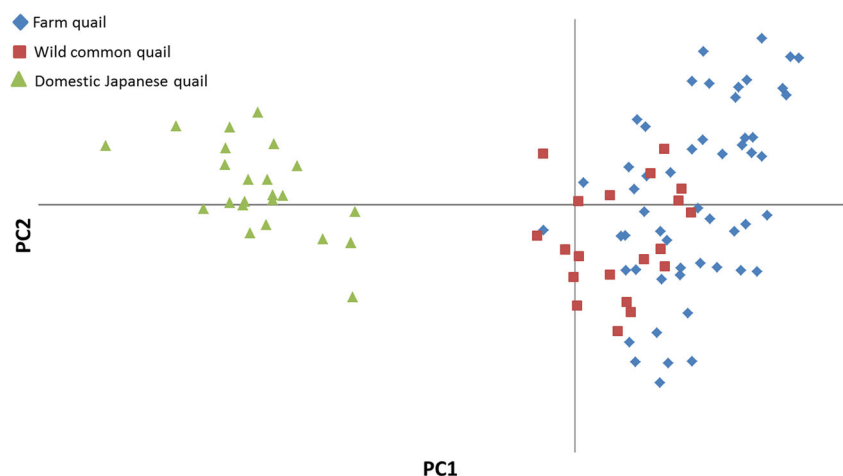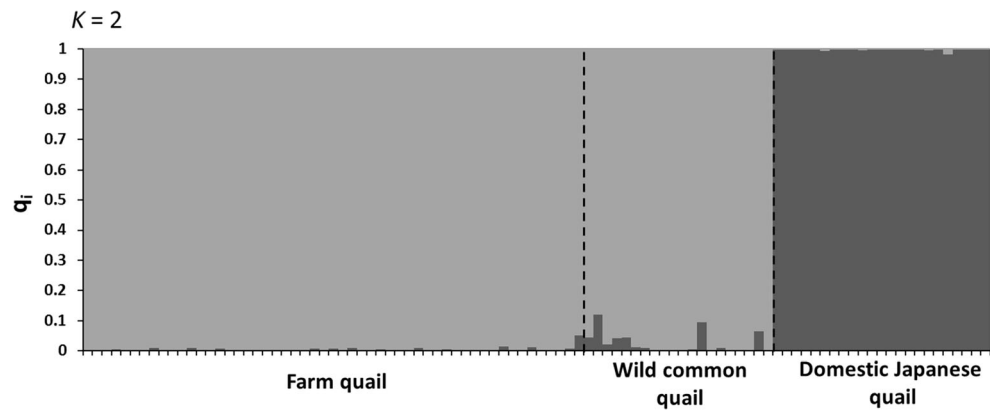
Fig. 2 Structure plot showing assigned clustering of individual genotypes into $K = 2$ clusters according to STRUCTURE. The colours within each column indicate the individual proportion of membership to each assigned cluster. Farm quail, $n = 53$; wild common quail, $n = 20$; domestic Japanese quail, $n = 23$

Italy). Genetic diversity of our study population was compared with two distinct reference groups of quail: domestic Japanese quail and wild common quail. Our results give three clear indications that the farm quail were markedly differentiated from the domestic Japanese quail population, and, importantly, had very similar allele frequencies compared to the wild common quail. First, the principal component analysis clearly showed that our farm quail clustered at the extreme of the primary axis relative to the domestic Japanese quail (Fig. 1) and this is further supported by the F_{ST} results. Second, the farm quail individuals were unequivocally assigned to the same cluster as the wild common quails and were clearly separated from the domestic Japanese quail cluster according to the STRUCTURE analysis (Fig. 2). Third, the mitochondrial DNA control region analysis also clearly placed the farm quail sequences within the wild common quail clade, ruling out any hybridisation with domestic Japanese quail along the maternal lineage (Fig. 3). Although we lack the direct phenotypic evidence, the mtDNA results also show that haplotypes from the study population are shared with migratory quail populations identified by Sanchez-Donoso et al. (2014b), and migration is a fundamental behavioural aspect separating common quail from domestic Japanese quail (Derégnaucourt et al. 2005). Altogether, our data indicate that the farm quails were derived from purebred common quail with no signs of admixture.
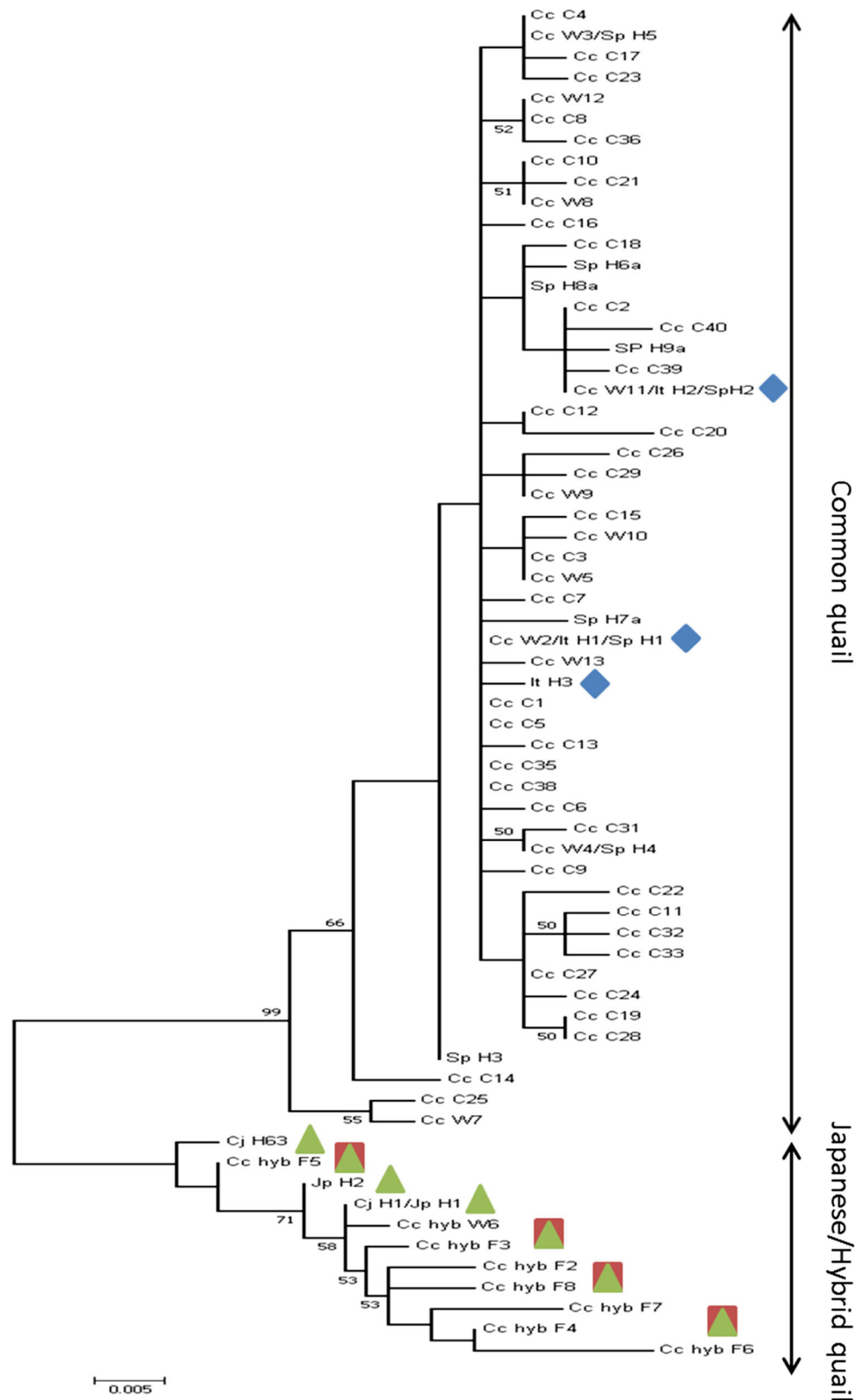
We also found that diversity levels for both the farm and wild common quail were much higher than the genetic diversity levels observed in the domestic Japanese quail. Our domestic Japanese quail individuals came from a single stock population and, at least to some extent, this could have contributed to a decrease in the diversity of the domestic population in our analyses. However, our results are consistent with previous work in which Japanese quail individuals were selected from various stock populations (Sanchez-Donoso et al. 2012). This result is best explained as a consequence of the selective breeding of domestic Japanese quail in Europe for meat and egg production using only a few founding lineages (Rizzoni and Lucchetti 1957). Therefore, different domestic lines of Japanese quails are likely to be genetically very

similar. Thus, it is improbable that the incorporation of domestic quails from additional stocks would have influenced our main findings.

It should also be noted that diversity levels within the farm quail were lower compared to the levels found in the wild common quail. This result is however not that surprising as genetic diversity is expected to be lower in reproductively isolated captive populations as compared to that in free-living populations (Jiang et al. 2005) likely due to founder effects and smaller effective population sizes. Another potential explanation, however, could be associated with differences in the migratory flows of the source populations. This might contribute to differences in the genetic architecture of quail populations using the Morocco-Spain route compared with populations using the Tunisia-Italy route (Guyomarc'h 2003). Direct experimental evidence would be needed to validate such a possibility. We also know very little about the inter-annual repeatability of migratory routes in the common quail. Large-scale and long-term genetic screening of wild-caught individuals across migratory flyways would be needed in order to assess intra-individual repeatability of migratory decision-making in this species.

Our study population of common quail was generated using only wild-caught individuals which were successfully bred in captive conditions (Gianni Pola *personal communication*). The possibility of breeding wild-caught quail in captivity is therefore biologically feasible and we point out that previous research did use populations of captive common quail sourced from wild founders to study physiological aspects of migratory and reproductive behaviour (e.g. Boswell et al. 1993; Derégnaucourt et al. 2005). Our data reinforce the importance of using appropriate genetic screening to control releases of quail for hunting bag supplementation as previously suggested (Sanchez-Donoso et al. 2012). For instance, genetic barcoding has already been used in Portugal to monitor farms producing red-legged partridges (*Alectoris rufa*). In fact, captive populations of red-legged partridge have historically been used for the production of artificial hybrids (e.g. chukar, *Alectoris chukar* grey × red-legged partridges; Blanco-Aguiar et al. 2008) leading to the introgression of hybrid genes into all European wild red-legged partridge

Fig. 3 Maximum likelihood tree from mtDNA control region haplotypes. The evolutionary history was inferred using the maximum likelihood method based on the Tamura 3-parameter model. Haplotypes labelled with an "It" and "Sp" prefix represent the farm quails from Italy and the wild common quails from Spain, respectively. The "Cc W" prefix denotes reference haplotypes of wild common quails, "Cj" and "Jp" prefixes denote haplotypes for Japanese quail, whereas "Cc hyb" are reference haplotypes for hybrids between common quail and Japanese quail. Codes after these prefixes correspond to the ones given in Sanchez-Donoso et al. (2014a) and the GenBank sequence name. Symbols in the figure denote different haplotypes similarly as in Fig. 1: blue diamonds, farm quail; green triangles, domestic Japanese quail; red squares and green triangles, hybrid quail; note that haplotypes without a symbol in the figure correspond to haplotypes of wild common quails (red squares in Fig. 1)

populations (Barbanera et al. 2010). It should be kept in mind, however, that genetic screening tools, such as microsatellite analyses, are useful in identifying potential hybridisation at the population level, but generally have reduced power at the individual level. Therefore, genetic inspections would need to be conducted on a regular basis using an unbiased sampling design

to accurately monitor potential changes in the genetic composition within the farm populations (e.g. due to uncontrolled introduction of breeders from different farms or from the wild).

Overall, our study also highlights the need to invest more resources and efforts in the policy making, planning, and management of game birds. Using common quail rather than domestic Japanese quail or hybrid individuals for restocking purposes could be a potential solution to mitigate the ongoing risk of genetic pollution in the native populations of quail. Future studies should also focus on assessing fitness proxies, including fertility rates and survival, of captive populations of pure common quails as these additional data would be extremely useful for farm managers and for improving the wildlife management of this species. We further recommend that the farm-reared common quail restocking should itself incorporate careful monitoring. Captive populations usually show reduced genetic diversity levels compared to wild populations and domestication practices could also introduce maladaptive alleles to the wild populations that could have an impact on fitness (Bekkevold et al. 2006). Simulations performed by Theodorou and Couvet (2004) suggested that wild populations exposed to repeated introductions from captive populations could be beneficial for population fitness when (1) the time length of the supplementation programme does not exceed a reasonable time frame, (2) introduction of captive individuals is kept at a low level, and (3) the size of captive population is reasonably large. Finally, we hope that our study will encourage enhancement of integration among different fields related to restocking of vertebrates and game management. The dialogues among farm managers, policy-makers, and conservation researchers are all necessary to ensure robust decisions are undertaken to protect biodiversity and to ensure the optimal use of available resources.

Compliance with ethical standards

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