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Cite this article: Forcina G, Tang Q, Cros E, Guerrini M, Rheindt FE, Barbanera F. 2021 Genome-wide markers redeem the lost identity of a heavily managed gamebird. *Proc. R. Soc. B* **288**: 20210285.
https://doi.org/10.1098/rspb.2021.0285

Received: 4 February 2021
Accepted: 2 March 2021

Subject Category:
Global change and conservation

Subject Areas:
genomics, ecology, molecular biology

Keywords:
Alectoris rufa, evolutionary potential, galliformes, genetic homogenization, introgression, wildlife management

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Electronic supplementary material is available online at https://doi.org/10.6084/m9.figshare.c.5340380.

THE ROYAL SOCIETY PUBLISHING

Genome-wide markers redeem the lost identity of a heavily managed gamebird

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Heavily managed wildlife may suffer from genetic homogenization and reshuffling of locally adapted genotypes with non-native ones. This phenomenon often affects natural populations by reducing their evolutionary potential and speeding up the ongoing biodiversity crisis. For decades, the red-legged partridge (*Alectoris rufa*), an intensively managed gamebird of conservation concern and considerable socio-economic importance, has been subjected to extensive releases of farm-reared hybrids with the chukar partridge (*Alectoris chukar*) and translocations irrespective of subspecific affinity. These practices have led to serious concerns that the genetic integrity and biogeographic structure of most red-legged partridge populations are irreversibly affected, as suggested by previous studies based on few genetic markers. Using over 168 000 genome-wide loci and a sampling across the entire *A. rufa* range, we detected unexpectedly limited and spatially uneven chukar introgression as well as significant intraspecific structure. We demonstrate that species widely feared to have irretrievably lost their genetic identity are likely to be much less affected by unsuitable management practices than previously assumed. Our results spell the need for a radical re-think on animal conservation, possibly restoring native status to populations long treated as compromised. Our study exemplifies how the application of innovative conservation-genomic methods is key to solving wildlife management problems dealing with introgressive hybridization worldwide.

1. Introduction

Intensive wildlife management can lead to genetic homogenization and reshuffling of locally adapted genotypes with non-native ones, which in turn may jeopardise natural populations by lessening their evolutionary potential and hastening the ongoing biodiversity crisis [1]. This phenomenon is often associated with forestry, fisheries and hunting-related restocking (i.e. the release of captive-reared individuals to supplement conspecific contingents [2]) practices [3,4], which not only often fail to boost target populations (e.g. [5,6]) but also represent an important pathway to homogenization by admixture among distinct gene pools [7,8].

Other than being culturally relevant to human society for including well-known poultry [9,10] and game species [11,12], the order Galliformes also hosts several taxa affected by large-scale human-mediated genetic introgression related to hunting activities. In Europe, the most popular study cases are represented by the common quail (*Coturnix coturnix* [13–15]) and the partridges of the genus *Alectoris* (e.g. [16,17]). Among the latter, the red-legged partridge (*Alectoris rufa*), a heavily managed gamebird native to southwestern Europe (Iberian Peninsula, central and southern France including Corsica, and northwestern Italy southwards to Tuscany) [18] and of considerable socio-economic importance [19,20], has been subjected for decades to management practices involving extensive releases of farm-reared hybrids with the chukar partridge (*Alectoris chukar*, a

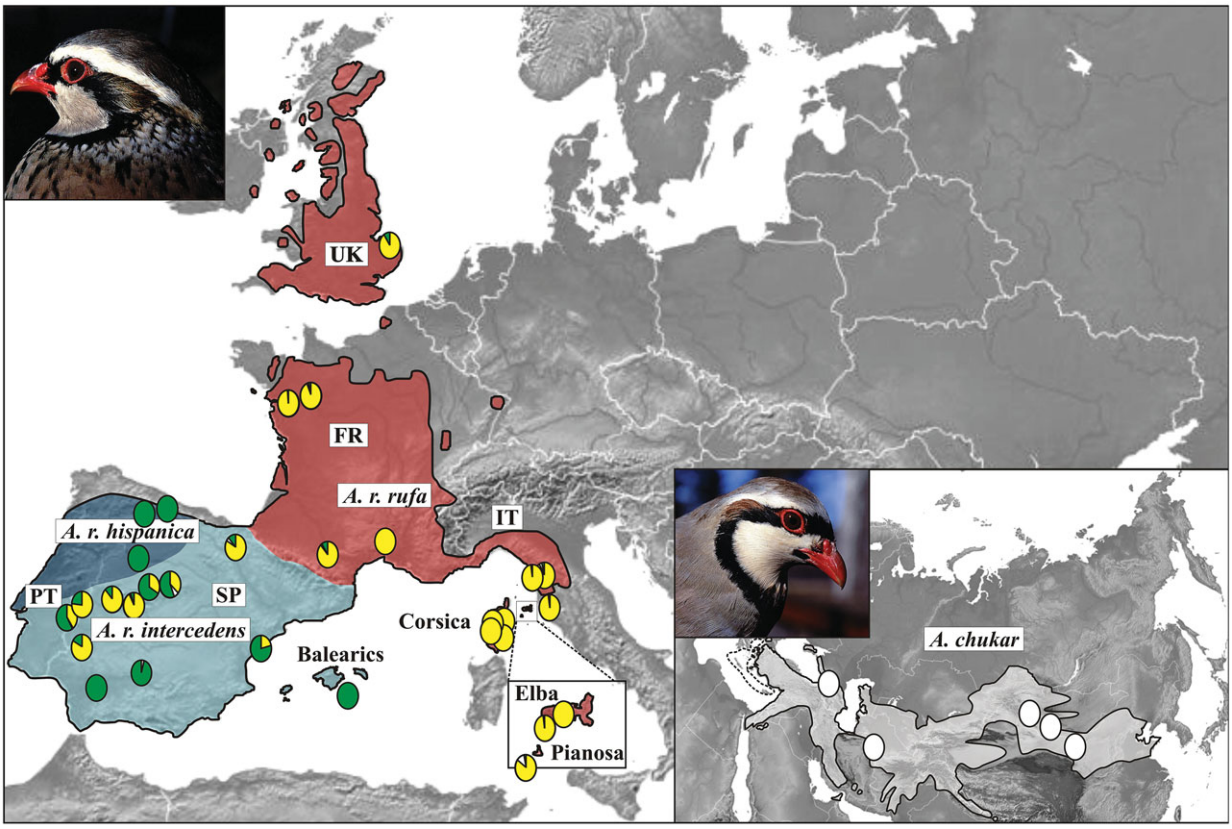

Figure 1. Distribution map of *A. rufa* and *A. chukar* (inset) with sampling localities and ADMIXTURE results at $K = 3$ based on 168 675 SNPs. The ranges of each taxon are given in different colours: *A. chukar*, white; *A. r. rufa*, red; *A. r. intercedens*, light blue; *A. r. hispanica*, dark blue [18]. Dotted lines indicate *A. chukar* distribution across the Eastern Mediterranean islands. Equal-sized pie charts show genotype assignment across different sampling localities (electronic supplementary material, dataset S1) with colours indicating allelic contributions typical of different genetic groups: white for *A. chukar*; yellow and green for *A. rufa*. Pictures of the two species are shown in the top left corner of their respective maps (author: F.B.). PT, Portugal; SP, Spain; FR, France; UK, United Kingdom; IT, Italy. (Online version in colour.)

largely Asian species found from Greece to Manchuria [18]) and to translocations irrespective of subspecific affinity [21–24]. Farmers are spurred to do this especially by the reward associated with the flourishing state of the better looking red-legged partridge hybrids, which are sold for restocking or as meat for human consumption [22]. These practices have led to serious concerns that the genetic integrity and spatial structure of most red-legged partridge populations are irreversibly affected, with widespread introgression adding to the list of traditional threats which include overharvesting, mechanized agriculture, pesticide use and rural abandonment [25–31]. These factors were indicated as the main causes underlying the severe decline of over 95% of the global *A. rufa* population since the 1980s [32,33], warranting the inclusion of the red-legged partridge in the list of threatened species under European Union legislation (79/409 CEE Ap.2/1, 3/I; BERN Ap.3), the status of Species of European Conservation Concern category 2 ('Vulnerable' [34]), and the upgrade to Near Threatened by the International Union for the Conservation of Nature and Natural Resources [35]. Previous studies based on few genetic markers (e.g. mitochondrial and microsatellite loci as well as random amplified polymorphic DNA) suggested that substantial chukar introgression may have primarily impacted nominate *A. r. rufa*, native to France and Italy (for which a virtual 'genetic extinction' has been hypothesized [22]), but also—to a lesser extent—*A. r. hispanica* and *A. r. intercedens* from northwestern Spain and the remainder of the Iberian Peninsula, respectively [17,21–24,36–40].

In this study, we used over 168 000 genome-wide markers and more than 80 *Alectoris* individuals, with a comprehensive sampling across the entire red-legged partridge's range, to assess its genomic identity and spatial structure. Specifically, we explored the degree and extent of chukar introgression as well as intraspecific structure at a much finer resolution than hitherto achieved. This work constitutes an innovative example of the importance of applying conservation-genomic methods to solving wildlife management problems involving introgressive hybridization worldwide.

2. Methods

(a) Field sampling and DNA isolation

A total of 97 *A. rufa* ($n = 87$) and *A. chukar* ($n = 10$) samples were collected between 1997 and 2012 across the range of the two species. However, single nucleotide polymorphism (SNPs) data used for downstream analyses were later obtained for only 81 samples (75 *A. rufa* and six *A. chukar*: figure 1; electronic supplementary material, dataset S1). The samples of *A. rufa* were assigned to morphological subspecies based on their collection locality (figures 1 and 2; electronic supplementary material, S1). In the case of shot partridges, no more than one sample was retrieved from each hunting trip to mitigate the risk of genotyping birds from the same covey. We carried out all DNA extractions at the Department of Biology (Zoology and Anthropology Unit, Zoology building) of the University of Pisa. We isolated DNA from blood and liver using the Puregene Core Kit-A (Qiagen, Hilden, Germany) following the manufacturer's instructions, and from feathers as in [22]. We determined DNA concentration and purity with an Eppendorf BioPhotometer (AG Eppendorf, Germany).

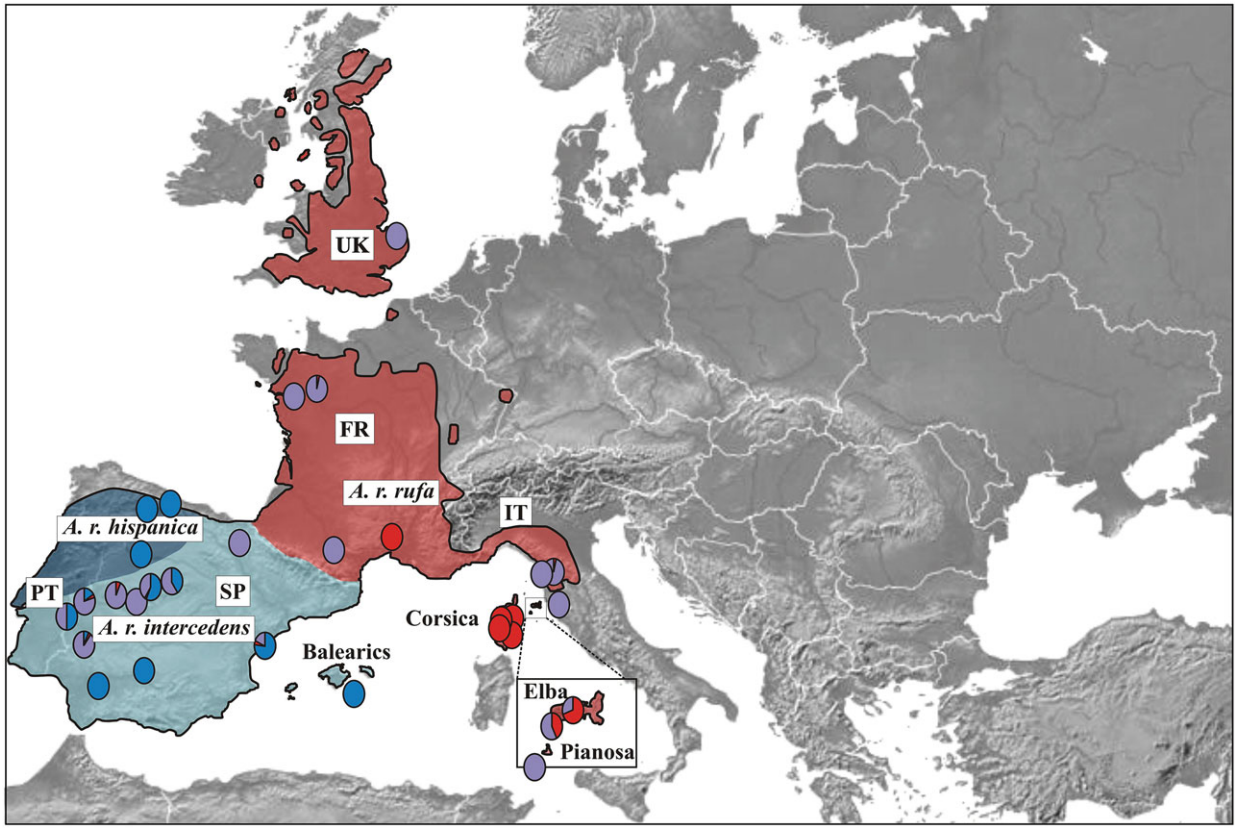

Figure 2. Distribution map of *A. rufa* with sampling localities and ADMIXTURE results at $K = 3$ based on 138 874 SNPs. The ranges of morphological subspecies are given in different colours: *A. r. rufa*, red; *A. r. intercedens*, light blue; *A. r. hispanica*, dark blue [18]. Equal-sized pie charts show genotype assignment across different sampling localities (electronic supplementary material, dataset S1) with colours indicating allelic contributions typical of different genetic groups: red for nominate *A. r. rufa*; blue for Iberian *A. r. intercedens* and *A. r. hispanica*; lilac for populations with a homogenized allelic contribution of captive-released stocks. PT, Portugal; SP, Spain; FR, France; UK, United Kingdom; IT, Italy. (Online version in colour.)

(b) Double digest restriction enzyme associated DNA sequencing library preparation

We prepared a double digest restriction enzyme associated DNA sequencing (ddRADseq) library as per [41], except for the last clean-up step that was replaced by a size selection to fulfill fragment size requirements. The collection of 250–600 bp long fragments and the downstream clean-ups were carried out using Sera-Mag magnetic beads (Thermo Scientific, USA). Sample concentrations were quantified using a Qubit 2.0 broad range DNA assay (Invitrogen, Carlsbad, USA) and DNA fragment size was checked with a fragment analyzer (Advanced Analytical Technologies, Inc., Ankeny, USA) prior to pooling. The library was then sequenced on an Illumina HiSeq 4000 platform (150 bp paired-end run) at Novogene AIT (Singapore) with a 20% PhiX spike to limit low nucleotide diversity issues.

(c) Data processing and single nucleotide polymorphism calling

We employed FastQC (Babraham Bioinformatics, UK) to examine sequence quality across all base positions. The *process_radtags* command in STACKS v.2.4 [42] was used for demultiplexing. We aligned the ddRADseq reads against the *A. rufa* genome scaffold-level assembly (B. Chattopadhyay, G. Forcina, K. M. Garg, M. Irestedt, M. Guerrini, F. Barbanera, F. E. Rheindt 2021, unpublished data) using BWA-MEM [43]. We initially called 431 070 SNPs using pipelines *ref_map.pl* and *population* in STACKS v.2.4 with all default parameters for 97 individuals (average stack depth: 34.1x) without prior population assignment. We discarded individuals and SNPs with more than 50% and 10% missing data, respectively, using PLINK v.2.0 [44], which left 81 individuals with 213 123 SNPs. We further removed physically linked loci using the *indep-pairwise* algorithm with a 25-SNP sliding window and 10 SNPs each step with an r^2 threshold of 0.95 to harvest the final dataset of 168 675 SNPs. We repeated the analysis described above on a second dataset including only *A. rufa* samples ($n = 75$) to obtain 138 874 SNPs.

(d) Population genetic analyses

We assessed population subdivision employing maximum-likelihood ancestry estimation in ADMIXTURE v.1.3 [45]. We ran the software with a range of presumed ancestral populations from $K = 1$ to $K = 15$. We explored population structure by generating a principal component analysis (PCA) of individual-based genomic differentiation in SNPRELATE [46], an R package based on a genetic covariance matrix calculated from genotypes. We repeated the same analyses for the reduced dataset excluding *A. chukar* individuals to investigate *A. rufa* population substructure at a finer scale. To explore genomic signatures of introgression, we first defined comparison groups on the basis of varying levels of *A. chukar* introgression (see Results), morphological subspecies affiliation, and features unique to given populations: (i) *A. chukar*; (ii) Corsica (*A. r. rufa* with no detectable introgression); (iii) northwestern Spain (Iberian *A. rufa* with no detectable introgression); (iv) Guadalajara (Iberian *A. rufa* with ample introgression); (v) Aigues-Vives (nominate *A. r. rufa* with ample introgression); (vi) Elba Island (the only historically self-sustaining Italian *A. rufa* population); and (vii) Pianosa Island (highest level of introgression). Hence, we calculated pairwise F_{ST} [47] values for each population pair across the genome in windows and steps of 20 000 bp and 5000 bp in size, respectively, using VCFTOOLS v.0.1.16 [48], and visualized them as Manhattan plots, a scatter plot widely employed in genome-wide association studies, using the R package *qqman* [49]. Furthermore, to illustrate the pattern of *A. chukar* introgression

across populations, we identified putative chukar-like sites with a two-step filtering based on Wright's [50] F_{ST} threshold of 0.6 (a value used to mark out substantial levels of population differentiation [51]). First, we pinpointed all the sites that differentiate the allegedly best preserved populations of *A. rufa* and *A. chukar* as those with $F_{ST} > 0.6$ in the two pairwise comparisons involving *A. chukar* versus (i) *A. r. rufa* from Corsica (four populations, $n = 12$) and (ii) *A. r. hispanica* from northwestern Spain (four populations, $n = 10$). Second, we inspected this pool of sites in the pairwise comparison between *A. chukar* and the *A. rufa* populations from Elba Island and Pianosa Island, flagging all those with $F_{ST} < 0.6$ as of putative *A. chukar* origin. We decided to take the latter two insular populations as examples by virtue of their specific features: while the former displays a limited but distinctive pattern of *A. chukar* introgression, the latter showed the highest level of admixture across the entire species' range (see Results). In parallel to the above method, we performed an analysis with *EILA* (efficient inference of local ancestry: [52]), a program using quantile regression and *k*-mean classifier to infer local ancestry across the genome, to further investigate patterns of *A. chukar* introgression. As the reference genome is at scaffold level, we selected scaffolds above 5Mb (57 from total 1618 scaffolds, which consist of 64.55% of the total SNPs) to input *EILA*. We used individuals of comparison groups (i) to (iii) and (iv) to (vii) as ancestry and admixed samples, respectively. We performed two *EILA* runs with *lambda* values set at either 15 or 30 for the different smoothness of the fused quantile regression.

3. Results

We obtained 168 675 genome-wide SNPs indicating distinct genetic groupings across 81 *Alectoris* individuals (75 *A. rufa* and six *A. chukar*: electronic supplementary material, dataset S1) from 36 sampling localities (electronic supplementary material, figure S1). The results of the optimum *K* value as determined with the lowest value of cross-validation error (electronic supplementary material, figure S2), $K = 3$, are shown in figures 1 and 2; electronic supplementary material, figure S3. One group (white in figure 1; electronic supplementary material, figure S3a) consisted of all *A. chukar*, while the other two (yellow and green or lilac in figure 1; electronic supplementary material, figure S3b, respectively) referred to *A. rufa* (electronic supplementary material, table S1). When looking at *A. chukar* representatives, we found they clustered in two groups consistent with the mitochondrial DNA (mtDNA) clades from the Middle and Far East found by [17] (electronic supplementary material, figure S4). Overall, the proportion of *A. chukar* genomic components in *A. rufa* individuals was quite limited, with a peak in the population of Pianosa Island (Italy) ($Q = 8.3$–13.6%: electronic supplementary material, table S1) leading to appreciably lower levels of differentiation between local birds and *A. chukar* as compared to all other *A. rufa* populations (figure 3*a*). This introgressive proportion decreased by half or more across other nominate populations from mainland Italy and France as well as the UK. When examining the amount of *A. chukar* introgression further west, we found it to be variable and unevenly distributed, with individuals from central (Toledo and Guadalajara: see the electronic supplementary material, dataset S1 and figure S1 for localities) and southwestern Spain (Andújar) as well as Portugal (Elvas) showing some signature of introgression to an extent more or less comparable with that found in many *A. r. rufa* populations. By contrast, individuals from multiple localities scattered across central and southern Spain (east to west: Mallorca, Castellón de la Plana, Madrid, Oropesa de Toledo, Sevilla, Badajoz) or in the northwestern corner of the country (Zamora, León, Cangas del Narcea) as well as in Portugal (Marvão) showed no detectable signs of introgression. Likewise, very limited or no such admixture emerged for representatives of nominate *A. r. rufa* from Elba Island and Corsica, respectively, at the easternmost edge of the species range.

Upon removal of *A. chukar* from analysis, three main population-genetic clusters emerged in *A. rufa*: (i) one mostly representing nominate *A. r. rufa* from Elba Island (Italy) and Corsica (red in figure 2; electronic supplementary material, figure S3b), (ii) another representing typical individuals of the two Iberian subspecies, with no subspecific discrimination (blue in figure 2; electronic supplementary material, figure S3b), and (iii) a third presumably corresponding to the homogenized allelic contribution of captive-released stocks at various levels of preponderance across the species range (lilac in figure 2; electronic supplementary material, figure S3b and table S1). The attribution of the latter genomic component to captive stocks is corroborated by two individuals of known captive provenance from mainland Italy (Scarlino) and western France (Chambretaud) displaying a 100% assignment to this component (figure 2). This homogenized allelic contribution emerged as dominant in many parts of the range of the *A. r. rufa* subspecies, especially in areas known to be subjected to restocking activity, such as the UK, western France and virtually all of mainland Italy, restricting the most unaffected areas of the nominate range to Mediterranean islands such as Corsica. Even so, a significant proportion of the genome of partridges from Elba Island ($Q3 = 30$–50%: electronic supplementary material, table S1) still carried considerable captive allelic contributions. In Spain and Portugal, a variety of populations exhibited a unique genomic signature presumably typical of the two Iberian subspecies (*A. r. intercedens* and *A. r. hispanica*), including birds from eastern and southern (Mallorca, Andújar, Sevilla) as well as northwestern Spain (electronic supplementary material, figure S3b) ($Q1 = 99\%$: electronic supplementary material, table S1), whereas birds from other localities (Castellón de la Plana, Guadalajara, Madrid and Elvas) displayed significant genomic components typical of captive stock ($50\% < Q3 < 97\%$: electronic supplementary material, table S1) (figure 4).

A genome-stratified visualization of pairwise F_{ST} (figure 4) suggested that the highest level of differentiation versus *A. chukar* occurred in red-legged partridge populations from Corsica (*A. r. rufa*) and northwestern Spain (*A. r. hispanica*). In comparison, *A. r. rufa* on Pianosa Island showed entire genome sections with low differentiation from *A. chukar*, probably reflective of wholesale introgression of entire linkage blocks (evidenced by the denser black dots in the lower section of the graph). Two of these, located around scaffolds 2 and 6, were not shared by other *A. rufa* populations showing *A. chukar* introgression (e.g. Aigues-Vives and Guadalajara in France and Spain, respectively; figure 4; electronic supplementary material, figures S6 and S7). Then, we explored patterns of potential *A. chukar* introgression (pairwise $F_{ST} < 0.6$) across populations with different management histories by inspecting 26 016 genome-wide sites that are clearly divergent (pairwise $F_{ST} > 0.6$) between *A. chukar* and the genomically preserved *A. rufa* populations from Corsica and northwestern Spain (electronic supplementary material, dataset S1). For instance, we detected 11 832 versus 6123 chukar-like sites on Pianosa and Elba Island, respectively. Only

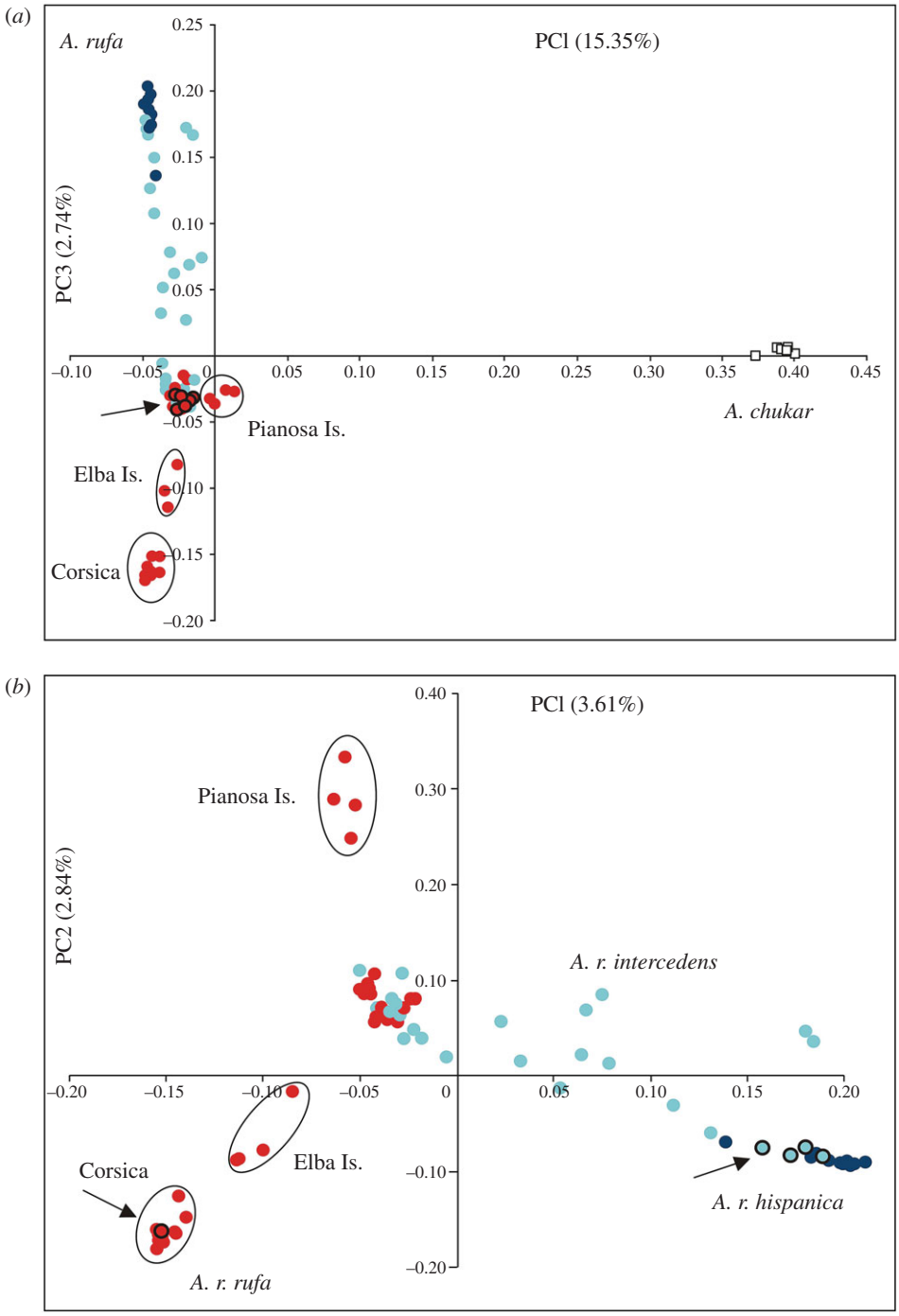

Figure 3. (*a*) Principal component analysis based on 168 675 SNPs extracted from restriction-associated DNA comparing *A. rufa* (circles, $n = 75$) and *A. chukar* (squares, $n = 6$). The colour scheme used is consistent with taxon distributions as shown in figure 1: *A. chukar*, white; *A. r. rufa*, red; *A. r. intercedens*, light blue; *A. r. hispanica*, dark blue. Individuals from Corsica and Elba Island (highly preserved *A. r. rufa* populations) as well as Pianosa Island (hosting an *A. rufa* population heavily admixed with *A. chukar*) are delimited by ovals. The arrow indicates captive individuals from Scarlino and Chambretaud (bordered black; electronic supplementary material, dataset S1). (*b*) Principal component analysis based on 138 874 SNPs extracted from restriction-associated DNA showing the intraspecific structure of *A. rufa* ($n = 75$). The colour scheme used is consistent with taxon distribution shown in figure 1: *A. r. rufa*, red; *A. r. intercedens*, light blue; *A. r. hispanica*, dark blue. Individuals from Corsica and Elba Island as well as Pianosa Island are delimited by ovals. The names of morphological subspecies are placed beside the groups of individuals on the basis of their geographical origin. The arrows indicate geographical outliers (see Discussion) flagged with asterisks in the electronic supplementary material, figure S3 (bordered black; electronic supplementary material, dataset S1). (Online version in colour.)

28.55% of the introgressed sites were shared between these two insular populations (electronic supplementary material, figure S5). Inference of local ancestry across the genome, using *EILA*, also indicated varying levels of *A. chukar* introgression across differently admixed populations (electronic supplementary material, figures S6 and S7). For instance, we detected 34 074 versus 8619 loci of *A. chukar* ancestry on Pianosa and Elba Island, respectively. In this case, only 17.07% of the loci with *A. chukar* ancestry were shared between these populations.

4. Discussion

Genetic homogenization associated with human-mediated wildlife reshuffling is listed among the main drivers of biotic impoverishment in the Anthropocene biodiversity crisis [53]. Game species often represent paradigmatic case studies to address key questions in wildlife conservation and management of interest to broader society (e.g. [54–56]). In this work, we used over 168 000 SNPs to investigate chukar

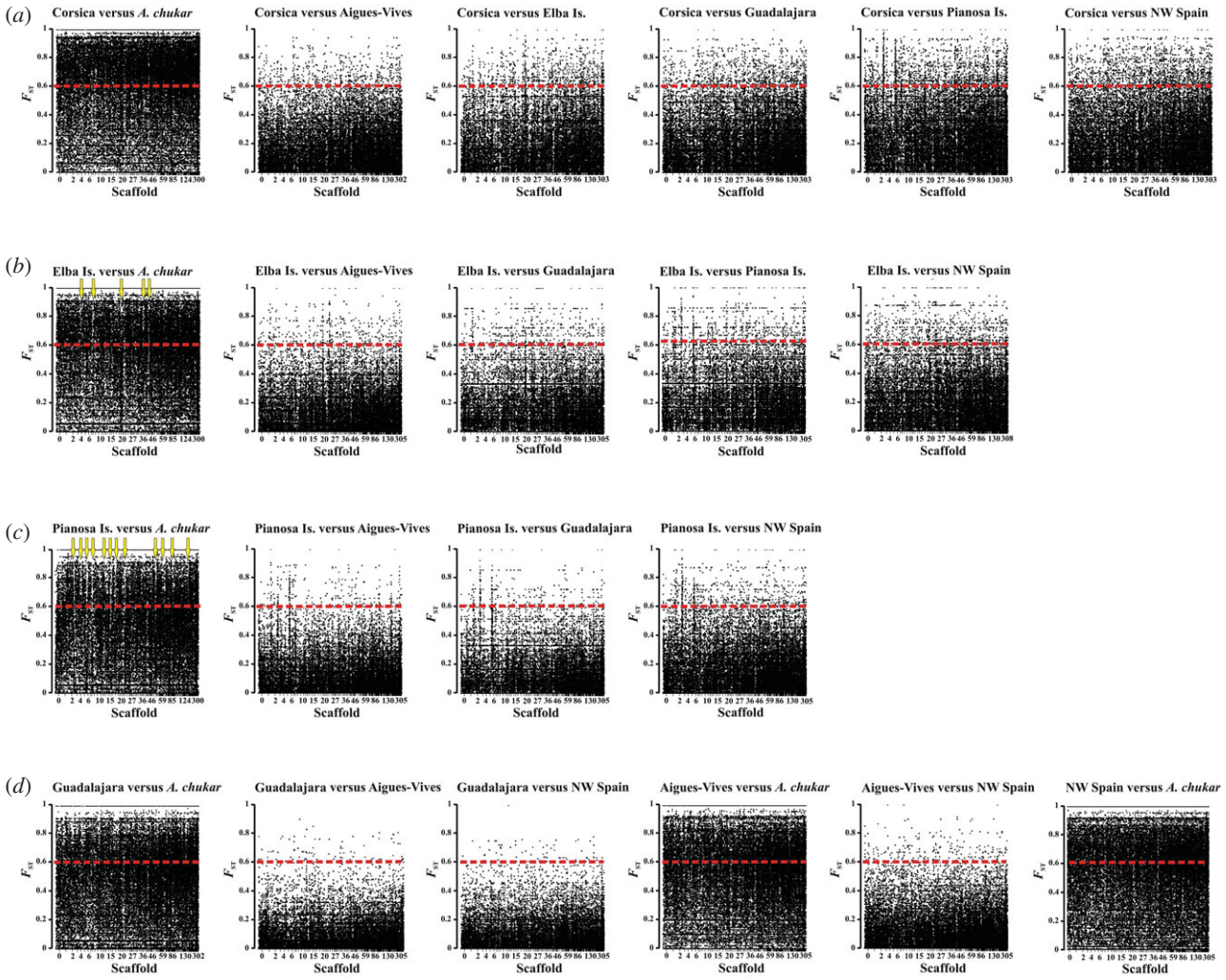

Figure 4. Manhattan plots showing genome-wide pairwise F_{ST} values between populations of interest. Dotted lines mark the threshold of $F_{ST} = 0.6$, above which differentiation is considered high. (*a*) Corsica versus others; (*b*) Elba Island versus others; (*c*) Pianosa Island versus others; (*d*) others versus others. NW Spain, northwestern Spain (Zamora, León, Cangas del Narcea: electronic supplementary material, dataset S1). The distinctive features of *A. chukar* introgression in Elba and Pianosa individuals are marked by yellow arrows. (Online version in colour.)

introgression and intraspecific structure in an intensively managed gamebird, the red-legged partridge. The strength of our methodological framework relies on comparing signatures of introgression by means of a genome-wide approach across the entirety of the *A. rufa* native range and the UK, hosting the most important introduced population of known origin (established with *A. r. rufa* from France in the eighteenth century [24,57,58]). Our results spell the need for a radical re-think on red-legged partridge conservation, restoring genomically preserved status to multiple populations long treated as compromised.

Overall, we discovered both the extent and the degree of *A. chukar* introgression to be far more limited than previously suggested [17,20–24,36–40,59], with a highly uneven pattern among and within different subspecies (figures 1 and 3; electronic supplementary material, figure S3a). Interestingly, while most populations within the ranges of *A. r. rufa* and *A. r. intercedens* showed a low yet detectable level of *A. chukar* introgression, those of *A. r. rufa* from Corsica and *A. r. hispanica* turned out to be probably unaffected. Rather than being a product of isolation-driven divergence, the present-day distinctiveness of *A. rufa* from Corsica is most likely related to the extensive erosion of native genetic structure of *A. r. rufa* populations across the continental portion of the range as a result of poor management practices. Indeed, the *A. rufa* population inhabiting Corsica is the product of a historic introduction that would place its natural divergence from mainland populations at the order of only 1400 years before present [60–62], and has been managed mostly with the local stock [23,36].

The fairly high genomic integrity of *A. r. rufa* on Elba Island is surprising since it contrasts sharply with a history of rampant chukar introgression (but see below), as disclosed by means of both microsatellite and mtDNA data [17,22,63,64], through local restocking activities carried out for over four decades but ceased with the institution of a national park. On the other hand, the overtly admixed nature of the *A. rufa* population inhabiting nearby Pianosa Island [21,22] is fully reflected in our results, which identify this population as the one most intensely affected by chukar introgression (electronic supplementary material, figures S4, S5 and S6). When examining the genomic makeup of *A. rufa* populations further west within the species' range, we interpreted the low but discernible signatures of introgression as an outcome of frequent restocking activities. Conversely, highly preserved genomes of individuals from northwestern Spain are probably associated with the lower levels of management in this region, where partridge hunting is less popular than elsewhere in the country (V. Piorno González 11 June 2020, personal communication to G. Forcina).

Our data suggest that the view of *A. rufa* as a game species whose genetic identity has been irretrievably spoiled by inappropriate relocations is probably an artefact of past reliance on traditional marker systems, particularly mtDNA, with high percentages of birds carrying the chukar mtDNA haplotype [23]. This approach suffers from serious methodological drawbacks. mtDNA works like a categorical marker that indicates the haplotype of the maternal line without reflecting different degrees of introgression. Moreover, mtDNA is under heavy selection [65] and can spread much faster within a native population than average genomic loci, further propelled by virtue of its 4-fold lower effective population size [66]. On the other hand, the use of either categorical (e.g. random amplified polymorphic DNA [17,22–24]) or probabilistic (microsatellites [17,36]) nuclear markers might have led to misleading inferences in that the loci employed could well be confined to 'islands' (i.e. discrete portions) of *A. chukar* DNA preserved within *A. rufa* populations, thus inflating estimates of introgression. This is probably also the case for the comparatively few SNPs tested in this species so far [67–69]. In the present study, based on a genome-wide approach which is orders of magnitude more informative than the loci used in the past, we suggest that the spread of *A. chukar* introgression into wild *A. rufa* populations could be constrained by negative selective forces lessening the fitness of hybrid partridges [70]. This hypothesis, proposed also for gene flow between *A. rufa* and another congener (the rock partridge, *Alectoris graeca* [71]), has indeed been confirmed in closely related gamebirds (e.g. *Coturnix* spp. [72–74]), even if stochastic processes are deemed accountable for the highly asymmetric nature of introgression in other study systems [75]. Hence, the virtual absence or limited extent of introgression in the *A. rufa* population from Elba Island might be indicative of similar habitat-driven negative selection against hybrids. Conversely, the xeric habitat and relaxed predation pressure on Pianosa Island might have favoured the spread and persistence of *A. chukar* genomic components in the local *A. rufa* population [21].

We also revealed that in spite of massive translocations using *A. rufa* stocks derived from different morphological subspecies, clear evidence of intraspecific structure remains (figure 3; electronic supplementary material, figure S3). In particular, we observed consistency between geographical origin and taxonomic affiliation as inferred from genomic clustering in the PCA of figure 3*b*, with *A. r. intercedens* in quadrant I, *A. r. hispanica* in quadrant II, *A. r. rufa* in quadrant III, and individuals with a heavily admixed genomic makeup in quadrant IV. In contrast to recent studies [17,40,64], Elba Island was found to host an overall fairly well-preserved population of the nominate subspecies, thus emerging with nearby Corsica as the last stronghold for this taxon. The close affinity between individuals from Corsica and a single consubspecific bird from southern France (Q2 = 99%: figure 2; electronic supplementary material, table S1) could be indicative of the alleged origin of the Corsican population as a relatively recent human-mediated introduction (sixth century AD [60–62]), which has resulted in a genomic backup of *A. r. rufa*. On the other hand, the other continental populations within the range of the nominate subspecies were all found to be devoid of their taxon-specific genomic signature, showing the clear effects of faunal relocation in the form of a homogenized allelic contribution typical of captive populations here represented by individuals from Scarlino (Italy) and Chambretaud (France), the latter being the origin (Vendée, western France) of the founders used for the Italian farm (figure 3*b*) [76]. Further west, we confirmed the distinctiveness of partridges of *A. r. hispanica* from northwestern Spain [77] and *A. r. intercedens* from the remainder of the Iberian Peninsula. Between these two, the higher proportion of captive allelic contributions in *A. r. intercedens* is the likely result of intensive translocations of *A. rufa* populations across central and southern Spain [20]. *Alectoris r. intercedens* outliers clustering with *A. r. hispanica* (figure 3*b*) come either from captivity (Andújar) or recreational areas (Sevilla and Mallorca) probably subjected to translocations with birds of non-local origin.

Our results are paralleled by other studies based on genome-wide loci which are disclosing a markedly different picture in terms of population structure of wild versus farmed gamebird stocks compared to those previously inferred with traditional markers [78,79]. Likewise, our results are in line with the conclusion of the only study supporting the persistence of an intraspecific structure in the red-legged partridge in defiance of intensive management [77] as opposed to many others pointing to the widespread loss of subspecific genetic signatures (e.g. [23,24,40,80]). However, the authors of that study [77], which was based on mtDNA, 20 microsatellites and detailed information on the management history of the investigated populations, did not include samples from areas subjected to restocking activities to lessen the occurrence of non-native genotypes. Here, we made no *a priori* assumptions about the native status of individuals but used a number of loci many orders of magnitude larger than that employed by others [77]. To sum up, our results pointed to *A. r. hispanica* (type locality: Galicia, northwestern Spain [81]) and *A. r. intercedens* (type locality: Málaga, southern Spain [82]) as the least and the most genomically compromised subspecies, respectively, while showing that *A. r. rufa* (type locality: northern Italy [81]) is characterized by highly eroded populations on mainland Europe and well-preserved populations especially on Corsica but also on Elba Island. The vast majority of *A. r. intercedens* in our sample were collected in areas subjected to intensive game management (see above). Further investigations, especially in protected areas—where no restocking is carried out—such as Doñana National Park in southeastern Spain, could identify genomically preserved populations of this subspecies.

Our research highlights the need to implement strategies aimed at preserving the genomic identity of regional population-genetic clusters of the red-legged partridge under an adaptive evolutionary conservation framework. Consequently, we strongly discourage wildlife managers from translocating stocks of a given *A. rufa* subspecies across the range of the others. Overall, this study exemplifies the great resolution provided by genome-wide markers as an essential prerequisite to properly assess the magnitude of introgressive hybridization, thus flagging candidate populations for faunal relocation, prioritising funding decisions and adequately informing stakeholders. This work also represents a blueprint for introgression-focused conservation studies that have long been based on incomplete molecular evidence drawing overly hasty and pessimistic conclusions. As such, management strategies might be in need of reappraisal in the genomic era.

Specifically, an approach such as ours may be a promising avenue to reassess the geographical extent and degree of admixture in wild populations of game species subjected to restocking with close relatives (i.e. different species or subspecies). Such

reappraisal is also needed for the fast-increasing number of closely related species whose admixture is being propelled by rampant climate change. Similar to the present work, population/individual-level estimates of admixture different from those inferred with traditional loci can be disclosed. Paradoxically, however, positive results such as ours might also be dangerous as they could reduce the focus of the scientific community and broader society on wildlife species which nonetheless continue to be in need of conservation and management efforts to secure their persistence in the long term.

Ethics. Research approved by the National University of Singapore and the University of Pisa to which are affiliated the two senior authors.

Data accessibility. The ddRADSeq data are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.q83bk3jh9 [83].

Authors' contributions. F.B. and F.E.R. conceived the study; E.C., M.G., and G.F. carried out laboratory work, Q.T. analysed the molecular data and performed the statistical analyses; G.F. and Q.T. prepared the figures; G.F. wrote the first draft of the manuscript; all authors contributed to discussions, review and editing.

Competing interests. We declare we have no competing interests.

Funding. This work was supported by the Erasmus Mundus project EURASIACAT (ref. no. 552067) coordinated by the University of Málaga (Spain) and a fellowship (FCT, PTDC/BAA-AGR/28866/2017) from the Portuguese Foundation for Science and Technology (FCT) to G.F., by a grant of the Provincia di Livorno, INTERREG III Toscana – Corsica – Sardegna (2002-2007), and by Università di Pisa with 'Progetto di Ricerca di Ateneo' grant no. PRA_2018_15.

Acknowledgements. Authors are deeply indebted to all the people who contributed to collecting the biological samples: N. Aebischer, R.A.H. Draycott, J. A. Ewald, R. Burrell, C. J. Wheatley, and the Norfolk gamekeepers for *A. rufa* from the UK; J. A. Dávila and J. J. Negro for *A. rufa* from Spain; C. Pietri, C. Cherbonnel, Fédération Départemental des Chasseurs de Charente-Maritime, A. Gregoire, and M. Nobili for *A. rufa* from France; R. S. Ceia for *A. rufa* from Portugal; F. Giannini, R. Giombini, F. Parri, and M. Arrighi for *A. rufa* from Elba Island; D. Scarselli and Agrofauna for *A. rufa* nearby Pisa; C. Valentini, S. Samadi, N. Liu, and S. Birks with the University of Washington Burke Museum of Natural History and Culture for *A. chukar* samples. Authors are grateful to J. J. Negro, R. Villafuerte and V. Piorno González for the fruitful discussions and information about *A. rufa* management in Spain. Elize Y. X. Ng and Kritika M. Garg are acknowledged for their assistance with laboratory activities.

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