

# Non-native and hybrid in a changing environment: conservation perspectives for the last Italian red-legged partridge (*Alectoris rufa*) population with long natural history



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## ARTICLE INFO

### Keywords:

*Alectoris* partridges  
 biotic homogenization  
 conservation of hybrids  
 Elba Island  
 introgressive hybridization  
 mitochondrial DNA

## ABSTRACT

The ever-increasing biotic homogenization - especially when associated with introgressive hybridization - raises concern for the reduction of the spatial component of diversity in wildlife worldwide. Nonetheless, there is a growing attention to the potential conservation value of hybridization in fastening the adaptive evolutionary responses to rapidly changing selective pressures. Under these premises, we investigated the genetic affinity of the red-legged partridge (*Alectoris rufa*) population inhabiting Elba Island (Italy) in the context of the overall species phylogeography and particularly of the nominate subspecies it is traditionally ascribed to. Although notoriously hybrid with the congeneric *A. chukar*, this island population is of undisputedly value because of its long natural history and self-sustainability. As such, its adaptive conservation management calls for a comprehensive knowledge including the assessment of its geographic origin. For this purpose, 110 fecal samples were collected across Elba, genotyped at their joint Cytochrome-*b* and Control Region genes (2,249 characters), and compared with 149 conspecifics from all over the species distribution range. We confirmed a widespread *A. chukar* mitochondrial DNA introgression in Elba partridges, whereas their expected formal assignment to the nominate subspecies from Italy and France was rejected, since these turned out to be closely related to conspecifics from the Iberian Peninsula. This counterintuitive result found support in a large variety of literary sources and compelling evidences from personal testimonies revealing recent intense management with farm-reared birds of Spanish origin. Although the nativeness of Elba partridges was disproved, we advise local authorities to keep warranting the ongoing conservation efforts - and especially restore the connectivity between the western and eastern island sub-populations - as this resource may still be conceived as the ultimate repository for part of the otherwise extinct Italian *A. r. rufa* genome. Indeed, the admixture with conspecifics from the Iberian Peninsula does not necessarily mean that the entirety of the native nuclear genome of Elba partridges has been wiped out. Furthermore, these latter represent an interesting case study in conservation biology to investigate the possible role played by introgressive hybridization in the adaptation to recent land use and vegetation cover changes associated with rural abandonment in an insular yet heavily anthropized context.

## 1. Introduction

Biotic homogenization - the progressive replacement of native biotas with locally expanding non-natives (McKinney and Lockwood, 1999; Olden and Poff, 2003; Olden et al., 2004) - is referred to as a leading driver of ecosystem impoverishment worldwide (Sax and Gaines, 2003). It consists of two main components, wildlife reshuffling and genetic homogenization, which often synergistically contribute to increase local diversity while decreasing disparity at global level (Olden and Rooney, 2006). In game birds, they are frequently associated with

relocations that have long been carried out more for ornamental or socio-economic rather than conservation purposes. Hence, lack of geographic and genetic similarity of the source populations being used often turned into widespread introgressive hybridization of local biotas (Lucio and Purroy, 1992a; Negro et al., 2001; Baratti et al., 2004; Barbanera et al., 2005, 2007, 2011; Barilani et al., 2007; Blanco-Aguilar et al., 2008; Sanchez-Donoso et al., 2014a; Söderquist et al., 2017; Forcina et al., 2018). Even if evidences of genetic rescue occur in some cases (Whiteley et al., 2015) - and there is reason to believe that the higher fertilization success of farm-reared hybrids in the wild (Sanchez-

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<https://doi.org/10.1016/j.zool.2019.125740>

Received 17 September 2019; Received in revised form 6 December 2019; Accepted 9 December 2019

Available online 18 December 2019

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Donoso et al., 2016) might well be involved in this process (as evidenced in natural hybrids: Chan et al., 2018) - the release of non-native individuals may cause detrimental changes. Among these, genetic impoverishment, alteration of population structure and behavioral traits (Frankham et al., 2002; Laikre et al., 2010; Champagnon et al., 2012; Sanchez-Donoso et al., 2014b) can eventually jeopardize the genomic integrity of a given species or population (Potts, 1989; Liukkonen-Anttila et al., 2002; Barbanera et al., 2005; Barilani et al., 2005; Amaral et al., 2007).

Management of introgressed populations deriving from natural as well as human-mediated or anthropogenic hybridization is a debated issue in conservation biology (Haig and Allendorf, 2006; Jackiw et al., 2015; Pielt et al., 2015; Hamilton and Miller, 2016; Todesco et al., 2016), with legislative gaps impairing the adoption of a concerted approach to this issue (Wayne and Shaffer, 2016; Erwin, 2017). Hybrids may offer an interesting opportunity to study how evolution works, bettering our understanding of the finest mechanisms underlying adaptation to new environments and/or to rampant global change in the attainment of the “keeping options alive” concept (Reid and Miller, 1989). Indeed, there is a growing body of research highlighting the conservation value of introgressive hybridization by virtue of its potential for fastening otherwise longer-term adaptive evolutionary responses to rapidly changing selective pressures (Hamilton and Miller, 2016). Not only would the admixed genotypes assist the recovery of declining populations, they would also facilitate the colonization of new niches thanks to the so-called “transgressive segregation” (Welch and Rieseberg, 2002; Dittrich-Reed and Fitzpatrick, 2013; Hamilton et al., 2013). Moreover, hybrids may still represent a valuable even though introgressed genomic resource - if not the last resort option available - to preserve genetic material of one or both their parental species (Crispo et al., 2011).

In this respect, the red-legged partridge (*Alectoris rufa*) is definitively one of the most emblematic species. Often referred to as the most socio-economically valuable among the small game of south-western Europe, this galliform is found from the Iberian Peninsula across central and southern France to north-western Italy (including most of the major western Mediterranean islands) (Madge and McGowan, 2002). Such species has been experiencing a sharp demographic decline over the last decades due to the combined effects of overharvesting, pesticide use and habitat loss following deep changes in Europe agricultural landscape and practices (Potts, 1980; Rands, 1986; Lucio and Purroy, 1992b; Aebischer and Potts, 1994). As a result, the red-legged partridge is listed as Species of European Conservation Concern (SPEC 2: species with core distribution range in Europe and declining populations) by BirdLife International (2004) and evaluated as threatened under European Union legislation (79/409 CEE Ap.2/1, 3/1; BERN Ap. 3). In the case of nominate *A. r. rufa* subspecies of Italy, since World War II massive releases of non-native and/or farm-reared hybrid birds with the exotic chukar partridge (*A. chukar*) caused widespread introgression of local populations. Unlike other similar cases (Sanchez-Donoso et al., 2014a, b), this phenomenon was quite fast due to the overall fairly good fitness of farmed hybrid individuals in the wild, turning into the vanishing of the original genomic *A. r. rufa* make-up (e.g., Spanò, 1992; Baratti et al., 2004; Barbanera et al., 2005, 2009, 2011; Barilani et al., 2007). Indeed, on the one hand the survival of hybrids is lower than that of wild pure birds, on the other hand the breeding success is not, with their larger clutches compensating for the higher mortality (Casas et al., 2012).

The *A. rufa* population found on Elba Island (Tuscan Archipelago National Park, Fig. 1A) is the most important of Italy in light of remarkable traits such as long natural history, absence of restocking over the last 25 years and self-sustainability (see Materials and methods). However, a recent demographic decline has raised major concern among wildlife ecologists, who are envisaging *ex situ* management to warrant its persistence (Baccetti and Gotti, 2016; see also below). Although previous studies relying on nuclear and mitochondrial DNA

markers revealed introgressive hybridization with *A. chukar* (Barbanera et al., 2009; Guerrini and Barbanera, 2009), the expected affiliation of Elba population to the nominate subspecies has never been investigated, yet it represents a fundamental information for the management of this natural resource within an adaptive conservation framework (Fraser and Bernatchez, 2001). In light of all these considerations, the aim of this study was to elucidate the kinship of Elba population in the context of the overall *A. rufa* distribution in order to provide the National Park with a comprehensive genetic picture. Therefore, we carried out a large-scale non-invasive sampling across the island to compare Elba partridges with conspecifics from the entire species range using mitochondrial DNA markers. We discussed the results relying on information drawn from a large variety of literary sources and compelling evidences from personal testimonies.

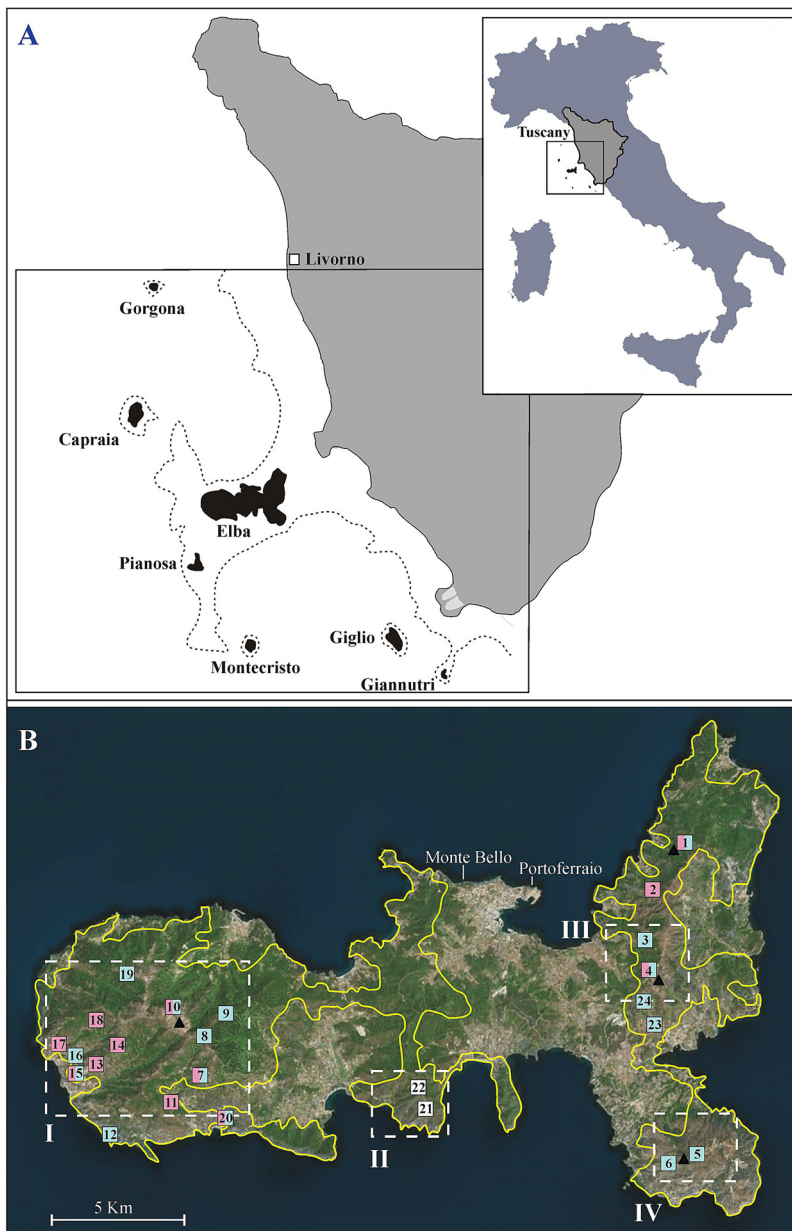
## 2. Materials and methods

### 2.1. Study site: Elba Island

The Tuscan Archipelago consists of seven main islands located midway between the Italian mainland and Corsica (France). This study was carried out on Elba (Province of Livorno, Tuscany), the third island of Italy by size (223.5 km<sup>2</sup>) and the largest in the archipelago, which is located c. 10 km off the coast in the Tyrrhenian Sea (Fig. 1A). Elba can be divided into three geomorphologically distinct areas (Fig. 1B). On the western side, characterized by harsh forms and the highest mountain range, long and deep valleys depart radially from the peak of the island (Monte Capanne, 1019 m: Fig. 1B, n. 10). In the central part, hills of moderate elevation, wide valleys and plain strips stretch out by only 4 km from north to south. On the eastern side, the landscape is dominated by the mountain range extending from Monte Strega (427 m: Fig. 1B, n. 1) southwards to Cima del Monte (n. 4, 516 m), Monte Castello (n. 24, 389 m) and Monte Val di Capanna (n. 23, 290 m) (Fig. 1B), with Monte Calamita (413 m: Fig. 1B, n. 5) in the extreme south-eastern corner. The dominant Mediterranean scenario notwithstanding, both the geological complexity (eastern side, 7 million years old; western side, up to 400 million years old) and climate variability provide Elba with a high degree of habitat diversity (Foggi et al., 2006) hosting 80% of the flora of the entire archipelago (Carta et al., 2018b).

### 2.2. The history of the red-legged partridge population of Elba Island

The occurrence of the red-legged partridge on the island is documented since the early XIX<sup>th</sup> century (Thiebaut de Berneaud, 1808; Salvadori, 1872). However, it cannot be ruled out that the species was established on Elba since late Pleistocene, when a connection to the Italian mainland was periodically available (most recently between 75,000 and 12,000 years ago). For instance, during last glacial maximum (c. 18,000 years ago), when the sea level was 100-120 m lower than today, a wide land bridge linked the islands of Pianosa and Elba to the Peninsula (Fig. 1A; Foresi et al., 2008). The occurrence of *Alectoris* in fossil remains of human meals found in Pianosa suggests that partridges might have reached this island (and most likely Elba too) in the course of such marine regressions (Baccetti and Gotti, 2016). Indeed, many species of the so-called “cold fauna” entered Elba and Pianosa from the mainland before insularity was definitively re-established about 12,000 and 15,000 years ago, respectively (Di Carlo, 1976; Azzaroli et al., 1990; Rustioni and Mazza, 1993; Barsotti et al., 2001; Zecchini, 2001). On the other hand, Pleistocenic climatic changes deeply shaped the phylogeography of *A. rufa* in most of its native distribution (Ferrero et al., 2011), and the congeneric rock partridge (*A. graeca*), which in Italy occurs on the Alps, the Apennines and in Sicily, was also present on Elba in the XIX<sup>th</sup> century (Arrigoni degli Oddi, 1904). However, the red-legged partridge was introduced to the nearby islands of Gorgona (mid-1700s, to supply the canteens of the Grand Duchy of Tuscany: Errico and Montanelli, 2000) and Montecristo



**Fig. 1.** A. Upper right inset. The study area in the Province of Livorno, Tuscany, central Italy. Lower left inset. The seven islands of the Tuscan Archipelago National Park are shown together with the paleogeographic outline during the last glacial period (c. 18,000 years ago: black dotted line): Pianosa and Elba were connected to the mainland through a wide land bridge (Foresi et al., 2008). B. Sampling localities on Elba Island (with elevation, a.s.l.). Eastern Elba: 1, Monte Strega (400 m); 2, Monte Capannello (350 m); 3, Volterraio (350 m); 4, Cima del Monte (400 - 500 m); 5, Monte Calamita (390 - 400 m); 6, Via Luperini - Elba Vortac trail (275 m); 7, Pietra Murata (550 m). Western Elba: 8, Le Calanche (800 m); 9, Monte Maolo (730 m); 10, Monte Capanne (800 m); 11, Vallebuia (300 m); 12, Punta Le Tombe (80 m); 13, Pomonte\_trail #104 (300 - 450 m); 14, La Terra (550 m); 15, Monte San Bartolomeo (430 m, summit); 16, Monte San Bartolomeo\_trail #103 (250 - 400 m); 17, Chiessi (100 m); 18 Pietragrossa (400 m); 19, Serraventosa (550 m); 20, Colonna Pisana (80 - 100 m); 23, Monte Val di Capanna (290 m, summit); 24, Monte Castello (389 m, summit). Central Elba: 21, Monte FONZA (270 - 295 m); 22, Monte Tambone (300 - 370 m). The yellow line shows the limits of the National Park while black solid triangles mark out Monte Capanne (n. 10), Monte Strega (n. 1), Cima del Monte (n. 4) and Monte Calamita (n. 5). For each locality, while red and blue indicate the occurrence of *A. rufa* and *A. chukar* mtDNA lineage, respectively, the white color refers to disclosure of *P. colchicus* only. White dotted lines indicate approximate boundaries of past game reserves: I, Monte Capanne ZRC; II, Monte FONZA ZRC; III, Volterraio ZRC; IV, Monte Calamita ZRC. The location of the main town of Portoferraio is reported together with the nearby site of Monte Bello. See text and Supplementary Table S1 for more information.

(1800s: Giglioli, 1881) (Fig. 1A). Hence, Masseti (2003) considers an event of this type as the most likely explanation for the recent occurrence of the species also on Elba.

The first information about the distribution of *A. rufa* on the island comes from Savi (1827–1831, 1873–1877), who reported the species as common on the southern slopes of Monte Capanne (Fig. 1B: n. 10). Before the establishment of the National Park (see below), Spanò et al. (1987) suggested the occurrence of 200–300 partridges in the post-breeding season, while Sposimo and Tellini (1995) estimated 100–200 breeding pairs, a value in line with that (300–330 birds) reported for 1994–1996 by Gariboldi (2006). The latter noted a remarkable negative trend of the species abundance across the whole island, especially near Monte FONZA (Fig. 1B: n. 21). A demographic collapse eventually took place by the end of 1990s (Arcamone, 1997), as later confirmed by both Centro Ornitologico Toscano and Parco Nazionale Arcipelago Toscano (2006), which assessed the occurrence of 30–50 pairs for the entire Elba, and Guerrini and Barbanera (2009). Ultimately, Chiantante et al. (2013) could not estimate any reliable population density value due to the rarity of *A. rufa* across the island.

Being a typical Mediterranean game species, the red-legged partridge has long been hunted also on Elba, and captive-reared birds have been largely used to supplement the local wild population at least since the early 1960s. In compliance with the national law 157/92, since 1992 the release of farmed partridges was carried out in four “Restocking and Capture Zones” (ZRC acronym in Italian, Fig. 1B: I to IV) created by the Province of Livorno to protect the small game species of the island before it was definitively stopped in mid-1990s (G. Burchianti and M. Arrighi, pers. com. to F. Barbanera, 20 January 2004 and 4 December 2018, respectively). After the establishment of the Tuscan Archipelago National Park (1996), the majority of Elba territory (128 km<sup>2</sup>: Fig. 1B) was set under strict protection while hunting was allowed in the remaining part of the island. Here, both introduced wild boar (*Sus scrofa*) and common pheasant (*Phasianus colchicus*) were the main species targeted together with the red-legged partridge. In 2003, the ban on *A. rufa* hunting was extended to the entire Elba Island.

### 2.3. Biological sampling

Sampling (2004-2006, 2018-2019) covered the whole island territory, both inside and outside the National Park limits. Former ZRCs, where occurrence of partridges was deemed most likely, were inspected with particular attention. We surveyed 24 localities (Fig. 1B, Supplementary Table S1) after post-breeding dispersal, namely between October and early April, when winter coveys are dissolved (Gariboldi, 2006). Sampling trips were performed following at least three days with no rain. Overall, 110 fecal samples were individually collected in plastic vials (no chemicals added) and stored at -40 °C at the University of Pisa. When a group of scats was discovered (i.e., at a covey night shelter), DNA was extracted from one fecal sample only to avoid duplicates from the same or a strictly related animal. As far as the entire *A. rufa* range is concerned, we used a large set of samples collected 2001-2011 from Portugal to Italy (n = 149, Supplementary Table S1) and preserved at the Department of Biology of the University of Pisa.

### 2.4. DNA extraction

A whole scat was used in each DNA extraction for Elba population using the QIAmp DNA Stool Mini-kit (Qiagen, Germany) and following the manufacturer's instructions (100 µl of final elution: Guerrini and Barbanera, 2009). All extractions from feces were carried out in a laboratory free of partridge DNA in the zoological building of the Zoology-Anthropology Unit (University of Pisa) and two blanks (no scat) were included in each session. DNA extractions from other *A. rufa* samples (blood, liver, muscle or feathers: Supplementary Table S1) were performed using the Puregene Core Kit-A (Qiagen) following the manufacturer's instructions as in Barbanera et al. (2010).

### 2.5. Mitochondrial DNA amplification and sequencing

A 503 bp-long fragment of the Cytochrome-*b* (Cyt-*b*) gene of the mitochondrial DNA (mtDNA) was initially amplified from Elba samples using primers CytL and SEMC575 and the PCR thermal profile adopted in Guerrini and Barbanera (2009). All PCR products were purified using the Genelute PCR Clean-up Kit (Sigma Aldrich) and sequenced in both directions on an ABI 3730 DNA automated sequencer at Genechro (Rome, Italy). When the mtDNA lineage corresponding to *A. rufa* was disclosed by blasting it against GenBank references, a longer portion of the Cyt-*b* (1,092 bp) and the entire Control Region (CR, c. 1,156 bp) of the mtDNA were amplified using the semi-nested protocol of Guerrini and Barbanera (2009). Instead, when the mtDNA did not correspond to *A. rufa* (e.g., *A. chukar* or *P. colchicus*, see Results), the sample was discarded from downstream analyses. Furthermore, we amplified and sequenced the same loci in 149 red-legged partridges sampled across the entire species range and following Barbanera et al. (2005) (see also Supplementary Table S2).

### 2.6. Genetic analyses

Forty-four combined (Cyt-*b* + CR) *A. rufa* sequences obtained from

Elba samples (see below) were aligned with those obtained from 149 conspecifics of Portugal (n = 12), Spain (n = 43), France (n = 20), Corsica (n = 48) and Italy (n = 26) using CLUSTALX (v. 2.1, Thompson et al., 1997). Downstream analyses were carried out by pooling together samples from Portugal and Spain into the Iberian Peninsula group. Indeed, the Pyrenees clearly mark out the separation between *A. r. rufa* and both *A. r. hispanica* and *A. r. intercedens*, the two westernmost subspecies (Madge and McGowan, 2002), whose geographic divide is far from being consistent across the molecular studies insofar carried out (Barbanera et al., 2011; Ferrero et al., 2011; Rodríguez-García and Galián, 2014). Conversely, we kept France, Italy and Corsica separated to determine the alleged genetic affinity of Elba population within the *A. r. rufa* range.

Summary statistics of genetic diversity (number of haplotypes, haplotype diversity or *h*, mean number of pairwise differences or *k*, and nucleotide diversity or  $\pi$ ) were calculated using DNASP (v. 6: Rozas et al., 2017) and ARLEQUIN (v. 3.5.2.2: Excoffier and Lischer, 2010). We built a haplotype network using the Median Joining method (Bandelt et al., 1999) as implemented in NETWORK v. 5.0.1.1 (Fluxus Technology). We investigated the partition of the mtDNA diversity (Analysis of the Molecular Variance, AMOVA) among and within groups (Elba Island, mainland Italy, mainland France, Corsica and Iberian Peninsula) using the  $\Phi_{ST}$  pairwise distance analogous to Wright's (1951) *F*-statistics (1,000 permutations) with ARLEQUIN. These values were plotted on the first two axes of a Principal Components Analysis (PCA) using STATISTICA v. 5.0/W (Statsoft Inc., USA).

Bayesian Analysis of Population Structure (BAPS, v. 6.0: Corander and Marttinen, 2006; Cheng et al., 2013) was used to cluster genetically similar individuals into panmictic groups using the module for linked molecular data and applying the codon linkage model, which is appropriate for sequencing data. The analysis was run using the entire *A. rufa* (Cyt-*b* + CR) mtDNA sequence set (n = 193) without prior information on geographic location with an upper bound of *K* = 10 and five replicates for each run. When the individuals were organized into clusters, an admixture analysis was carried out to infer the ancestral source of each individual [1,000 simulations, 200 reference individuals from each group (i.e., geographical region), and 50 iterations used to estimate the admixture coefficient for the reference individuals]. The admixture coefficient values for the assignment to each cluster were computed for any investigated group and for western and eastern Elba sub-populations.

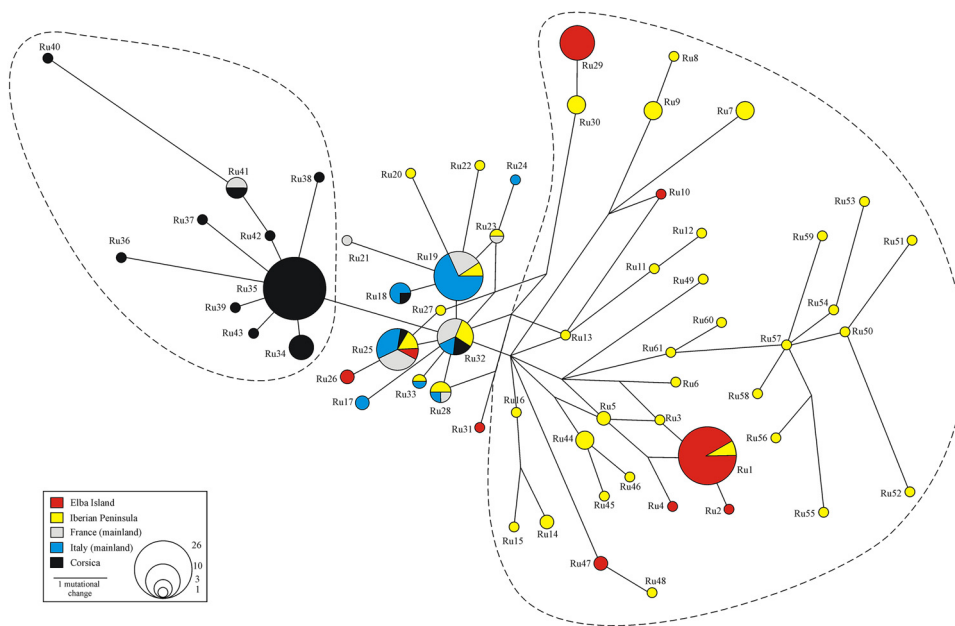
## 3. Results

During 2004-2006 and 2018-2019, we spotted red-legged partridges in both western and eastern Elba, while scattered individuals were observed very rarely outside the National Park (e.g., in Monte Bello, F. Barbanera, pers. obs., 12 January and 9 February 2019) and no records were found for both Monte Fonza and Monte Calamita areas (Fig. 1B, Supplementary Table S1). Among the 103 samples belonging to the *Alectoris* genus, the 42.7% and 57.3% held *A. rufa* (nine haplotypes, Table 1, Fig. 2) and *A. chukar* (single haplotype: 100% identity with GenBank sequence AM850755 from Barbanera et al., 2009) mtDNA

**Table 1**

Estimates of genetic diversity computed for each *A. rufa* group. Legend: n, sample size; N, number of haplotypes; S, number of polymorphic sites; *h*, haplotype diversity; *k*, mean number of pairwise differences;  $\pi$ , nucleotide diversity (%); SD, standard deviation.

	n	N	S	<i>h</i> ± SD	<i>k</i> ± SD	$\pi$ ± SD
Elba Island	44	9	21	0.633 ± 0.069	5.388 ± 2.647	0.239 ± 0.131
Elba Island (Western)	35	7	16	0.484 ± 0.099	3.559 ± 1.853	0.158 ± 0.092
Elba Island (Eastern)	9	3	12	0.556 ± 0.165	4.944 ± 2.656	0.219 ± 0.134
Italy (mainland)	26	7	8	0.717 ± 0.079	1.391 ± 0.883	0.062 ± 0.044
France (mainland)	20	8	11	0.874 ± 0.041	2.184 ± 1.435	0.097 ± 0.071
Corsica	48	13	19	0.604 ± 0.081	1.476 ± 0.966	0.066 ± 0.048
Iberian Peninsula	55	40	50	0.987 ± 0.006	6.424 ± 3.249	0.286 ± 0.160



**Fig. 2.** MtDNA network computed using all *A. rufa* haplotypes (Ru). A scale to infer the number of haplotypes for each pie is provided together with a length bar to compute the number of mutational changes. The color of each geographic area and the number of each haplotype are indicated. See Supplementary Table S1 for more details. For the sake of clarity, only the two most overt haplogroups are indicated, while the third is intuitable as a complement of the first two.

lineage, respectively. The *A. chukar* introgressed samples from the western side of the island (50.7%,  $n = 36$ ) were disclosed around the massif of Monte Capanne (Fig. 1B:  $n$ : 7-10, 12, 15, 16, 19 and 20), while the entirety of scats collected around Monte Calamita and Monte Fonza held either *A. chukar* or *P. colchicus* (single haplotype: 100% identity with GenBank sequence KF833639 from Harris et al., 2014) mtDNA lineage, respectively. Overall, eastern Elba hosted the highest regional frequency (71.9%,  $n = 23$ ) of *A. chukar* introgressed samples when compared to its western counterpart (50.7%,  $n = 36$ ) (Supplementary Table S1).

The alignment (2,249 characters, with indels) included 193 (Cyt-*b* + CR) joint mtDNA sequences (Elba, 44; entire *A. rufa* range, 149). We found 61 *A. rufa* haplotypes (Ru1-Ru61, Supplementary Table S1; GenBank accession codes, Supplementary Table S2), with the lowest values of mean number of pairwise differences and nucleotide diversity found in mainland Italy and France as well as Corsica (Table 1). Nine haplotypes were disclosed in Elba representatives (western side:  $n = 7$ ; eastern side:  $n = 3$ ; Table 1): haplotype Ru1 ( $n = 25$ , 71.4%) and Ru29 ( $n = 6$ , 66.7%) were the most frequent in the western and eastern part of the island, respectively, with only one (Ru29) being shared between the two sides (Supplementary Table S1). Although private to western Elba, haplotype Ru1 was also the most frequent (56.8%) across the whole island, and it was shared only by an individual from Spain within the entire *A. rufa* distribution range (Fig. 2; Supplementary Table S1). While the genetic diversity was higher in eastern ( $h = 0.556$ ,  $k = 4.994$ ,  $\pi = 0.219\%$ ) than in western ( $h = 0.484$ ,  $k = 3.559$ ,  $\pi = 0.158\%$ ) Elba, there was no substantial difference over time in the whole island, with five (from 20 samples) and six (from 24 samples) haplotypes disclosed in 2004-2006 and 2018-2019, respectively.

The Median Joining network (Fig. 2) pointed out to the occurrence of three major haplogroups. The first included almost the whole sample from Corsica; the second comprised all the individuals from mainland Italy and France, some from the Iberian Peninsula and eight from Elba Island and Corsica ( $n = 4$ , each); the third consisted of almost all partridges from the Iberian Peninsula and Elba Island ( $n = 40$ ) (Supplementary Table S1). Overall, three haplotypes (Ru19, Ru25 and Ru32; Fig. 2) were shared by all groups, but only one (Ru25) was found also on Elba (Supplementary Table S1). On the contrary, all of Elba haplotypes included in the group hosting the very large majority of Iberian representatives turned out to be private to the island (except Ru1, see above; Fig. 2).

The 61.9% of the total mtDNA variability was partitioned within the

five groups (Elba, Italy, France, Corsica and Iberian Peninsula) and 38.1% across such regions ( $AMOVA$ :  $\phi_{ST} = 0.381$ ,  $P < 0.001$ ). When the  $\phi_{ST}$  pairwise distance values were plotted on a PCA, the first two axes explained the 91.9% of the total diversity. Italian and French continental representatives clustered together, Elba partridges were very close to those from the Iberian Peninsula, and Corsica was the most diverging group (Fig. 2). The  $\phi_{ST}$  distance value computed between Elba and the Iberian Peninsula ( $\phi_{ST} = 0.165$ ,  $P < 0.001$ ) was the lowest when compared to those obtained for Elba-mainland Italy and Corsica-mainland France pairs ( $\phi_{ST} = 0.405$  and  $0.529$ , respectively: see Table 2 for all comparisons).

The Bayesian clustering method performed on the mtDNA dataset defined six genetically distinct clusters ( $P = 1$ , optimal partition, log likelihood = -1,507.4; Fig. 4, Supplementary Table S3). The red-legged partridges from Elba were included in four distinct genetic clusters (1 - 4), with three of these (1, 3 and 4) hosting also individuals from the Iberian Peninsula but not from Italy, France and Corsica. When the analysis was repeated by dividing Elba partridges into western and eastern sub-populations, we found that birds from the eastern side were mostly assigned to cluster 1 (69.6%), whereas the very large majority of those from the western one were included in cluster 3 (76.8%). Only a few individuals ( $n = 3$ , 6.8%) from Elba were fully assigned to cluster 2, the only one including representatives from all investigated *A. rufa* groups. Almost all red-legged partridges from Italy and France were assigned to cluster 2 (100% and 90%, respectively), the 91.7% of those from Corsica fell into cluster 5, and cluster 6 included only Iberian individuals (Fig. 4, Supplementary Table S3).

**Table 2**

$\phi_{ST}$  pairwise distance among all *A. rufa* groups. All probability values were highly significant ( $P < 0.001$ ) except for Italy (mainland) vs. France (mainland) ( $P = 0.07$ ).

	Elba Island	Iberian Peninsula	France (mainland)	Italy (mainland)	Corsica
Elba Island	–				
Iberian Peninsula	0.165	–			
France (mainland)	0.336	0.141	–		
Italy (mainland)	0.405	0.191	0.068	–	
Corsica	0.585	0.441	0.529	0.659	–

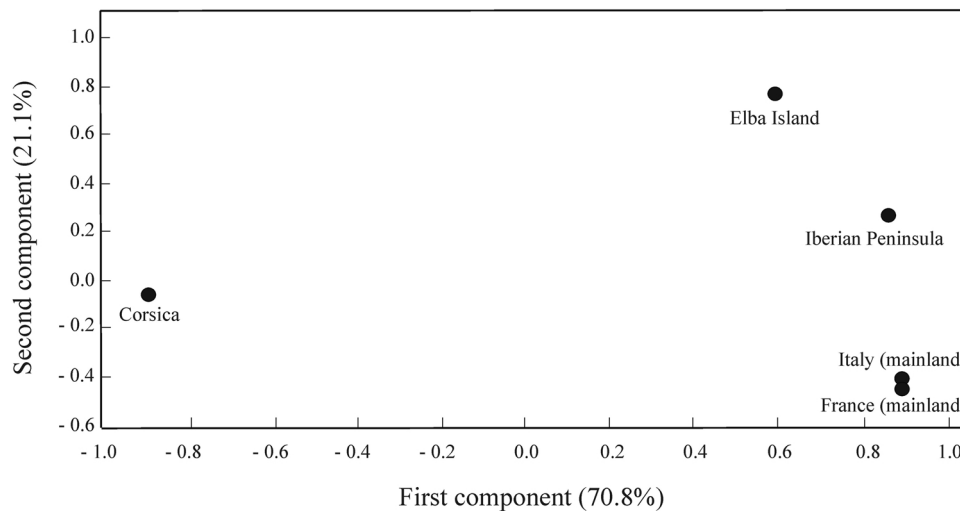


Fig. 3. PCA performed using the  $\phi_{ST}$  pairwise distances calculated for all mtDNA haplotypes of Elba Island, Italy (mainland), France (mainland), Corsica and Iberian Peninsula.

#### 4. Discussion

As a consequence of the large-scale reshuffling for hunting purposes, no more *A. r. rufa* populations free from admixture with chukar genes occur. In this study, the extensively *A. chukar*-introgressed nature of Elba partridges was confirmed, whereas their affiliation to the nominate subspecies - hence its nativeness - was definitively disproved. Nevertheless, the conservation of this population of undisputed natural-historical value is still advisable other than feasible, as it may be conceived as the ultimate repository for at least part of the otherwise extinct Italian *A. r. rufa* genome. Importantly, Elba partridges deserve interest in light of their potential adaptive introgression as potential resource to warrant their demographic rescue.

##### 4.1. Genetic make-up of Elba red-legged partridge

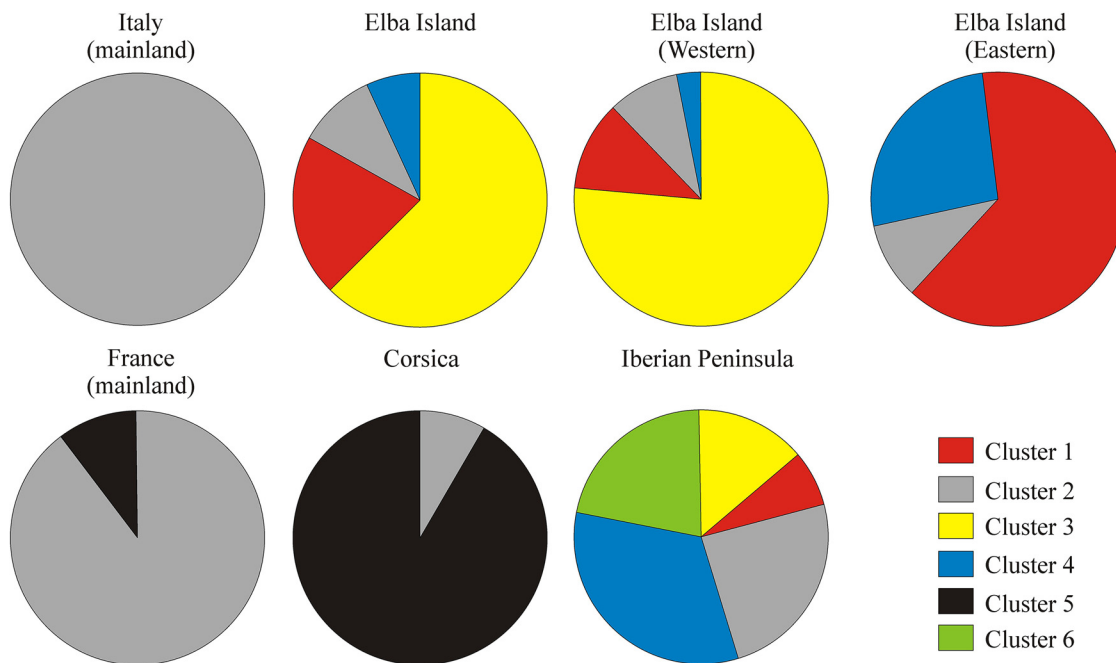
Overall, 42.7% and 57.3% of our samples were found to host *A. rufa* and *A. chukar* mtDNA lineages, respectively. Maternally introgressed birds were particularly abundant in the eastern side (69.0%,  $n = 59$ ) and represented the entirety of the samples from Monte Calamita, where recent demographic data had indicated a sharp decline. All the samples from Monte Fonza turned out to be pheasants, thus confirming the absence of partridges as reported in the last official census (Centro Ornitologico Toscano and Parco Nazionale Arcipelago Toscano, 2006). When compared with records from this survey, our data confirmed the persistence of partridges only around Monte Capanne and along the Monte Strega-Monte Val di Capanna mountain range in the western and eastern side of the island, respectively (Fig. 1B, Supplementary Table S1).

Genetic analyses carried out on *A. rufa* maternal lineage evidenced a high diversity across the island, with values similar if not higher than those from the other, much wider, geographic areas investigated in this study (Table 1). This is clear when looking at haplotypes shared with individuals from each of them (Fig. 2) as well as AMOVA results. When partridges from the western and the eastern side of the island were compared, the latter turned out to be genetically more variable in spite of their lower numbers. Likewise, this sub-population showed stronger evidences of *A. chukar* gene introgression. Overall, the comparatively high mtDNA genetic diversity of Elba partridges would not be expected in an insular landbird population, which was most likely established a few centuries ago and experienced a founder effect, unless constantly supplemented with conspecifics of non-local origin. Indeed, studies on sedentary species which were imported to other Mediterranean islands much before revealed lower values of mtDNA genetic diversity (e.g.,

Guerrini et al., 2007; Barbanera et al., 2011; Forcina et al., 2014) or even haplotype fixation (Guerrini et al., 2015).

It is known that partridges from breeding facilities located in northern (e.g., Fratelli Lanata stock-farm, Genova) and central (e.g., L'Incrociata stock-farm, Livorno) Italy were released in high numbers since the 1960s. Even though the exact origin of the stocks being used was generally unknown, it is widely acknowledged that their founders were mostly from outside Italy (G. Burchianti and P. Casanova, pers. com. to F. Barbanera, 20 January and 27 October 2004, respectively). At that time, genetic analyses were lacking and phenotypic inspection sketchy, with *A. rufa* x *A. chukar* hybrid birds remaining largely undetected. Even worse, reiterated attempts to introduce *A. chukar* were a common practice across Elba (e.g., Moltoni and Di Carlo, 1970; Di Carlo, 1976). Overall, the intensity of restocking is testified by the high-density value (16 birds/km<sup>2</sup>, on average) recorded within the ZRCs, with a maximum of 29 birds/km<sup>2</sup> at Volterraio in 1994-1996 (Fig. 1B; III; Gariboldi, 2006).

Notwithstanding the extensive *A. chukar* mtDNA introgression confirmed by our study, the overt and phylogeographically counter-intuitive genetic affinity of Elba partridge with conspecifics from the Iberian Peninsula is the new and most intriguing result. A number of evidences support this affiliation, the most striking being the exclusive sharing of the most abundant haplotype in western Elba with an individual from Spain (Fig. 2) and the comparatively lower genetic distance values between birds of these two areas (Table 2). Also, the haplotypes private to Elba were mostly embedded among those from Iberia, thus suggesting a tight kinship (Fig. 2). Concordantly, the PCA (Fig. 3) and the Bayesian clustering (Fig. 4) strongly pointed to the affinity between these two groups. Overall, these results can be interpreted on the basis of the *A. rufa* historic management that we reconstructed in detail relying on a series of documents found in the Archive of the Province of Livorno. In the mid-1950s, a protection plan adopted for *A. rufa* across Elba included the census of areas where extensive conifer reforestation had started shortly before (Intrieri, 1953). Nevertheless, only a few years later the species had almost disappeared with the exception of Monte Calamita area (Fig. 1B; Bichecchi, 1962). Here, a private game estate (Le Ripaltes) was operating since 1947. Following the yearly release of a high number of red-legged partridges, birds were impressively abundant in the property (more than 4,000 in 1965) and dispersed to relatively distant places. Noteworthy, these birds were imported from foreign countries (e.g. 80 partridges from Spain: Bichecchi, 1963; 80 partridges from a stock-farm in Genova that was known to employ Spanish founders: Bichecchi, 1965 and L. Ciuffardi, pers. com. to F. Barbanera, 7 December 2018; 100



**Fig. 4.** The genetic structure of Elba Island, Italy (mainland), France (mainland), Corsica and Iberian Peninsula red-legged partridges as inferred using BAPS admixture analyses is given by means of a pie chart with segments proportional to the estimated membership to the  $K$  clusters. The analysis on Elba Island was performed also by dividing the local population into western and eastern groups (see Supplementary Table S3 for more details).

partridges from the Pyrenees: Censi, 1967). By the end of 1960s and early 1970s, partridges of admittedly Spanish origin were released also in the western part of the island (Monte Capanne, Fig. 1B; R. Giombini, pers. com. to F. Barbanera, 14 March 2003 and 21 November 2018). Overall, this international trade involving the westernmost *A. rufa* subspecies is not surprising since importations of stocks from Spain - and particularly from Andalusia and Castilla-La Mancha (Ghigi, 1968) - to Italy were a common practice at that time (Leporati, 1970; Casanova et al., 1993; Masseti, 2003). Even in mid-February 2005, 42 partridges were exported from Andújar (Andalusia) to the Tuscan public farm of Scarlino in an attempt to create a genetically pure stock (Barbanera et al., 2010). At the present-time, *A. rufa* partridges imported from one of the most important European breeding centre located near Burgos (Spain), are sold and released across the Italian Peninsula (e.g., Tuscany and Piedmont). Likewise, a recent study unveiled the occurrence of Spanish genes in an *A. rufa* population from northern Italy (Negri et al., 2013). Therefore, the higher genetic diversity in birds from the eastern part of Elba Island (Table 1, Fig. 4) could be interpreted as the result of supplementation with multiple stocks favoring the persistence of a higher number of *A. rufa* mtDNA haplotypes. In conclusion, the intense restocking with Spanish birds explains the overt and overriding genetic contribution from the Iberian Peninsula found in Elba partridges which, however, show evidence of another one likely attributable to a less encompassing (but carried out over a longer period) restocking with allopatric conspecifics of different origin. As such, there is reason to believe that the alleged autochthony of *A. rufa* on the Elba Island was compromised well before 1968 (G. Burchianti, pers. com. to F. Barbanera, 20 January 2004).

Even though the overall genetic picture is the most likely result of recent restocking events, the genetic affinity between Iberian and Elba partridges might be also the consequence of an historical introduction. The eastern part of this island started to host Spanish military posts around the beginning of the XVII<sup>th</sup> century to eventually fall under the dominion of the Spanish crown later and till the beginning of the XVIII<sup>th</sup> century. Elba was part of the so-called “State of the Presidi”, a strategic dominion in the Mediterranean on the route between Barcelona and Naples. Importations of wildlife from the XVII<sup>th</sup>-XVIII<sup>th</sup> Italian

kingdoms to Spain and between Spanish dominions in Italy is well documented (Clavero et al., 2016) and included game birds such as the black francolin (*Francolinus francolinus*: Oriani, 2014; Forcina et al., 2015) and *P. colchicus* (Archivo General de Simancas, Estado 258:1, folio 14, provided by M. Clavero; Archivo General de Simancas, Estado 1358, folio 27, provided by A. Pacini). The likelihood of this prospect for the red-legged partridge is supported by the benevolent attitude of the Spanish crown to its subjects in the State of the Presidi and the animosity between Spain and the Grand Duchy of Tuscany, possibly limiting trade between the latter and the nearby Spanish dominions. This translated into a flourishing trade of goods, livestock and possibly game directly from Spain to its Tuscan strongholds (Capezzuoli, 1979). However, only further analyses would allow to assess whether the recent releases using partridges from Iberia have masked historical importations of the same kind other than to quantify the portion of the native gene pool still preserved (see below).

#### 4.2. Conservation perspectives

Notwithstanding the efforts made by local managers over decades to establish a healthy and stable partridge population, the 1990s censuses evidenced a negative demographic trend across Elba. Apparently, the decline continued even after the institution of the National Park and the adoption of special measures to preserve the species also outside its boundaries. Several factors were invoked as causing the negative demographic trend and their persistence discouraged from carrying out further restocking to secure a self-sustaining population (Gariboldi, 2006). In fact, such intervention would have most probably turned into worsening the introgressive hybridization of local partridges. Taking into account that alternative measures, even if readily adopted, would have produced benefits within some years and that this time lag might have witnessed a further demographic decline hampering the recovery potential, an *ex situ* program to secure the persistence of local gene pool was strongly advised (Centro Ornitologico Toscano and Parco Nazionale Arcipelago Toscano, 2006). The almost entirely uninhabited and nearby island of Pianosa was identified as the most suitable venue to host a management initiative of this type for the benefit of Elba

partridges. In fact, Pianosa is home to a hybrid and allegedly non-native *A. rufa* population of recent introduction (1985: Baratti et al., 2004; Barbanera et al., 2005) that is currently being eradicated (Baccetti and Gotti, 2016). Provided that this translocation will allow to secure a backup of Elba partridges, we recommend to focus also on *in situ* actions aimed to improve connectivity between western and eastern Elba sub-populations (15 km away from each other) with the establishment of ecological corridors. Indeed, our results point to a limited gene flow between them (one shared haplotype, Ru29), thus raising conservation concern for their preservation in the long run.

Even though both the autochthony and genomic integrity of Elba *A. rufa* population is definitively rejected, its conservation interest is not under question. Far from wanting to promote the value of an overtly jeopardized genetic resource at all costs, we call for the advisability of warranting its protection in line with tenets of conservation management for introgressed yet somehow irreplaceable populations. The concept of adaptive introgression is getting increasing attention among wildlife managers as a potential resource to warrant the evolutionary rescue of threatened species or populations by counteracting the negative demographic and genetic effects of small population size (Carlson et al., 2014; Hamilton and Miller, 2016). The enhanced fitness exhibited by introgressed genotypes may eventually lead to habitat divergence (Rieseberg et al., 1999; Gompert et al., 2006), which is a desirable trait in an epoch of increasing environmental stochasticity (e.g., Kremer et al., 2012). Noteworthy, the potential evolutionary interest of hybrid *Alectoris* populations in terms of adaptive potential had been already evidenced by previous studies in insular as well as mainland environments. Specifically, the *A. rufa* x *A. chukar* hybrid swarm once thriving on Pianosa Island was thought to benefit from some selective advantage warranting adaptation to the xeric habitat of the island (Baratti et al., 2004), unusual for *A. rufa*. Even more surprisingly, Negri et al. (2013) reported an *A. rufa* population consisting of mostly *A. chukar* introgressed-individuals to colonize riverine areas - notoriously unsuitable for this species - in north-western Italy over just a few years. Although the last official census of Elba partridges pointed to a concerning population decline (Centro Ornitologico Toscano and Parco Nazionale Arcipelago Toscano, 2006), it dates back to more than a decade ago, and personal observations by one of the authors (F.B., 2017-2019 on a monthly basis) suggest that a demographic recovery might be ongoing. Well aware of the possible downsides of genetic admixture (Rhymer and Simberloff, 1996; Muhlfeld et al., 2014) and far from promoting it intentionally for the mere purpose of testing the adaptive potential of the hybrids, in light of the above considerations we think that Elba red-legged partridges well deserve the present and future conservation efforts. Indeed, this could provide managers with useful information about how this overtly non-native and hybrid game bird population will cope with ongoing environmental change in an insular - hence easily monitorable (Wardle, 2002) - context over time. Elba has been experiencing deep changes in terms of land use and vegetation cover, with digital aerial photographs taken over the second half of the last century showing a progressive and marked shift from intensively cultivated to widely naturally vegetated island (Carta et al., 2018a). Decreased habitat patchiness following rural abandonment in the context of the broader shift from an agriculture-based towards a tourism-based economy occurred in Elba as well as in the whole Tuscan Archipelago (Arrigoni et al., 2003) and was likely detrimental to the red-legged partridge. Indeed, abandonment of traditional agricultural practices was pointed out as one of the main causes for the local extinction of this species in nearby rural areas of the Italian mainland (Spanò, 1985) and has long been targeted among the main drivers of *A. rufa* decline across its entire range (Aebischer and Potts, 1994). The increased cover of Mediterranean maquis and woodlands at the expense of garrigue and agriculture patches on Elba has likely hampered partridge dispersal and gene flow among the four nuclei (ZRCs) hosting the species. This would explain the early vanishing of Monte FONZA (Fig. 1B: 21) sub-population, surrounded by the sea on three sides and unable to

connect with the others due to the increase of vegetation cover on the fourth. Indeed, reforestation (since 1950s) in the central part of the island made the habitat of this area unsuitable for the red-legged partridge creating a barrier between the western and eastern sub-populations. Moreover, unlike other Mediterranean islands affected by a similar rural abandonment, Elba did not experience any decrease of human presence (Sedlar et al., 2017), rather it saw a massive increase along its coasts and the main urban centres (Carta et al., 2018a). If connectivity among partridge sub-populations were restored, the adaptive potential of the Elba introgressed partridges might be properly evaluated, although long-term studies suggest that adaptation and evolutionary rescue may indeed take some generations to trigger demographic recovery (Hwang et al., 2011). Moreover, since both *A. chukar* and Iberian *A. rufa* genetic material inferred with mtDNA in local partridges is a quite recent event, it is likely that vast and well-preserved portions of the original *A. rufa* nuclear genome still reside in these birds. Indeed, while the mtDNA is much faster to be replaced due to its uniparental inheritance and lack of recombination, the nuclear genome would need way more generations to lose its identity. A long-term monitoring in the field is highly recommended along with the ongoing genome-wide investigation of adaptive other than neutral diversity to get a reliable picture of evolutionary resilience and persistence capacity of this historically valuable insular game bird population.

## Acknowledgments

Authors wish to thank Parco Nazionale Arcipelago Toscano (Delibera Commissariale n. 307, 31 Dicembre 2003) and Dr.ssa Francesca Giannini for the qualified support to the fieldwork carried out between 2004 and 2006. Authors are deeply grateful to Isp. Sup. SC. Renato Giombini (former Corpo Forestale dello Stato, Marciana Marina), Ass. Scelto Mauro Arrighi and Ass. Scelto Giancarlo Burchianti (Polizia Provinciale, Portoferraio), Prof. Paolo Casanova (previously at Università di Firenze), Dr.ssa Paola Meneganti (Archivio della Provincia di Livorno), Dr.ssa Elena Amadori (Cooperativa Microstoria, Livorno), Brig. Ca. Federico Parri (Reparto Carabinieri, Parco Nazionale Arcipelago Toscano, Portoferraio), Ass. Ca. Luca Balducci (Carabinieri Forestali, Livorno) and Dr. Luca Ciuffardi (Ambito Territoriale di Caccia GE2 Levante, Genova) for their valuable support to collect information about *Alectoris rufa* on Elba Island. Authors are also deeply thankful to R. Giombini, F. Parri and their colleagues at former Corpo Forestale dello Stato in Marciana Marina, M. Arrighi, F. Giannini and G. Grasseschi (Ecological Research and Services for the Environment, Viareggio) for their help in the sampling (2004-2006). We thank Dr. Daniele Scarselli (Agrofauna, Livorno) for *A. rufa* samples collected in the countryside of Pisa. For *A. rufa* sampling outside Italy, authors are deeply indebted with all colleagues acknowledged in Barbanera et al. (2015). Authors are also thankful to Dr. Miguel Clavero (Estación Biológica de Doñana, EBD-CSIC, Sevilla) and Prof. Arturo Pacini (Dipartimento di Civiltà e Forme del Sapere, Università di Pisa) for sharing information and material on historical wildlife trade between Spain and Italy. Finally, authors are grateful to Dr. Fabrizio Erra and Francesco Paolo Frontini (Università di Pisa) for preparing line drawings and laboratory work, respectively. This work was supported by Università di Pisa with "Progetto di Ricerca di Ateneo" grant #PRA\_2018\_15.

## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.zool.2019.125740>.

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