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Genetic diversity and conservation status of the Red-legged Partridge *Alectoris rufa* in NW Italy (Genoa Province, Liguria)

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Habitat modifications, increase of alien species and translocation in the wild of farmed animals can generate negative effects in terms of genetic pollution and extinction of natural populations due to hybridization (Rhymer & Simberloff 1996). The gene mixture and the introgression of alien alleles can destroy local adaptations, lead to a decline in the fitness of individuals and ultimately affect the size and distribution of natural populations (Templeton 1986). The risks of genetic pollution are affecting both common and threatened species: the anthropogenic hybridization has been documented in several bird species including Galliformes, such as those belonging to the genus *Alectoris* (Rhymer & Simberloff 1996).

The partridges of the genus *Alectoris* include seven potentially interfertile species distributed in Eurasia, northern Africa and southern Arabia (Spanò 1975, Johnsgard 1988); their distribution is largely allopatric, with the exception of two partially sympatric species in Arabia and central China (Barbanera 2014). Natural hybridization in the overlap areas of the different species has been described, for instance, in the Maritime Alps and in the Southern French Alps (Spanò 1978, Bernard-Laurent 1984), in the Rhodope Mountains in Bulgaria and Greece (Dragoev 1974).

The Red-legged Partridge *Alectoris rufa* extends its distribution range from the Iberian Peninsula through France to central-northern Apennine, including the islands of the Tuscan Archipelago, Corsica and the Balearics. On the basis of the phenotypic characters three subspecies of Red-legged Partridge are known: *A. rufa rufa* in Italy and central-southern France including Corsica; *A. rufa intercedens* in south-eastern Spain including the Balearic Islands; *A. rufa hispanica* in Portugal and north-western Spain (Madge & McGowan 2002, Spanò 1986, 2010, Barbanera *et al*. 2011). *Alectoris chukar*, on the other hand, originates from a large area between the Aegean Sea and eastern Asia, where it is present with 16 subspecies (Madge & McGowan 2002, Barbanera *et al*. 2009).

The Red-legged Partridge is the object of intense hunting and restocking activities along its entire distribution area in the Iberian Peninsula, France and Italy (Aebischer & Potts 1994, Ciuffardi & Spanò 2013). Restocking became a common practice especially after the Second World War (Goodwin 1986): in captivity *Alectoris chukar* is the most prolific breeder within the genus *Alectoris*, and the crossbreeding with Red-legged partridge is therefore productive (Barbanera *et al.* 2010). As a result of these releases, the genetic integrity of *Alectoris rufa* is seriously threatened by extensive introgression, which is already underway (Potts 1989).

In recent years, in order to verify the conservation status of the Red-legged partridge molecular investigation techniques have increased and spread: in fact, in spite of the general morphological similarity, the *Alectoris* partridges are genetically well differentiated and can be clearly identified (Randi *et al*. 1998).

In Italy, Baratti *et al*. (2004) and Barbanera *et al*. (2005) highlighted, through the use of genetic techniques, the presence of hybrids between *Alectoris rufa* and *A. chukar* in the population of Pianosa Island within the Tuscan Archipelago National Park. Based on these results Barbanera *et al*. (2005) concluded that the management of species subject to hybridization, such as the Red-legged Partridge, requires the identification and elimination of hybrids not only on a phenotypic but also genetic basis, in

order to favor the expansion of genetically pure animals in agreement with Frankham *et al*. (2002).

In the following year, the French research group of Vallance *et al*. (2006) developed a genetic test based on a set of 10 SNP markers, in order to evaluate the level of hybridization between Red-legged Partridge and Chukar: the subsequent application of the analysis to Red-legged Partridge groups from French farms ascertained that 50% of the breeders were hybrids.

Barilani *et al*. (2007) affirmed that from a genetic point of view the introgression with Chukar has now spread along the entire distributional range of the Red-legged Partridge, even arriving in the area of spontaneous hybridization between Red-legged and Rock partridge in the southern French Alps. If in unmodified conditions the natural hybridization would be limited to three small contact areas, today instead the anthropogenic spread of the hybrids seems to be much more extensive.

Also Barbanera *et al*. (2009) ascertained a rather compromising situation: the natural population on the Island of Elba, for example, showed a high percentage of hybrids between *A. rufa* and *A. chukar*. The authors, also in this case, concluded that the Italian population of Red-legged partridge can be considered wholly polluted by introgression with the Chukar.

From the comparison between specimens preserved in museum collections (dating back to 1856-1934) and today's individuals, Barbanera *et al*. (2010) found that all ancient animals had a Cyt-b mitochondrial line fully attributable to *Alectoris rufa*; compared to the entire distributional range of the Red-legged Partridge from the Iberian Peninsula to the north-west of the Italian Peninsula, currently in Italy there would be the highest percentage of specimens with a Chukar maternal line, compared to those found in France and in Iberian Peninsula.

Finally, Barbanera *et al*. (2011) also found the presence of hybrids between *A. rufa* and *A. chukar* on the island of Corsica, albeit with a lower frequency than in Italy. The authors concluded by observing how the frequent repopulation plans with farmed animals along the entire distributional range of the Red-legged Partridge probably contributed to the reduction of the genetic diversity originally present among the different subspecies of *A. rufa*.

Aware of the situation described above, the Hunting Territorial Zone (the Italian acronym is A.T.C.) Genoa 2 East activated a genetic study aimed at ascertaining the genetic diversity of *Alectoris rufa* and at evaluating the level of introgression with the Chukar in the area of its competence. During the hunting season of 2017, hunters took a foot from shot down Red-legged Partridges, delivering it promptly to the A.T.C. Genoa 2 office; here each foot was placed in a tube containing pure ethyl alcohol, marked with a unique identification code and then placed in a freezer waiting to be transferred to the laboratory. The use of the feet allows the extraction of DNA from the toe-pads preserved in 96% ethanol (see Barbanera *et al*. 2010, 2015).

Biological samples were also collected from live partridges originating from two farms (an amateur from the Province of Genoa and a professional one called "Azienda Agricola Venelia" located in the Province of Massa-Carrara): in this case some growing feathers with trophic quill were taken from each animal. Each of the six breeders was equipped with a colored ring to the leg for individual identification. At the end of the sample each feather was placed in ethyl alcohol, coded in association with the relative ring and then placed in a freezer. Samples were sent to Antagene Laboratory (France) which carried out the genetic analyses through the technique applied by Vallance *et al*. (2006); unlike the original test, which started from a set of 10 nuclear DNA markers, Antagene studied the samples sent by A.T.C. Genoa 2 through the use of following 19 SNP markers: 01-AGC1; 02-M01-Aru1I68; 03-M02-Aru1F32; 04-M03-Aru1V16; 05-M09-ACLY; 06-M10-AK; 07-M11-ENO; 08-M12-FGB; 09-M13-HNFA1; 10-M16-Rho; 11-N01-VIM; 12-N02-RPL7A; 13-N03-CKB; 14-N04-CDC2L1; 15-N05-CRYB; 16-N10-RAG1; 17-PTH; 18-TCF1; 19-THSB1. The Laboratory used also a mitochondrial DNA marker (D–loop).

SNP markers are polymorphic, or show variations in the genetic material, related to a single nucleotide. From the moment of the evolutionary separation between the lines that led to *Alectoris rufa* and *Alectoris chukar*, antecedent to the glaciations of the Quaternary, the various SNP markers have differentiated between the two taxa, thus making possible to distinguish unequivocally the chromosomes of the two species (Vallance *et al*. 2006).

Overall 50 Red-legged Partridge samples were sent to the laboratory: n. 39 feet from specimens shot down during the hunting season 2017 and n. 11 tubes with growing feathers from bred individuals. For each specimen, the genetic analysis produced a percentage rate of hybridization indicating the introgression level with *Alectoris chukar*.

From the specimens shot during the hunting season of 2017, represented by wild animals or individuals released as a result of restocking, there was an average hybridization rate (± standard deviation) equal to 3.73 ± 3.99%, with a range between 0.00% (pure *A. rufa*) and 17.14% of a single specimen (Tab. 1). Among farmed animals, the breeders from the Genoese farm had an average hybridization equal to 4.78 ± 3.43%, with an interval between 0.00 and 8.11% (Tab. 1); the partridges bred in the Province of Massa-Carrara, on the other hand, showed an average hybridization rate of 2.05 ± 1.15%, with a range of 0.00 and 2.56% (Tab. 1).

Table 1. Descriptive statistics on the hybridization rate of the studied Red-legged partridges, calculated on the basis of all the markers (SNP and D-loop).

Percentage rate of hybridization	Shot down during the hunting season 2017	Coming from the farm located in the Province of Genoa	Coming from the farm located in the Province of Massa-Carrara
Sample size	39	6	5
Average ± SD	3.73 ± 3.99	4.78 ± 3.43	2.05 ± 1.14
Median	2.70	5.41	2.56
Minimum value	0.00	0.00	0.00
Maximum value	17.14	8.11	2.56

Comparison of mitochondrial DNA between partridges shot down during the hunting activity showed a percentage of hybrids equal to 15% of the total. The comparison conducted with all the markers, however, ascertained a percentage of hybrids equal to 64% compared to 36% of pure specimens (with a hybridization rate of 0.00%). An overall comparison between the different hybridization entities (obtained through an equanimous and arbitrary subdivision in six different classes ranging from 0.00% to 17.99% of hybridization) observed that 59% of the killed specimens fell into the category with no or minimal introgression (hybridization class between 0.00% and 2.99%); 20% of the Red-legged partridges fell in a degree of slight hybridization (hybridization class between 3.00% and 5.99%) and 10% in a medium compromising class (hybridization class between 6.00% and 8.99%); the remaining 11% was on the level of medium-high or very severe introgression (classes between 9.00% and 17.99%; Fig. 1).

The conservation status recorded through genetic analysis in the A.T.C. Genoa 2 appears partially satisfactory, with less than 80% of the shot down animals included within a maximum individual rate of 5.99% of introgression with Chukar (Fig. 1).

When compared to the international situation, the result obtained in the study area appears to be in agreement with previous research. In particular Barbanera *et al*. (2010, 2011) suggested that in Italy there would be the highest percentage of introgression with Chukar compared to what is recorded in France and in the Iberian Peninsula. The average individual hybridization rate equal to 3.73% recorded in the study area, appears higher than that recently ascertained in Upper Corsica, where an average hybridization value of 1.14% was observed with a maximum peak

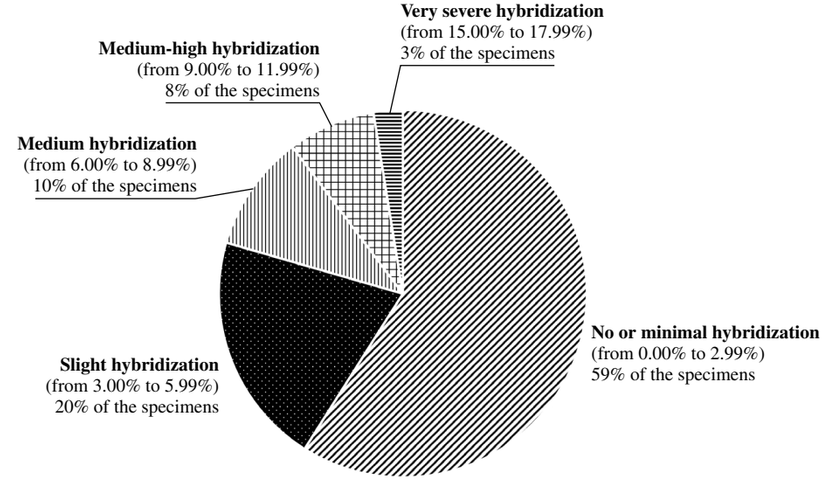

Figure 1. Comparison between the different intervals of the hybridization level found in the shot down specimens.

of 2.29% in the Niolu area (Fédération Départementale des Chasseurs de la Haute-Corse 2017). Still in Corsica, Barbanera *et al*. (2010), however, reported introgression rates of up to 67%, in line with the overall level of hybridization detected in the A.T.C. Genoa 2 (equal to 64%).

In continental Europe the situation seems slightly better: Vallance *et al*. (2006) showed for the south of France phenomena of hybridization with Chukar in 30% of the Red-legged Partridge populations investigated; in the Iberian Peninsula, 45% of the wild populations of *Alectoris rufa* as affected by genetic introgression (Blanco-Aguiar *et al*. 2008). In northern Italy, in the perifluvial areas of the Province of Alessandria (Negri *et al*. 2013), introgression frequencies were observed ranging from 17.60 to 32.20% for mitochondrial DNA, compared to what registered (equal to 15%) in the present study.

Noteworthy is the average value of the hybridization rate (equal to 2.05% with a maximum peak of 2.56%) that emerged about the farm "Azienda Agricola Venelia" of the Province of Massa-Carrara: this result is largely within the parameters that in France satisfy the ARC Criteria (*Alectoris rufa* Certifié: average rate of individual hybridization of less than 3% and absence of partridges with a percentage higher than 10%), imposed on "virtuous" farmers of Red-legged Partridge.

The situation affecting the Genoese farm is different: in this case the average value of hybridization and the maximum peak are higher (respectively equal to 4.78 and 8.11%) and require the immediate replacement of four reproducers out of six.

In conclusion, the genetic conservation status of *Alectoris rufa* monitored in the study area (A.T.C. Genoa 2) seems to be in agreement with the recent research, with an Italian situation tending to be worse than that observed in the other zone of the originating area. In the Italian context, the level of introgression of the population living in the A.T.C. Genoa 2 seems to be similar with the overall situation, with hybridization rates probably a bit more positive than the general trend observed in other Italian areas.

Starting from this assessment, it appears necessary to proceed towards a further reduction of the individual levels of hybridization both of the restocking specimens and consequently of the wild population, so as to implement the fitness of the partridges living on the territory and thus ensure an increase in the ability to adapt to the natural environment and reproductive success (see also Potts 1989).

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