Biometric characterization of the Red-legged Partridges *Alectoris rufa* of northwestern Italy

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Abstract - The biometrics of the Red-legged Partridge *Alectoris rufa* has been relatively well studied in the western part of its distribution range (Iberian Peninsula and France), especially due to the available large hunting bag samples. Conversely, the Italian population is poorly characterized. We analysed a sample of 254 live birds trapped and ringed in the northern Apennines (province of Alessandria, northwestern Italy), by measuring body mass and the length of wing, eighth primary, tail, tarsus and bill. We found significant differences in some biometric measurements between sex and age classes. A subsample of birds (n = 112) was genetically tested for introgressive hybridization with *A. chukar,* an introduced species, showing that 16.1 % of free-living partridge are hybrid individuals. When we analysed separately "pure" and "introgressed" birds we found only small biometric differences in body mass of adult males. Finally, our results were compared with the biometry of other populations from the whole natural range of the species (France, Spain and Portugal) showing a negative biometric trend of wing length from westernmost to southeastern birds of the range. Introgressed birds found in the studied population were not easily distinguishable with biometric criteria from pure *A. rufa rufa,* so genetic analysis is highly recommended when planning reintroductions or restocking.

Keywords: *Alectoris rufa*, biometry, *Alectoris chukar,* hybridization, Red-legged Partridge

INTRODUCTION

The Red-legged Partridge *Alectoris rufa* is a ground-dwelling species occurring in Mediterranean habitats, whose distribution is restricted to the Iberian Peninsula, southern and central France, Corsica, and northwestern Italy. According to del Hoyo and Collar (2014), the subspecies occurring in Italy and France is *A. rufa rufa* (Linnaeus 1758), while *A. r. hispanica* (Seoane 1894) is distributed in the northern and western Iberian Peninsula, and *A. r. intercedens* (A.E. Brehm 1857) is present in southern and eastern Spain as well as in the Balearic Islands. The species was also introduced in southeastern England around 1770 (Witherby 1924).

The Italian *A. rufa* population occupies the easternmost part of the species range, overlapping with the Rock Partridge *Alectoris graeca saxatilis* in the Maritimes Alps (France-Italy border), where cases of natural hybridisation have been described (Martorelli 1913, Bernard-Laurent 1984). Hybrids of *A. rufa* with *A. chukar* are only known in captivity or in introduced populations since the natural geographical ranges of the two species do not overlap (del Hoyo & Collar 2014).

The Red-legged Partridge is an important game-bird species in the western Palearctic, and it is classified as SPEC 2 (Species of European Conservation Concern), having a marked population decline and a population range limited to Europe (Staneva & Burfield 2017). Additionally, the species is classified as Near Threatened (NT) by the IUCN Red list, due to the significant population decrease observed in the last years (BirdLife international 2021). The reduction of *A. rufa* populations was compensated by increasing the release of bred individuals for hunting purposes. Although the origin of some stocks can be local, it has always been common practice to breed, relocate, and release partridges without taking into account their geographical origin and genetic differences (Martínez-Fresno et al. 2008). Many captive breeding stocks are of unknown origin and the use and occurrence of captive hybrids with *A. chukar,* has been documented throughout the species range (Barilani et al. 2007, Martínez-Fresno 2008, Barbanera et al. 2009, Negri et al. 2013), even if a recent study suggests that the genetic integrity of *A. rufa* seems to have been maintained notwithstanding restocking activities (Forcina et al. 2021). Intensive management of the species has been documented also in the province of Alessandria, (Piedmont, northwestern Italy) since the beginning of the past century (Arrigoni degli Oddi 1929) but the proportion and origin of the individuals used for restocking is unknown.

Considering the limited information so far available on the Italian population, with the present study, we aim at providing new information on the biometric features of *A. rufa* from a poorly studied area, namely northwestern Italy, where it showed signs of recent spreading (Tizzani et al. 2013) and where genetic studies have confirmed the absence of hybrids in several subpopulations (Negri et al. 2013).

Specifically, the measurements were used to achieve the following objectives: a) improving and expanding the limited Italian biometric available data, b) highlighting a scaling effect of age and sex on size, c) comparing the biometry of the Italian population with the information available for other European countries (including different subspecies), and d) testing for biometric differences between genetically pure and hybrid individuals.

MATERIALS AND METHODS

From 2009 to 2011, 254 full-grown partridges were captured during the period December - February, with a modified pheasant box trap in seven protected areas (the so-called "Zone di Ripopolamento e Cattura" - ZRC), in the Alessandria province (northwestern Italy). The study area includes the municipalities of Serravalle Scrivia, Cassano, Tortona, Casasco, Novi Ligure, Cerreto Grue, Stazzano (Fig. 1) which are characterized by the presence of rivers basins, gravel beds, dry grassland, hills with badlands, and vineyards, preferred habitat of *Alectoris rufa* (Spagnesi & Serra 2004) hosting relevant bird communities (e.g., Silvano et al. 1988, Silvano & Boano 2012, Treggiari et al. 2013).

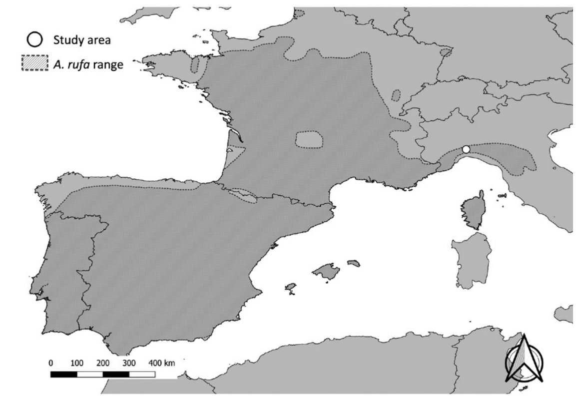

Figure 1. Red legged Partridge distribution range and location of the study area (redrawn from Arroyo et al. 2020).

All captured birds were ringed (with a unique metal ring) according to the Italian ringing centre rules and measured following Svensson (1992) methodology including seven morphometric variables: wing length (measured as maximum chord; ± 0.5 mm), eighth primary feather length numbered descendantly (± 0.1 mm), tail length (± 0.5 mm), tarsus length (± 0.1 mm), bill length from the tip to the skull (± 0.1 mm), head length from the nape to bill tip (± 0.5 mm) and body mass measured with a Pesola® (precision scale ± 1 g). All measurements were taken by the same field operator (F. Silvano) in order to reduce as much as possible bias linked to subjective variability. Birds were classified as young (EURING code 3 = hatched in the current calendar year or code 5 = hatched in the previous calendar year), or adults (all the others), on the basis of retained juvenile feathers, juveniles do not moult the two outer primaries, P9 and P10, until the second moult cycle, starting in late June of the second calendar year (Cramp 1980). The presence of knob-like spurs on the tarsus was used as a criterion of sex differentiation (Witherby 1924): birds with pointed or rounded spurs on both legs were considered as having spurs and classified as males while, birds lacking spurs or with spurs on one leg or flattened spurs on both legs were considered spurless and classified as females (Pépin 1985). For each bird we plugged two-three axillaries or undertail feathers or we collected blood samples (~40 µl) by puncturing the brachial vein. Feather samples were stored in test tubes containing 95% ethanol and blood samples in Longmire buffer (Longmire et al. 1997), both were preserved at −20 °C until being analysed. Each bird was safely released at the capture site within 15 minutes. Approval of the capture and manipulations of animals was provided by the Department of Environment, Province of Alessandria, Auth. n° 2009/003/1830,

The presence of introgressive hybridization with *A. chukar* was evaluated in a subsample of 112 individuals by mtDNA control region sequences and microsatellite loci as reported in a previous study (Negri et al. 2013). Briefly, hybrids were identified by the mtDNA haplotype shown (maternal inheritance) and by Bayesian admixture analyses on multi-locus genotypes at eight microsatellites (biparental inheritance). The microsatellites panels could allow the 100% correct identification of simulated first and second generation (F1 and F2) hybrid partridges but could also underestimate the first backcrosses (Barilani et al. 2007). In order to improve the accuracy of hybrids identification Negri et al. (2013) employed a strict threshold value of the individual proportion of membership (qi = 95 %; 90% confidence interval (CI)): hybrids were detected when an individual proportion of membership was partitioned and lower than 95% to each genetic group.

We conducted statistical analyses on three datasets: the first including all the individuals, the second including the pure *A. rufa* individuals only and the last one including the individuals showing introgression with *A. chukar* only.

Kruskal-Wallis tests were used to assess differences between groups (i.e., sex, age and place of capture). Data on recaptured birds were not included in the statistical analysis, to avoid lack of independence between observations. We compared our data with the biometric measurements of *A. rufa* from other European wild populations sampled in autumn (game season) from southern France (Pépin 1985), northern (Chalaux 2005) and southern Spain (Rubiales 1983), Portugal (Magalhães et al. 2001) and few birds (sampled in February) from Tuscany, Italy (Scarselli et al. 2013). Lastly, we used t-test for between-population comparisons that however, was not always possible because of the lack of useful published statistics.

RESULTS

Two hundred and fifty-four *A. rufa* were captured, measured, and safely released (Tab. 1). One hundred and five individuals were captured in 2009, 124 in 2010, 25 in 2011 with 92% of the birds captured in January. All the individuals were successfully sexed resulting in a proportion of 47% females (n = 117) and 53% males (n = 137). Moreover, we identified the age of the 94.5% of the birds where 97 were adults, 143 young while 14 remained not classified (Tab. 1).

Table 1. Biometric data of the sampled Red-legged Partridge individuals in Alessandria Province, northwestern Italy (n = 254) (lengths were given in mm; body mass in g).

Variable	sex	age	n	mean	SD	min-max
Wing length	F	J	77	153.58	3.11	146-164
	F	A	34	153.18	3.35	145-161
	M	J	66	161.77	3.45	154-168
	M	A	63	164.26	3.78	151-174
8thprimary length	F	J	76	108.79	3.39	101-119
	F	A	34	109.15	3.28	103-116
	M	J	66	114.02	3.58	106-120
	M	A	63	116.52	2.98	107-124
Tail length	F	J	77	90.09	3.79	78-102
	F	A	34	91.91	2.66	86-96
	M	J	65	96.35	3.96	87-104
	M	A	63	100.02	3.97	88-114
Tarsus length	F	J	77	41.3	1.37	39-47
	F	A	34	41.56	1.88	39-48
	M	J	66	45.04	1.37	42-48
	M	A	63	45.47	1.36	42-49
Bill from skull	F	J	76	20.36	1.52	17-25
	F	A	33	20.65	1.33	17-24
	M	J	65	22.35	1.69	19-27
	M	A	61	23.15	1.6	19-27
Nape-bill tip	F	J	77	50.43	1.71	46-56
	F	A	34	50.51	1.54	47-54
	M	J	66	53.75	2.02	48-59
	M	A	62	53.65	2.22	49-59
Body mass	F	J	77	442.36	27.56	370-500
	F	A	34	444.12	30.21	380-510
	M	J	66	524.38	32.82	440-600
	M	A	63	541.35	38.41	450-630

Genetic analysis of a subsample of birds (n = 112) allowed us to distinguish the pure *A. rufa* from those that presented introgression with *A. chukar* identifying 94 genetically pure *A. rufa* individuals and 18 hybrids *A. rufa* x *A. chukar* (i.e., 16.1% of the examined samples). The "hybrid" and "pure" birds were biometrically indistinguishable except for the adult male body mass, apparently greater in hybrids (Kruskal-Wallis test $\chi^2 = 4.819$, df = 1, P = 0.028; for all other comparisons P was always > 0.52).

When we compared the biometric measurements of pure *A. rufa rufa* we found no significant differences by sex in biometric measures of juveniles (Tab. 2 and Tab. 3), while significant differences were found in adults for the length of wing, eighth primary, tail, and body mass (Fig. 2). Age-related difference in males were statistically significant for wing length (χ^2 = 5.747, df = 1, P = 0.016), 8thprimary length (χ^2 = 6.814, df = 1, P = 0.009), tail length (χ^2 = 9.6481, 1, 0.002) and body mass (χ^2 = 4.969, df = 1, P = 0.026) but not in females (P was always > 0.069).

Regarding the comparison of *A. rufa* biometry with published data of other populations, our sampled individuals showed remarkable differences mainly with the southern Spanish population of *A. r. intercedens* (Tab. 4). A cline of bigger versus smaller male adult birds in relation to wing length and body mass, seems to occur from north-east to south-west (Fig. 3).

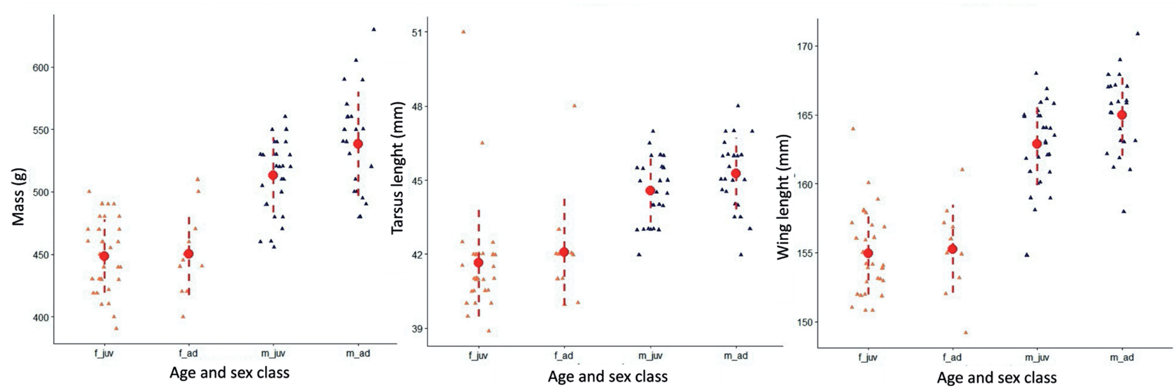

Figure 2. Comparative scatterplots of body mass, tarsus and wing length (Max. chord) measurements for genetically "pure" *A. rufa* presented according to age (juv = Juvenile, ad =Adult) and sex classes (F = Female, M= Male). Small dots and triangles represent single values, while the big dots and triangles with dotted line represent mean and standard deviation values respectively.

DISCUSSION

The main objectives of our analysis were to provide new information on the biometry of the Italian Red-legged Partridge population in the northwest of Italy and to test possible differences related to age, sex, and as a secondary objective to test biometry differences between pure and hybrid with *A. chukar.* This latter, is an introduced game species often used in restocking programs for improving the tolerance to captivity of *A. rufa*, by increasing laying period and producing heavier birds (Tejedor et al. 2008). Of particular interest are the observed differences in biometric measures between our sampled population and other *A. rufa* subpopulations showing a cline from northeast toward southwest through the distribution range. More in particular, considering the different objectives of our analysis, the main discussion points are presented below.

The data presented in this study has significantly increased the number and accuracy of the biometric measurements up to now available for the Red-legged Partridge Italian population. The range of measures in

Table 2. *Alectoris rufa rufa* descriptive summary of the biometric measures presented by sex (Female and Male) and age class (Juveniles and Adults). Only birds genetically tested are presented (n = 94) (lengths were given in mm; body mass in g).

Variable	sex	age	n	mean	SD	min-max
Wing	F	J	31	154.94	3.00	151-164
	F	A	11	155.27	3.20	149-161
	M	J	28	162.89	3.01	155-168
	M	A	24	165.00	2.96	158-171
8^{th}primary	F	J	30	109.28	3.24	101-116
	F	A	11	110.09	3.05	105-116
	M	J	28	114.46	2.95	108-120
	M	A	24	116.25	2.49	110-120
Tail	F	J	31	92.45	4.69	86-112
	F	A	11	92.91	2.95	86-96
	M	J	28	97.16	4.01	89-104
	M	A	24	101.12	3.98	95-114
Tarsus	F	J	31	41.64	2.18	39-51
	F	A	11	42.09	2.17	40-48
	M	J	28	44.57	1.29	42-47
	M	A	24	45.25	1.44	42-48
Bill from skull	F	J	31	20.66	1.75	17-25
	F	A	11	21.18	1.33	20-24
	M	J	28	22.91	1.62	20-27
	M	A	23	23.59	1.38	21-27
Nape-bill tip	F	J	31	50.11	1.53	46-54
	F	A	11	50.73	1.54	49-53
	M	J	28	52.58	2.02	48-57
	M	A	24	52.6	3.09	49-57
Mass	F	J	31	448.55	29.36	390-500
	F	A	11	450.45	33.35	400-510
	M	J	28	513.25	30.03	456-560
	M	A	24	538.33	41.49	480-630

Table 3. *Alectoris rufa x chukar* descriptive summary data for the biometric measures by sex (age combined). Only birds genetically tested (n = 18) are shown (lengths were given in mm; body mass in g).

Variable	sex	n	mean	SD	min-max
Wing	F	8	152.88	2.1	150-155
	M	10	165.10	4.53	158-174
8thprimary	F	8	107.94	3.03	103-111
	M	10	115.70	5.66	107-124
Tail	F	8	88.88	2.75	84-92
	M	10	100.60	4.79	90-108
Tarsus	F	8	40.56	1.08	39-42
	M	10	45.35	1.81	43-49
Bill	F	7	20.86	0.75	20-22
	M	10	22.70	1.09	21-25
Nape-bill tip	F	8	49.88	1.71	47-51
	M	10	52.10	1.41	51-56
Mass	F	8	402.50	22.52	370-450
	M	10	549.50	36.7	470-600

Table 4. Comparison among adult Red-legged Partridge from northwestern Italy (see Tab. 2) and of some other European populations (t-test): *A. rufa rufa*, Tuscany, Italy (Scarselli et al. 2013), southern France (Pépin 1985); *A. rufa hispanica*, northern Spain (Chalaux 2005); *A. rufa intercedens*, southern Spain (Rubiales 1983) (lengths were given in mm; body mass in g).

Population	Variable	age	N	mean	SD	t-test	df	P	Sign.
Tuscany (I)	Mass	m	17	508	73.76	1.679	39	0.101	n.s
		f	9	426	34.07	1.616	18	0.124	n.s
South France	Wing	m	142	164	4	1.17	164	0.243	n.s
		f	16	157.7	4	1.676	25	0.106	n.s
	Mass	m	27	523.1	21.8	1.667	49	0.102	n.s
		f	16	429.1	23	1.974	25	0.059	n.s.
North Spain	Wing	m	57	163.51	4.15	1.594	79	0.115	n.s
		f	35	156.63	2.21	1.593	44	0.118	n.s
	Tarsus	m	43	47.16	1.64	4.768	65	0	**<0.01**
		f	34	44.57	1.46	4.326	43	0	**<0.01**
	Mass	m	39	502.15	48.67	3.025	61	0.003	**<0.01**
		f	25	435.2	37.37	1.163	34	0.252	n.s.
South Spain	Wing	m	327	160.4	4.36	5.08	349	0	**<0.01**
		f	360	151.25	4.51	2.932	369	0.004	**<0.01**
	Tarsus	m	327	44.17	1.88	2.754	349	0.006	**<0.01**
		f	219	41.41	1.62	1.335	228	0.183	n.s.
	Tail	m	306	96.61	5.66	3.827	328	0	**<0.01**
		f	226	90.22	5.11	1.73	235	0.085	n.s
	Mass	m	234	480.34	45.82	5.953	256	0	**<0.01**
		f	192	408.41	42.04	3.256	201	0.001	**<0.01**

our sample does not differ from previously published data (Brichetti & Fracasso 2004), and in particular, we found no statistical differences in body mass between a comparable sample of wild *A. rufa* from Tuscany suggesting that (Scarselli et al. 2013).

The biometric differences detected between age/sex classes are more influenced by sex than by age, with between-sex differences being greater than those between age classes. The scaling effect of age and sex on biometric measurements is appreciable for wing length, 8th primary length, tail length and body mass which are consistently greater in adult versus young males, but not between juvenile and adult females. This finding is probably influenced by the sampling period (January), as body mass can change from autumn to winter and especially in the first winter, when, the young female, unlike males, have probably almost reached the definitive adult body size, as suggested by faster growth curve (Cramp 1980, Rubiales 1983). A greater scaling effect of age was found by Nadal et al. (2018) in Spain probably because they included birds harvested during the hunting season (autumn) when the juveniles were at a less advanced stage of development compared to our population.

We found no significant biometric differences between our samples and the other *A. rufa rufa* populations; in particular, no statistical differences were detected with the populations in southern France (Pépin 1985). However, Spanish birds seem to be slightly different in size, especially the ones from southern Spain (*A. r. intercedens*) which are consistently smaller in some parameters (Tab. 4, Fig. 3). Although only few significant differences have been found between subpopulations, the presence of northeast toward southwest cline for wing length and body mass is remarkable. The difference between the Italian and the southern Spain populations (mean difference for wing length: 5mm and approximately 50g for body mass), suggests a longitudinal trend throughout the whole population range despite limited information is available from three sample sites in Portugal (Magalhães et al. 2001). Compared to all the other examined populations, the Portuguese birds (with the larger sample size, n = 39) are also smaller in size for wing and tarsus length, as well body mass for both sexes, but the lack of differentiation between adults and juvenile makes unreliable any comparison.

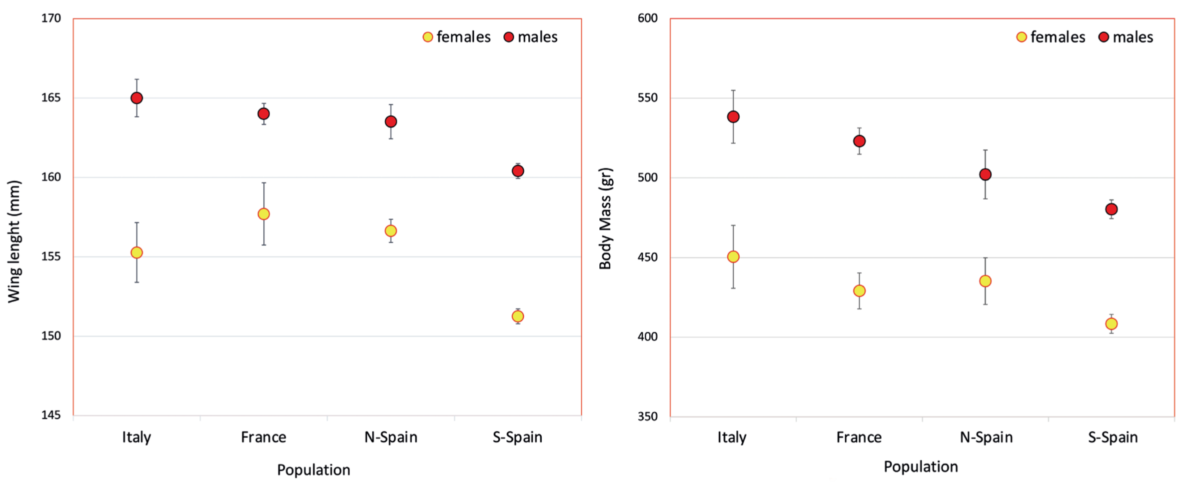

Figure 3. Comparison of wing length and body mass in adult males and females in four populations of Red-legged Partridge from West to East Europe.

Casas et al (2013) found ''pure'' *A. rufa* males being larger than hybrid ones (and no difference in size according to genotype in females) and explained the observed differences as a consequence of artificial selection in farms favouring smaller and less aggressive males. On the contrary, we observed that the body mass of hybrids was higher than pure *A. rufa* birds, and this was somehow expected as some *chukar* subspecies or populations are known to reach slightly heavier weight than Red-legged Partridge (Glutz von Blotzheim 1994). However, it must be stressed that our hybrid sample is very small and that the selection criteria in different game bird farms can be very different. Considering that no clear information is available on the presence of hybrids in the European populations sampled in other studies (Forcina et al. 2021), we cannot exclude the fact that the presence of hybrids might act as confounding factors and thus biometric comparisons among populations should always be carefully carried out.

At first look, the sample size presented in our study may appear limited for the declared purposes, and consequently for the robustness of our findings on the species biometric variability by sex, age and geographic regions. On the other hand, it is important to consider the numbers of measured individuals in our work in light of the lower abundance and smaller range of distribution in our study area when compared to Spain and France (Arroyo et al. 2020). Finally, an additional value of the biometric measurements taken in this study is the parallel genetic verification carried out, that allowed the identification and differentiation of the data related to genetically pure animals and hybrids.

Overall, the information reported in this study has increased the knowledge on the biometric variability of *A. rufa* and might serve as a useful reference for future studies. Additional measurements taken at different times of the year, and over a wider area would be important to confirm our findings and allow to understand better the temporal and spatial biometric variability of *A. rufa* across its range. According to our results, biometric criteria *per se* are insufficient to differentiate *rufa* x *chukar* hybrids from pure *A. rufa* birds. In case of planned reintroduction or restoking activities, we recommend to carry out an accurate morphological screening of plumage characteristics, or even better, a genetic analysis. This weak morphological differentiation makes difficult to identify hybrids in case of eradication projects. Indiscriminate restocking using allochthonous and hybrids individuals should be avoided and banned not only because it compromises the genotypic and phenotypic integrity of wild populations (e.g., Negri et al. 2013, Ottenburghs 2021) but also because it can cause the dissemination of infectious diseases in wild Galliformes populations (Fanelli et al. 2020a, 2020b, Tizzani et al. 2020).

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Fabrizio Silvano, a true enthusiastic expert of the Red Partridge in his territory, has sadly passed away before the completion of this paper, after having conceived the work, carried out the field sampling and biometric measurements, and participated in the manuscript drafting.

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