

# Caratterizzazione genetica delle popolazioni autoctone di starna in Italia: l'approccio biomolecolare in sinergia con i dati storici.

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Coordinatore beneficiario



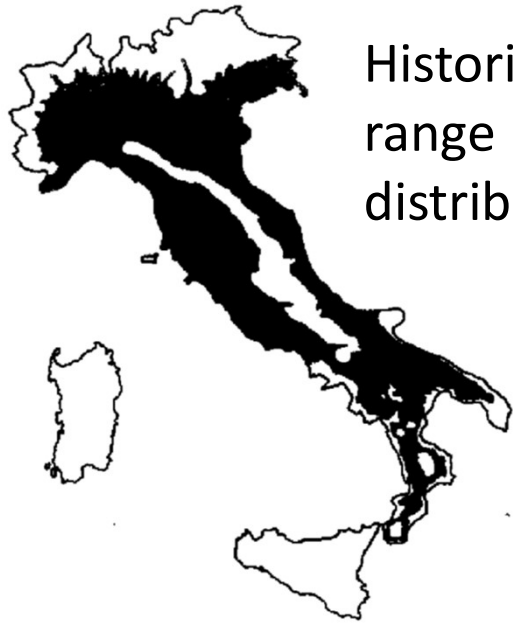
Beneficiari associati



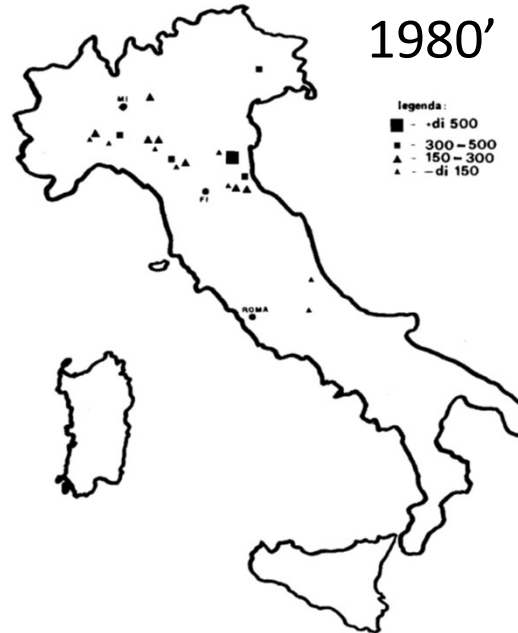
Cofinanziatore



## Historical Demographic Decline



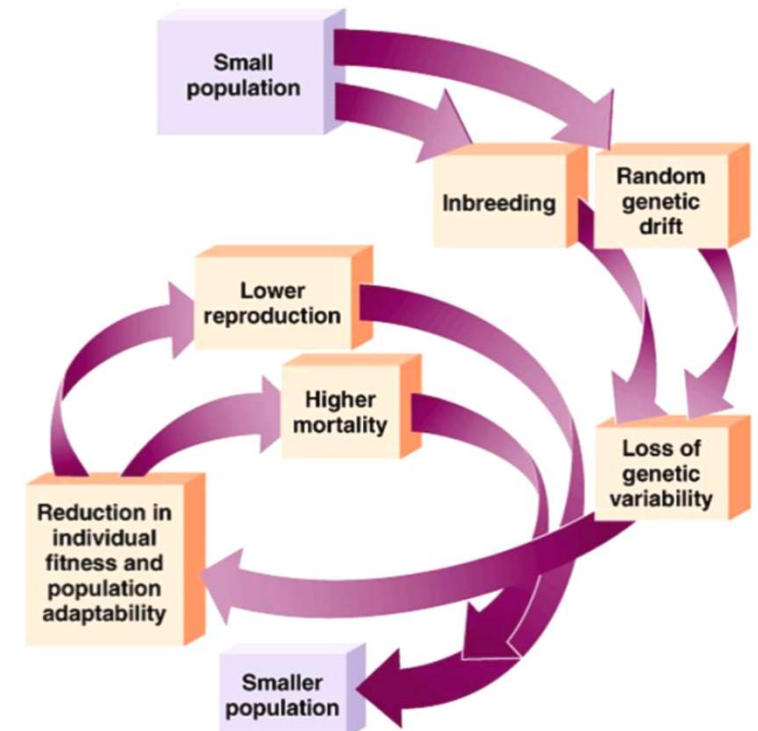
Historical  
range  
distribution



Declared extinct in the wild from 2015 due to:

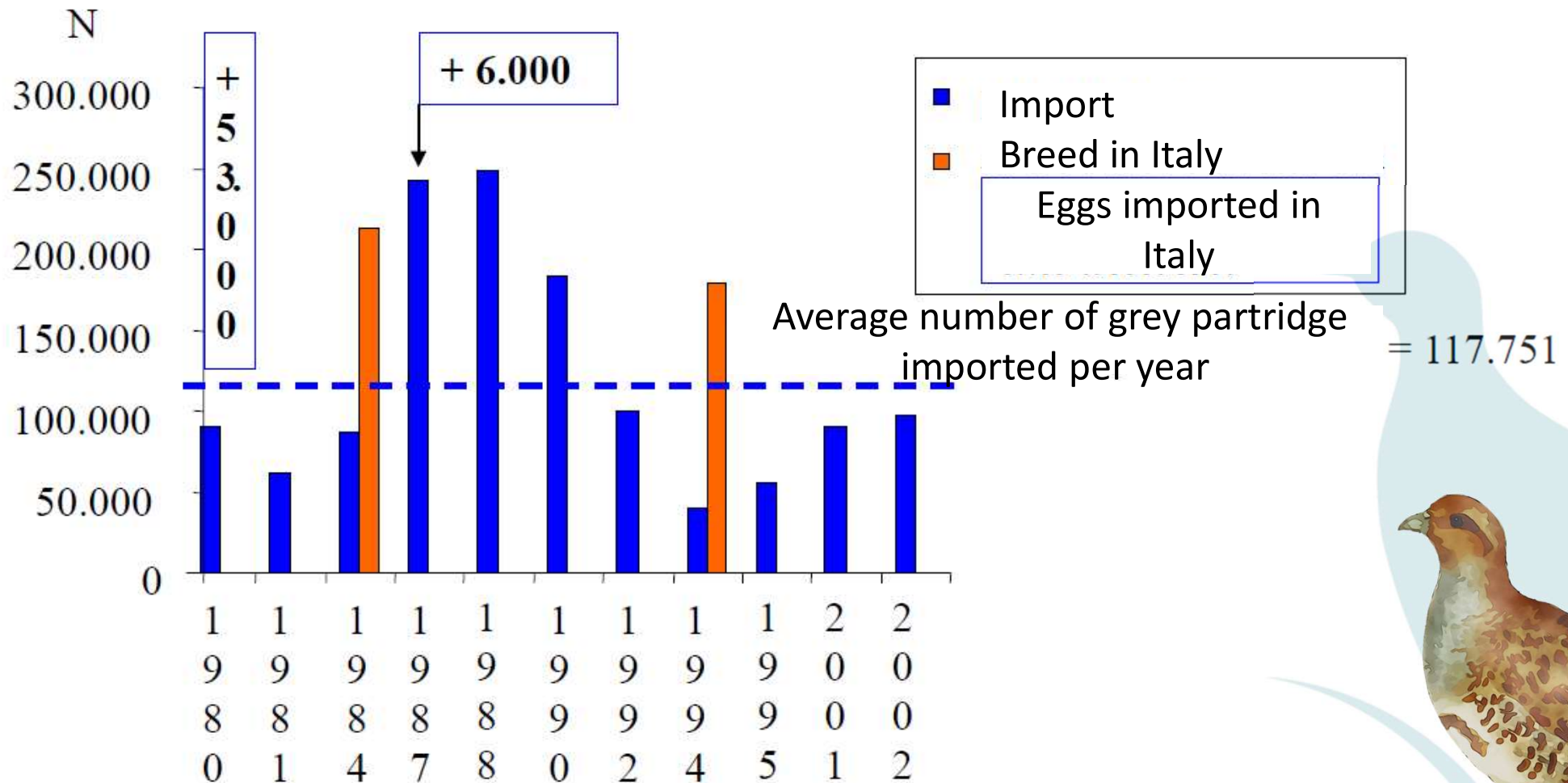
- ❖ Hunting pressure
- ❖ Habitat loss and fragmentation
- ❖ Releasing of allochthonous individuals

### The Extinction Vortex:



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## High pressure of introductions in the near past: Number of individuals introduced from 1980 to 2002







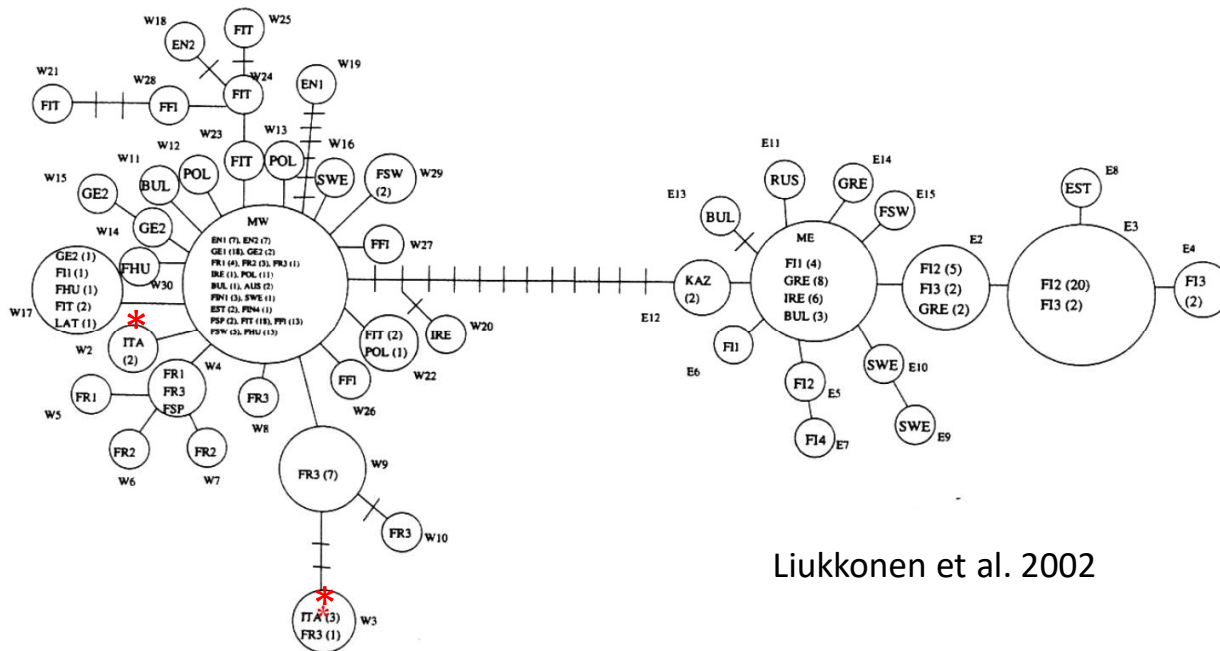
## High pressure of introductions in the near past: Countries of origin of grey partridges imported in Italy from 1987 to 2002

1987	%	1988	%	2001	%	2002	%
Danimarca	93,9	Danimarca	92,8	Danimarca	76,7	Danimarca	46,7
Francia	2,9	Polonia	1	Francia	22,6	Francia	46,7
Polonia	1,6	Francia	5,4	Slovenia	0,7	Romania	3,6
Ungheria	1,6					Slovenia	3,1

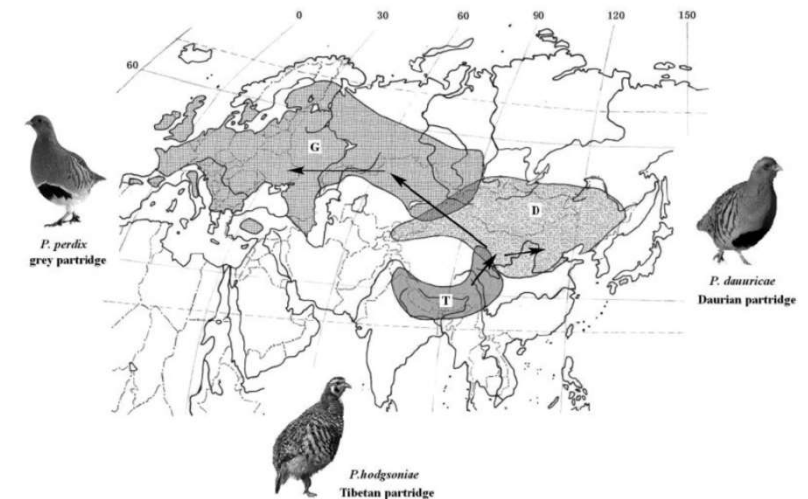
Information for the preceding period covering from the beginning of the 20th century until the 1980s need to be better investigated...



## Available biomolecular information on the species



Liukkonen et al. 2002



**Network** connecting CR1 partridge haplotypes with the least possible number of mutations, built on the basis of segregation sites. In brackets the number of individuals belonging to each haplotype, whose name is indicated in block letters. Each line between two lines or circles represents a mutation. Note in particular the placement of the **5 Italian museum samples (ITA\*)** and the **26 bred (FIT)**.

Identification of phylogenetic lines characteristic of Italian populations and present before the massive releases of alien specimens into nature.

## MAIN GOALS

- ✓ Outline the effect of **introducing non-native individuals** on the native genetic population composition.
- ✓ Trace a **timeline of introductions** to define periods of null, minor, and maximum influence of the releasing of allochthonous individuals on the native genetic pool.
- ✓ Gather **previously published data** from GenBank pinpointing a unique haplotypes identification.





## Materials & Methods Documentary Research: Examination of Hunting Periodicals

Title	Periodicity	Distribution	Examined time frame
Andando a caccia	Bimonthly	National	1947-1956
Diana	Bimonthly	National	1930-2012
Gazzetta di caccia	Bimonthly	National	1951-1961
Il cacciatore italiano	Bimonthly	National	1912-2006
Italia venatoria	Bimonthly	National	1946-1973
Venatoria	Bimonthly	National	1931-1938
Liguria Venatoria	Quarterly	Regional	1939-1941
Bologna venatoria	Quarterly	Local	1961-2006



Hunting magazines title list and periods coverage retrieved in the **collection of periodicals from the ISPRA library, Ozzano.**

In alphabetical order starting from National distribution, followed by regional and local.



# **Materials & Methods Documentary Research:**

## **Information gathering on Partridge Trade, Introduction, and Release**

Careful reading focusing on keywords “starna” and “pernice” and searching for pictures we selected over **300 documents** of different types subdivided into the four following categories and their relative numerical consistency:

**Photo (PH) – 24** Pictures sent by hunters are often not linked to notes; they also included a cartoon and a cover of Diana magazine.

**Game Launches (LC) – 105** Game launches in regional hunters’ associations reports.

**Advertisements (PB) – 43** Promotional materials of breeders, distributors, and importers.

**Text documents (TX) – 136** texts, articles, opinions, and experiences on game management.





# Materials & Methods ancient DNA: Museum sampling and biomolecular analyses

- ✓ Identification of museum sample collection, invitation letter, ANMS
- ✓ Provide museal bird sampling protocol to avoid contamination
- ✓ Collection and conservation at the ISPRA Biobank of Ozzano E. (BO)
- ✓ Analysis of a fragment of mitochondrial DNA in Italian museum specimens collected from the late 19th to early 20th centuries
- ✓ Networking ISPRA, University of Eastern Piedmont - Agreement protocol 16/09/2019, 5y; University of Perugia - Agreement protocol 14/01/2020, 5y to merge all the available sample datasets;
- ✓ Comparison with available sequence published and from GenBank.

Agreement protocol  
Prot. 105025/XI.A.6  
4/09/2003 INFS - Dep.  
Productive Activities,  
Province Lucca



# Documentary Research Results: timing and occurrence of introduction in Italy

Geographic location	Region	N° release	N° individuals	Time frame
North Italy	Friuli-Venezia Giulia	9	43	1930-1965
	Liguria	52	10.217	1930-1971
	Lombardy	60	9.024	1913-1971
	Piedmont	46	34.378	1932-1971
	Trentino-Alto Adige	3	12	1931-1959
	Veneto	37	3.476	1928-1970
	<b>TOTAL N</b>	<b>207</b>	<b>57.150</b>	
Central Italy	Abruzzo	5	906	1966-1971
	Campania	14	2.436	1932-1970
	Emilia-Romagna	43	9.135	1932-1969
	Lazio	13	4.476	1930-1960
	Marches	8	3.161	1932-1971
	Tuscany	44	6.849	1951-1970
	Umbria	17	1.973	1939-1969
	<b>TOTAL C</b>	<b>144</b>	<b>28.936</b>	
South Italy	Basilicata	3	106	1955-1970
	Calabria	5	628	1950-1971
	Molise	2	410	1942-1965
	Apulia	5	310	1932-1963
	Sicilia	3	415	1932-1970
	<b>TOTAL S</b>	<b>18</b>	<b>1.869</b>	

Data recorded on numbers and time of releases and individuals all over the country. One row for each region, subdivided into North, Central and South Italian peninsula, reporting: the number of release events detailed **from 1913 to 1971**, the number of individuals released, and the time frame considering the first and last year of documented release.



## Documentary Research Results: Grey Partridge Trade, Introduction, and Release in Italy

- ✓ **over 50 game farms and distributors** operating in Italy. Most provided foreign specimens primarily from Central Europe (Hungary, Germany, Austria, Bulgaria, Romania, Denmark).
- ✓ **more than 300 episodes of grey partridge releases** were documented from 1915 to 1971 and involved **almost 90,000 individuals** throughout the peninsula.
- ✓ **we collected information on introduction activities from 1915**, and before this date, only an introduction event was documented in our bibliographic research in Italy.
- ✓ **First evidence** of grey partridge introduction in Italy was reported in **1913**.
- ✓ Massive introductions were recorded **after 1945**.





# Museal Sampling Results: extensive collection in the ISPRA biobank

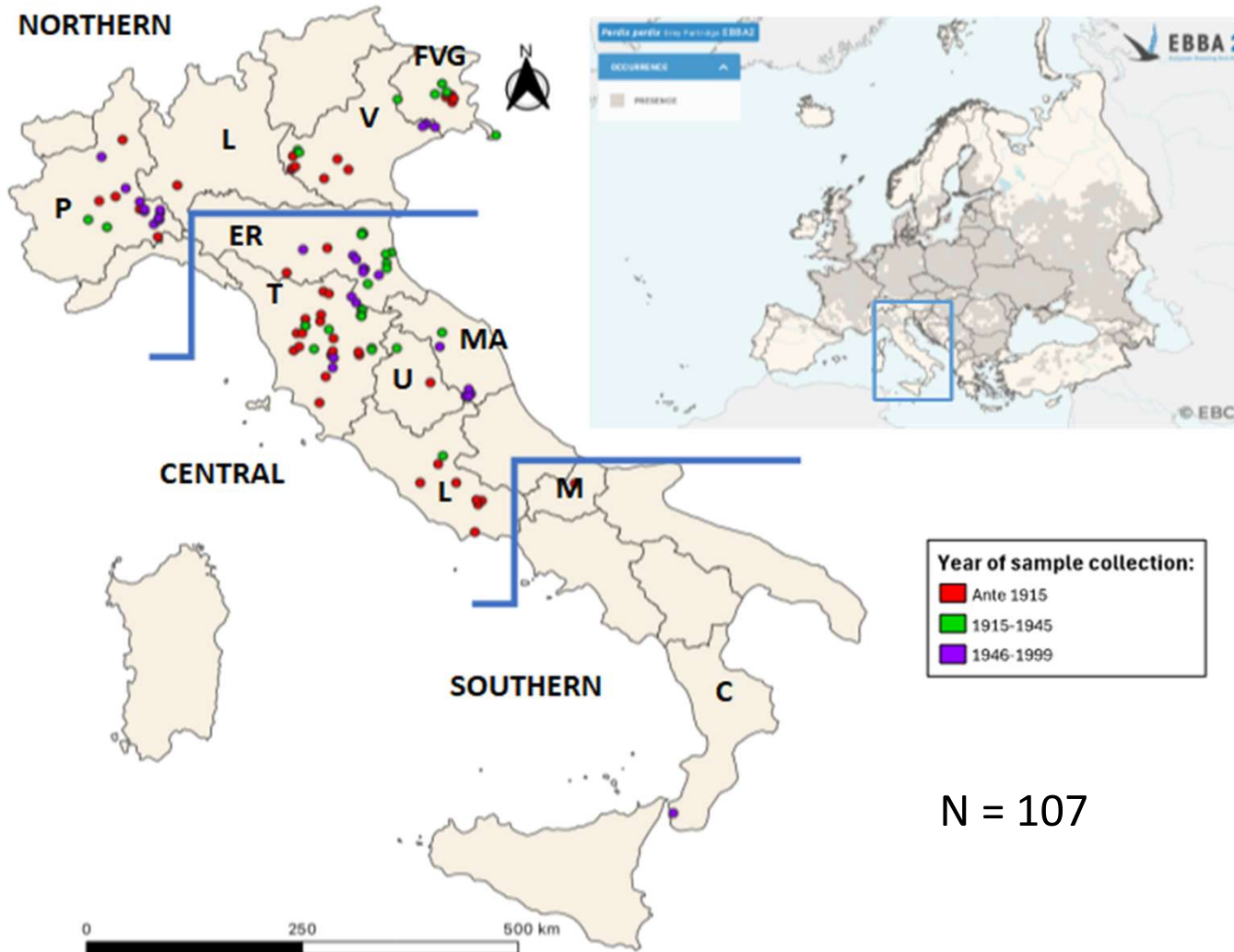
Museum name	N° samples	Period
Civic Museum of Zoology - Roma	26	1892-1927
Museum Malmerendi - Faenza	8	1948-1971
Museum of La Specola - Firenze	15	1876-1949
Museum of Natural History and Territory - Pisa	10	1835-1923
Museum of Natural History and Territory Città della Pieve	1	unknown
Museum of Natural History of Friuli	4	1923-1935
Museum of Stazzano - Alessandria	15	1950-1999
Museum of the Accademia dei Fisiocratici - Siena	7	1884-1910
Museum of Zoology University of Bologna	4	1897-1907
Natural History Museum of Casalina - Perugia	3	1855-1857
Natural History Museum of Pavia	1	1887
Ornithological and Natural Science Museum of Ravenna	5	1915-1946
Ornithological Museum Foschi - Forlì	10	1933-1956
Regional Interdisciplinary Museum Palazzo d'Aumale, Sicily	11	1900-1954
Zoological Collection ISPRA Ozzano dell'Emilia - Bologna	6	1895-1949
Private Collection - Marche	4	1982
Private Collection - Piedmont	1	1950
Federaccia - Reggio Calabria	1	1956
Federaccia - Imola	2	1960-70
Private Collection - Umbria	6	unknown
<b>Total</b>	<b>140</b>	

Selected museum dataset included in the study. We had to discard samples with incomplete or inconsistent data and duplicate ones.

List of the 15 museums and 5 private collections that provided historical samples of grey partridge. For each one, the number of samples and the collection period is indicated.



# Documentary Research Results: definition of 3 periods with null, low-medium and high influence of releasing

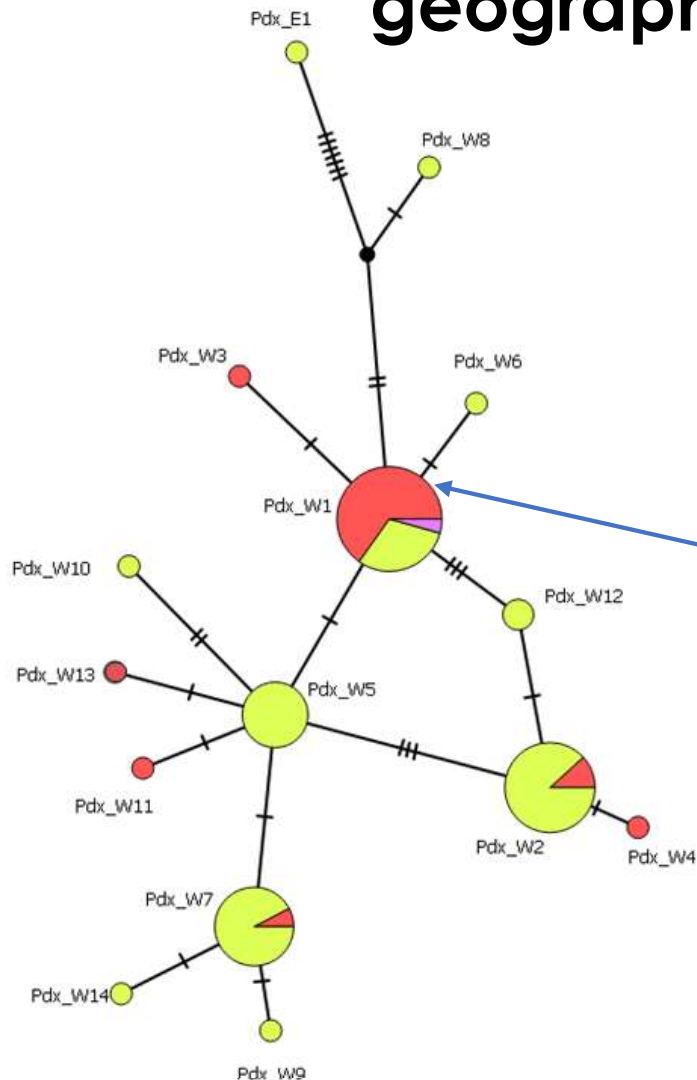


Geographical distribution of collected museal samples in Italy. Samples colour based on the collection year: **before 1915**; **from 1915 to 1945**; **from 1946 to 1999**.

Map from European Breeding Bird Atlas - EBBA2 of the occurrence of *Perdix perdix* in Europe in the upper right corner.



# mtDNA CR Results: network of museum samples identifies a geographical distribution of haplotypes



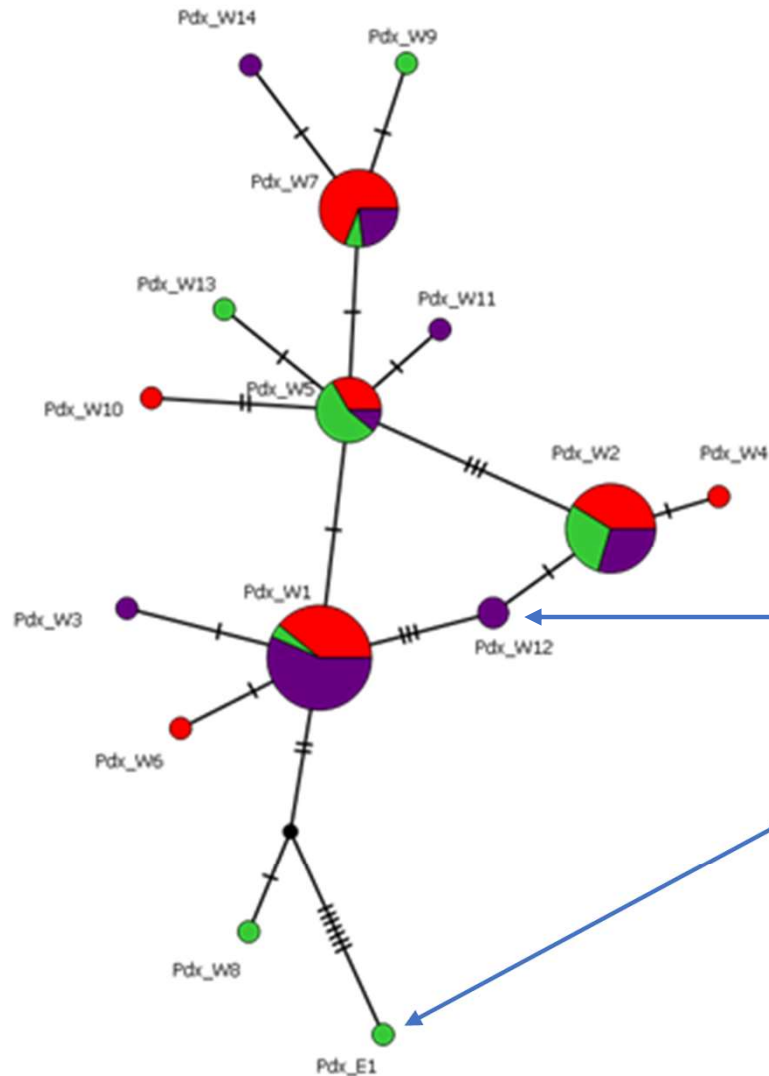
- North Italy
- Central Italy
- Unknown origin

- ✓ The lack of samples in South Italy is probably due to species distribution
- ✓ The central position and wider distribution of Pdx\_W1: ancient haplotype





# mtDNA CR Results: network of museum samples identifies a different distribution of haplotypes during 3 time periods



- ✓ Unresolved nodes due to low fragment length
- ✓ Presence of Eastern haplotype Marche region, 1933
- ✓ Some Italian haplotypes present in the first period were lost in the second and third periods



# mtDNA CR Results: Different distribution of haplotypes in Italy in the three time frames

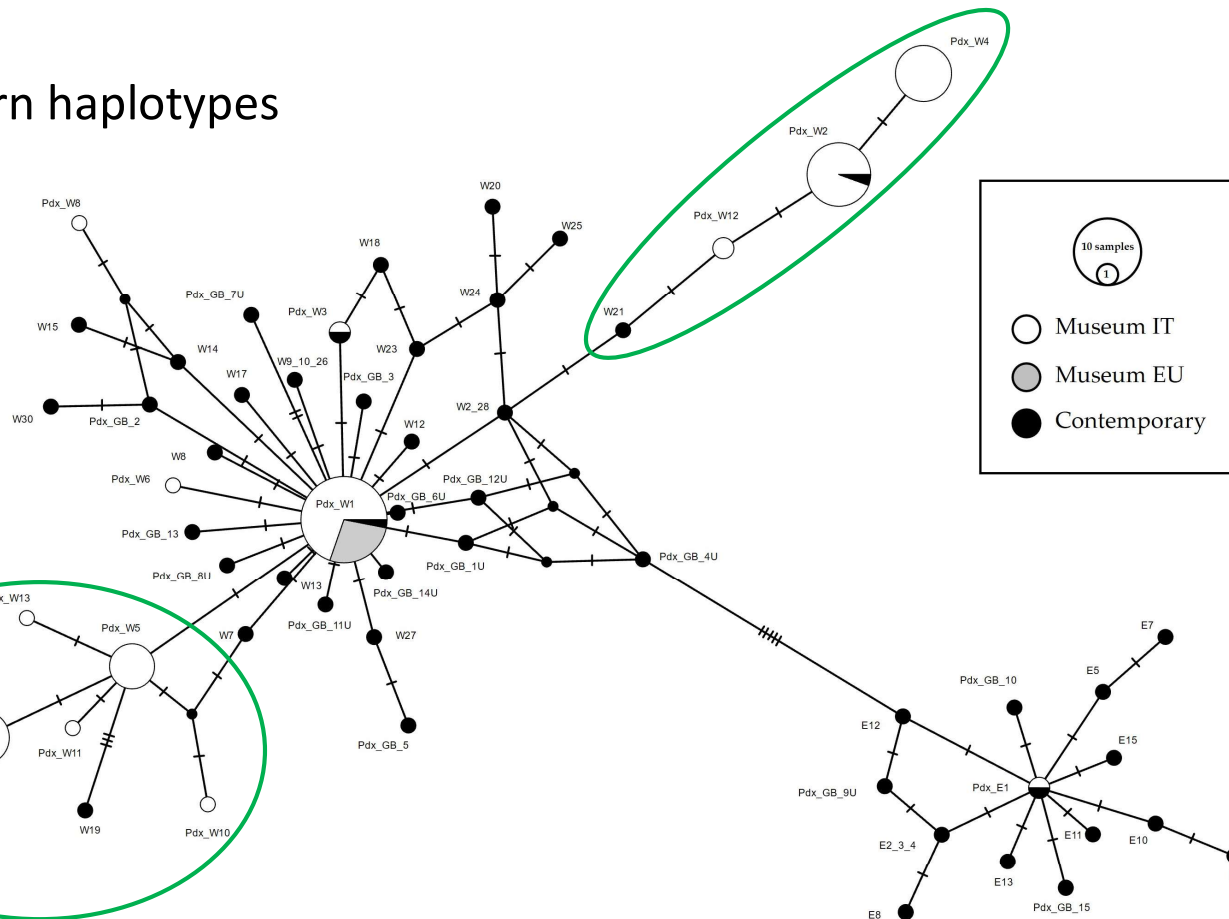
Haplotype	Italy	1835-1914	1915-1945	1946-1999
Pdx_W1	23	9	1	13
Pdx_W2	17	7	5	5
Pdx_W3	1			1
Pdx_W4	1	1		
Pdx_W5	9	3	5	1
Pdx_W6	1	1		
Pdx_W7	13	9	1	3
Pdx_W8	1		1	
Pdx_W9	1		1	
Pdx_W10	1	1		
Pdx_W11	1			1
Pdx_W12	2			2
Pdx_W13	1		1	
Pdx_W14	1			1
Pdx_E1	1		1	
Haplotypes n°	15	7	8	8
Samples n°	74	31	16	27

*The choice of threshold was due to information on the first releases and the starting of massive reintroductions (after the Second World War). Each colour indicates a haplotype identified only in a specific time frame: **ante 1915**, **between 1915-1945**, **post 1945***



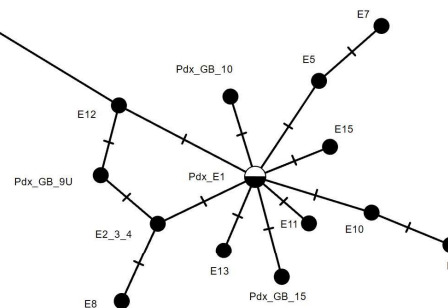
# mtDNA CR Results: network of museum samples and sequence downloaded from Genbank

## Western haplotypes



- ✓ Comparison: 74 museal Italian sequences versus 113 from GenBank and 45 published (Liukkonen et al. 2002)
- ✓ Central position and wider distribution of Pdx\_W1: ancient haplotype
- ✓ Isolation of Italian haplogroups in two main branches: probably two isolation events

## Eastern haplotypes





# mtDNA CR Results:

## Haplotype names harmonization and publication in GenBank

name	175	176	184	188	189	196	211	217	219	220	236	240	242	243	244	245	259	294	302	308	309	310	GenBank A.N.
Pdx_W1	G	C	C	T	C	A	T	T	T	A	A	T	C	C	T	A	G	T	C	C	C	C	PQ299011
Pdx_W2	.	.	.	.	T	.	C	.	.	G	.	.	.	.	.	.	.	.	.	T	.	.	PQ299012
Pdx_W3	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	PQ299013
Pdx_W4	.	.	T	.	T	.	C	.	.	G	.	.	.	.	.	.	.	.	.	T	.	.	PQ299014
Pdx_W5	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	PQ299015
Pdx_W6	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	PQ299016
Pdx_W7	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	T	.	.	PQ299017
Pdx_W8	.	.	.	.	.	G	.	.	.	.	.	.	.	T	.	.	.	.	.	.	T	.	PQ299018
Pdx_W9	.	.	.	.	.	.	.	.	.	.	.	.	G	.	C	.	.	.	.	T	.	.	PQ299019
Pdx_W10	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	G	.	.	.	T	.	.	PQ299020
Pdx_W11	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	T	.	.	PQ299021
Pdx_W12	.	.	.	.	T	.	C	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	PQ299022
Pdx_W13	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	T	.	.	PQ299023
Pdx_W14	.	.	.	.	.	.	.	.	.	.	G	.	.	.	C	.	.	.	.	T	.	.	PQ299024
Pdx_E1	A	.	.	.	T	.	.	.	.	T	.	.	.	T	.	.	A	C	A	.	T	C	PQ299025

List of new haplotypes identified in our study, mutations and relative positions and GenBank Accession numbers, compared with the over 150 published or deposited from other authors in GenBank.



Article

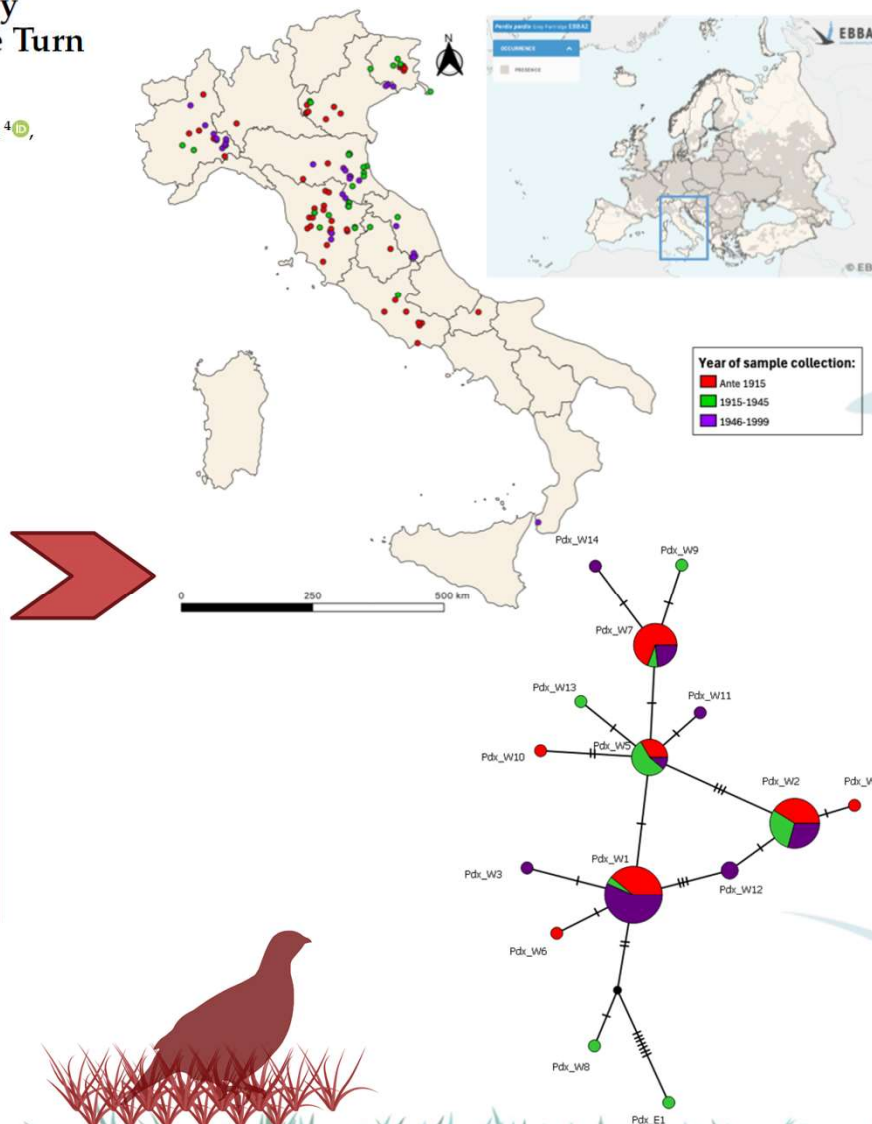
# Combining Historical and Molecular Data to Study Nearly Extinct Native Italian Grey Partridge (*Perdix perdix*) at the Turn of the Twentieth Century

Claudia Greco <sup>1,\*</sup>, Cristiano Tabarroni <sup>1</sup>, Irene Pellegrino <sup>2</sup>, Livia Lucentini <sup>3</sup>, Leonardo Brustenga <sup>4</sup>, Lorenza Sorbini <sup>5</sup> and Nadia Mucci <sup>1</sup>

## DNA sequencing of Museum samples



## Historical documentation checking



## Conclusions

- ✓ **Multidisciplinary approach and networking** improve deep understanding of phenomena and enforce data quality.
- ✓ Documentation of **massive releases with exponential increase after 1945** mainly in Northern and Central Italy, thanks to hunting magazines.
- ✓ Early effect of reintroduction: **eastern haplotype's presence** after 1915.
- ✓ Harmonization of published data by identifying and renaming identical haplotypes with a unique code; great relevance of this process for future studies and applications.







**Many thanks to all the contributors:  
Collectors, Museums, Students...**

Coordinatore beneficiario



Beneficiari associati



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