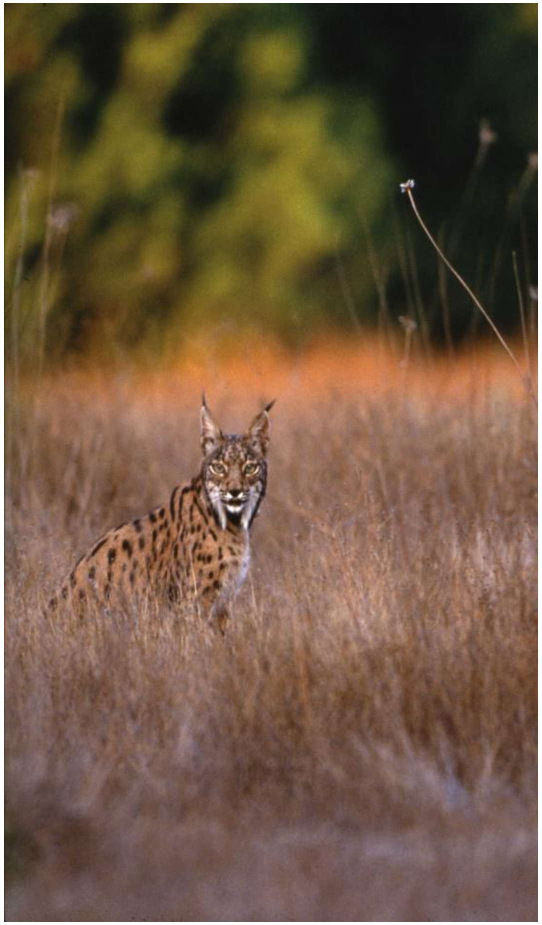
Herintroducties: van genen tot landschappen
(Reintroductions: from genes to landscapes)
Brussels, 4 October 2020



The reintroduction of the Iberian lynx in Spain and Portugal

A conservation success despite extreme genetic erosion

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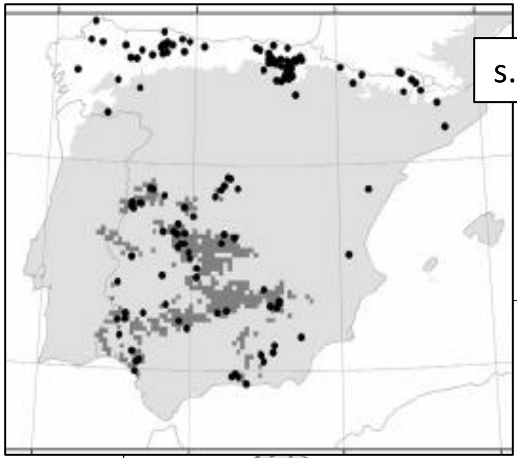
GOBIERNO DE ESPAÑA
MINISTERIO DE CIENCIA, INNOVACIÓN Y UNIVERSIDADES

CSIC
CONSEJO SUPERIOR DE INVESTIGACIONES CIENTÍFICAS

Estación Biológica Doñana

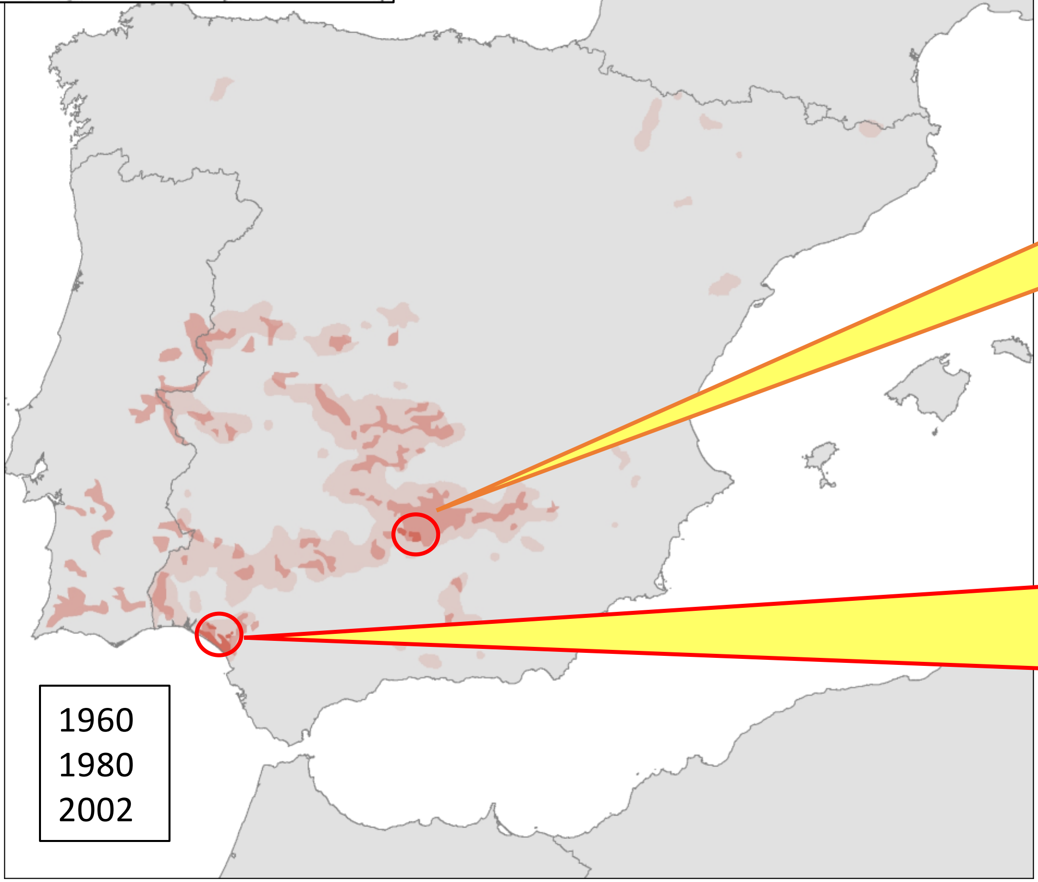
lynxconnect

Iberian lynx decline and fragmentation



s. XVI-XIX

Clavero & Delibes, 2013



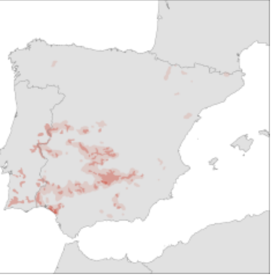
1960
1980
2002

Sierra Morena (SMO)
53 ind.
18 territorial females

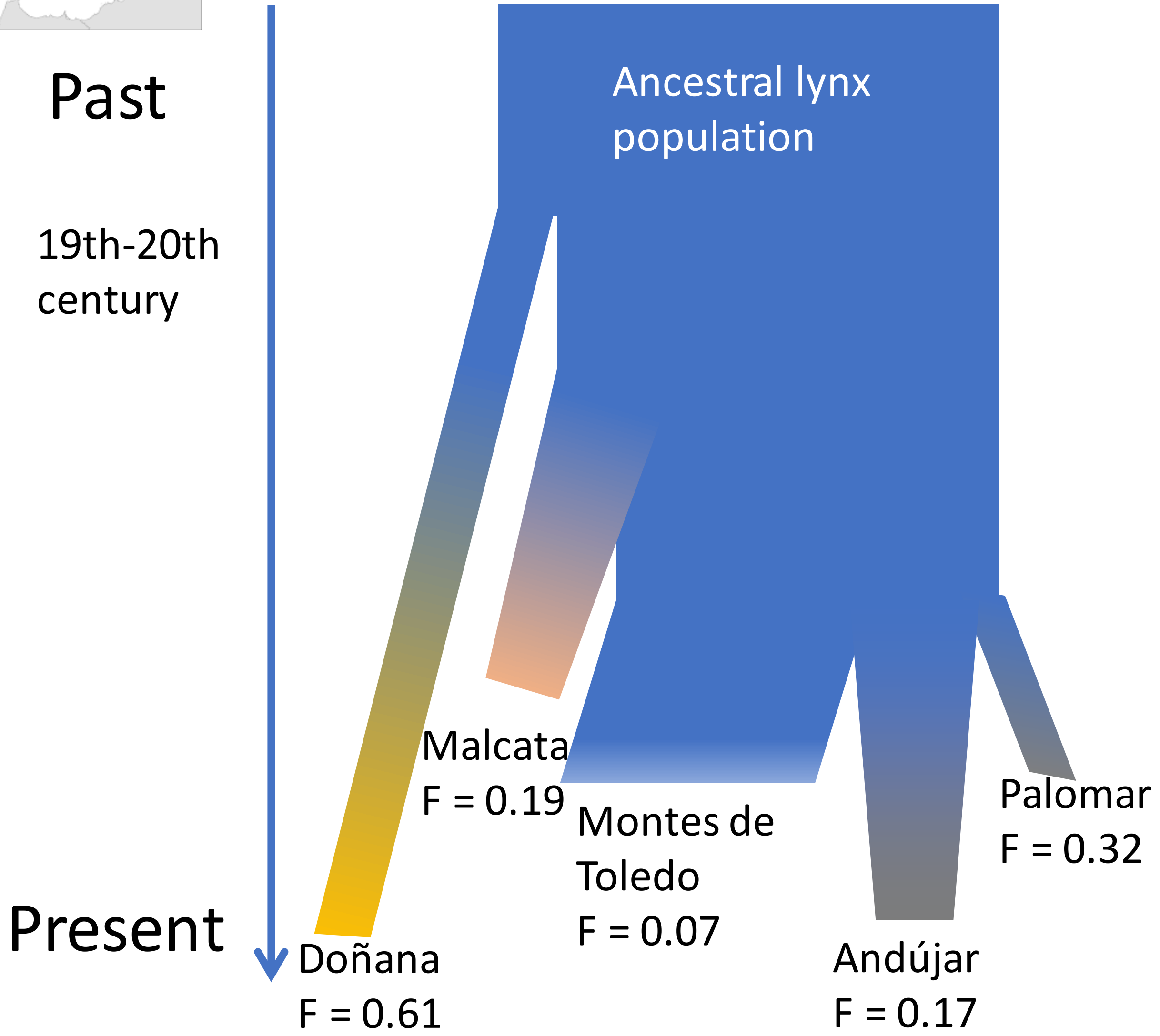
2002 census

Doñana (DON)
41 ind.
9 territorial females

(Rodríguez & Delibes, 1990, 1992, 2002; Castro & Palma, 1996; LIFE, 2007)



Genetic history: recent erosion



Past

19th-20th century

Present

Ancestral lynx population

Malcata
F = 0.19

Montes de Toledo
F = 0.07

Palomar
F = 0.32

Doñana
F = 0.61

Andújar
F = 0.17

Likely panmictic and **relatively** diverse population

Genetically structured metapopulation with populations differing in diversity and inbreeding

$H_{Global} = 0.602$

Two isolated and highly inbred populations

$H_{Doñana} = 0.306$ (50%)
$H_{Andújar} = 0.506$ (84%)

$H_{Global} = 0.54$

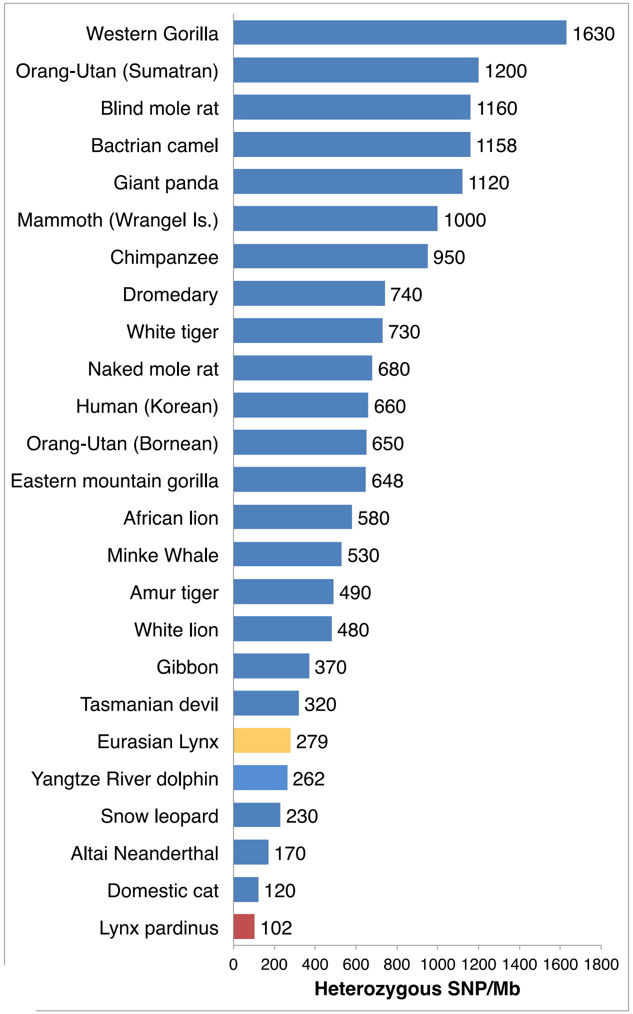
Casas-Marcé M, Marmesat E, Soriano L, *et al.* (2017) Spatio-temporal dynamics of genetic variation in the Iberian lynx along its path to extinction reconstructed with ancient DNA. *Molecular Biology and Evolution* **34**, 2893–2907.

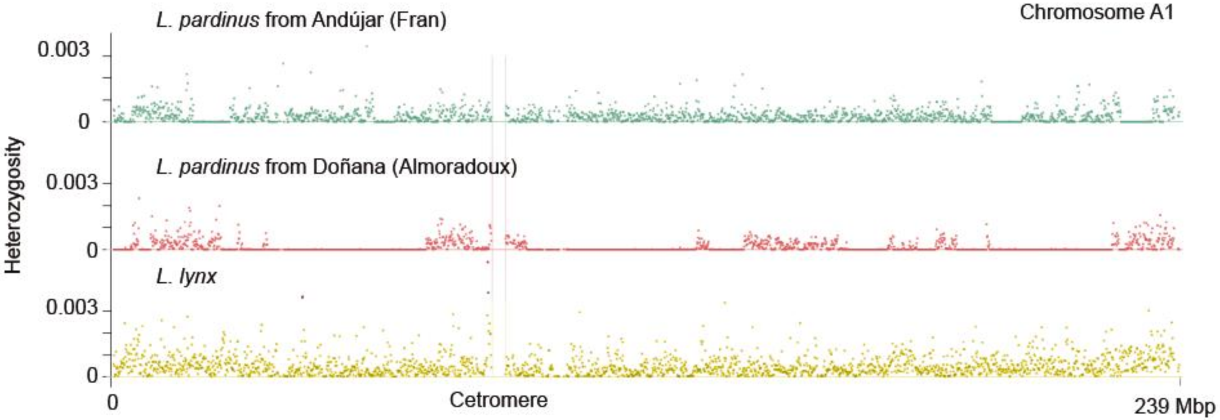
Extreme genetic erosion in remnant populations

- Among the lowest species-wide genetic diveirsity ever reported
- High recent inbreeding
- Small effective size
- Most dramatic in small and isolated Doñana population
- High genetic differentiation: **Fst=0.22**

	Doñana	**Andújar**
F_h	0.47	0.06
F_{roh}	0.32	0.16

	Ne
DON	8.4 (4.9-13.7)
SMO	18.6 (15.0-23.3)





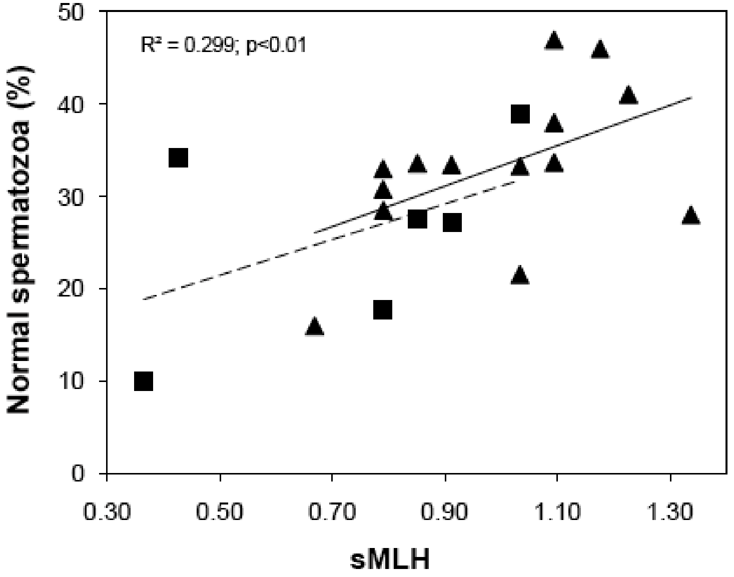
Abascal *et al.* (2016) Extreme genomic erosion after recurrent demographic bottlenecks in the highly endangered Iberian lynx. *Genome Biology* **17**, 251.

Impact on fitness?

- High incidence of genetic? disorders
  - Membranous glomerulonephritis (Jiménez et al., 2008)
  - Lymphoid depletion (Peña et al., 2006)
  - Idiopathic epilepsy (Minguez et al., 2021)
  - Cryptorchidism (Martínez, et al., 2013)
  - High susceptibility to infectious diseases
- High rate of non-traumatic mortality
  - Feline Leukemia Virus epidemics in 2007 (12 infected, 7 died)
- Recent reduction of average litter size in DON
  - 1993-2001: 3.1 kittens (n=18, SE= 0.18, range=2-5)
  - 2006-2008: 2.2 (n=9, SE= 0.15, range=2-3).

Palomares F (2012) Possible extinction vortex for a population of Iberian lynx on the verge of extirpation. *Conservation Biology* **26**, 689-697.

Heterozygosity-fitness correlations for semen quality



Ruiz-Lopez MJ, *et al.* (2012) Heterozygosity-Fitness Correlations and Inbreeding Depression in Two Critically Endangered Mammals. *Conservation Biology* **26**, 1121-1129

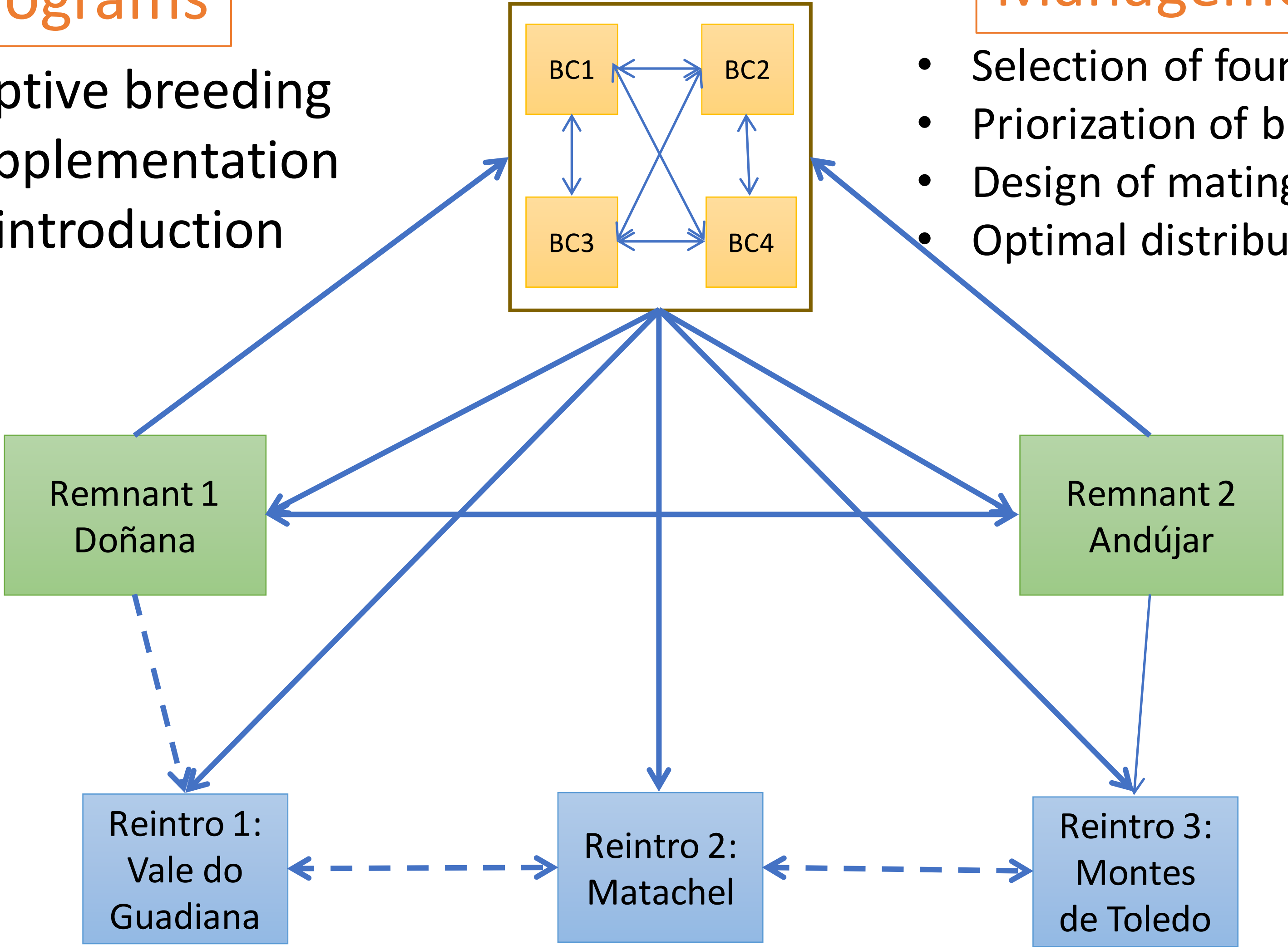
Iberian lynx conservation

Programs

- Captive breeding
- Supplementation
- Reintroduction

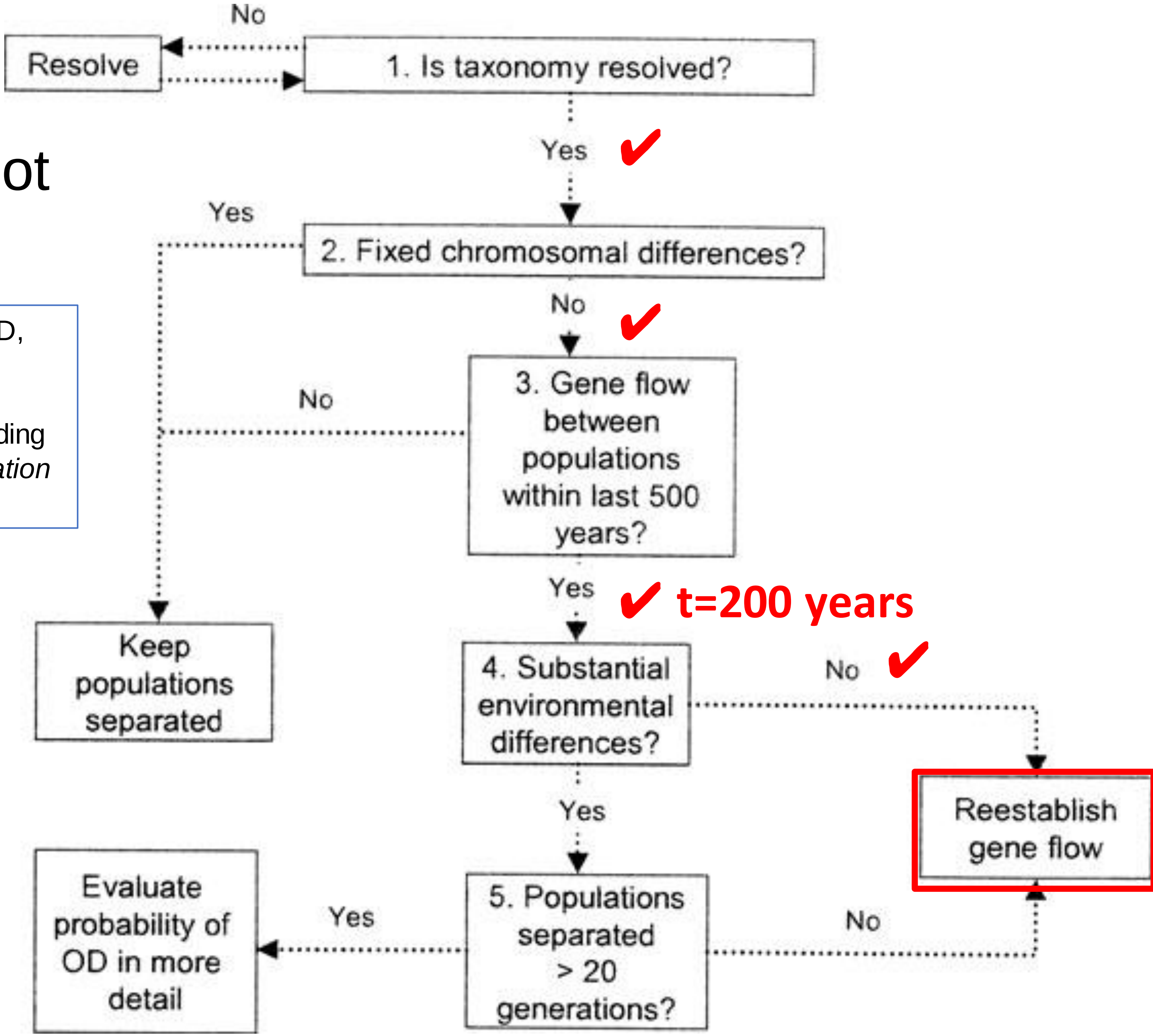
Management

- Selection of founders
- Priorization of breeders
- Design of matings schemes
- Optimal distribution



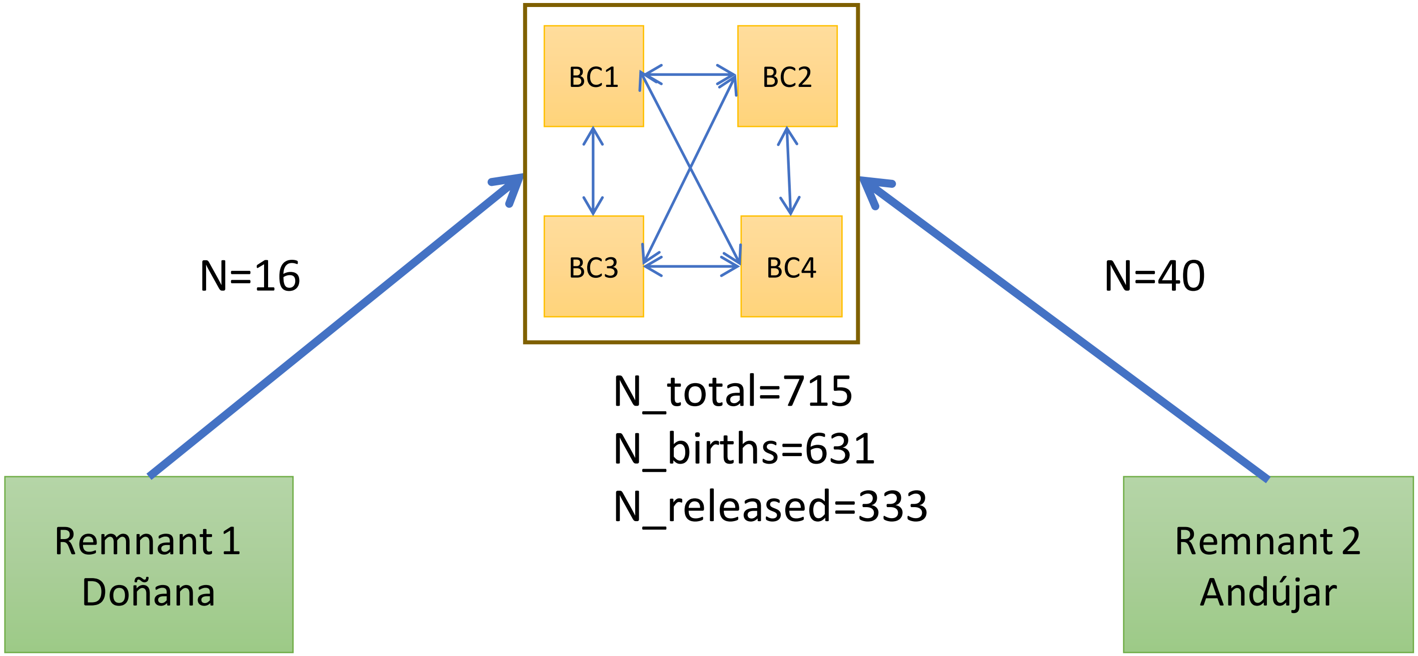
To mix or not to mix?

Frankham R, Ballou JD, Eldridge MDB, *et al.* (2011) Predicting the Probability of Outbreeding Depression. *Conservation Biology* **25**, 465-475



t=200 years

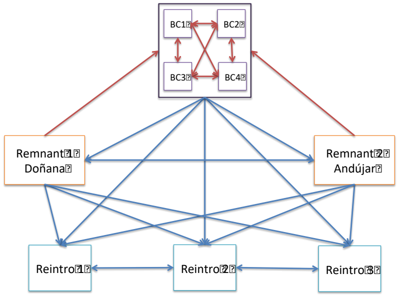
Captive breeding (2005-2021)



N_total=715
N_births=631
N_released=333

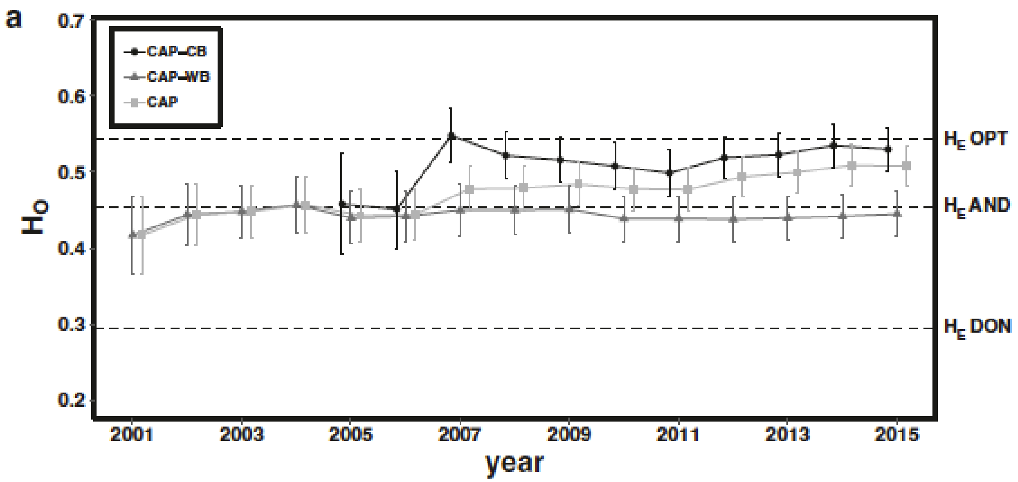
- Current status (2021): N_living=144; N_rep=95; N_cubs=48
- 20-25 attempted matings per year
- 30-45 kittens per year surviving > 2 months (avge = 39,5)
- Most are liberated at reintroduction sites since 2012
- Genetically managed based on minimum kinship

Genetics of captive population

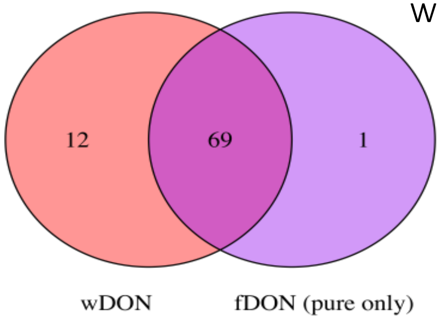

BC1
BC2
BC3
BC4
Remnant 1 Doñana
Remnant 2 Andújar
Reintro 1
Reintro 2
Reintro 3

Wilcoxon signed rank test with continuity correction

	wDON (N=50)	fDON_pure (N=9)	V	p
H_O	0.313	0.358	150	0.056
H_E	0.294	0.300	244	0.945
AR	2.250	1.944	36	0.012



wDON vs. fDON (pure only)



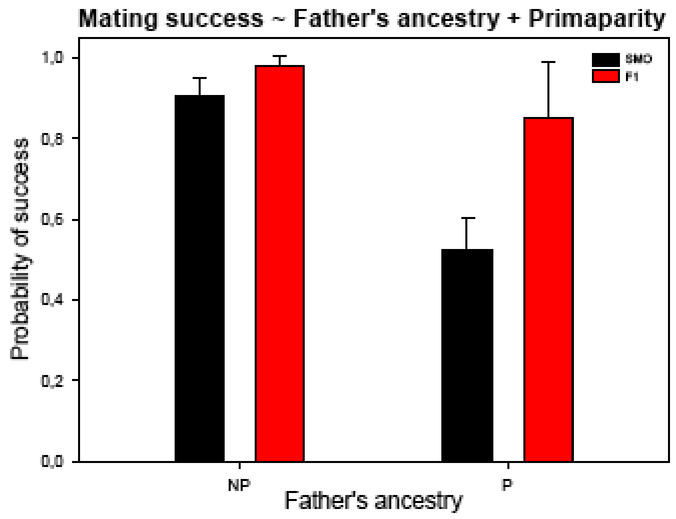
$$\boxed{F_{ST} = 0.005}$$

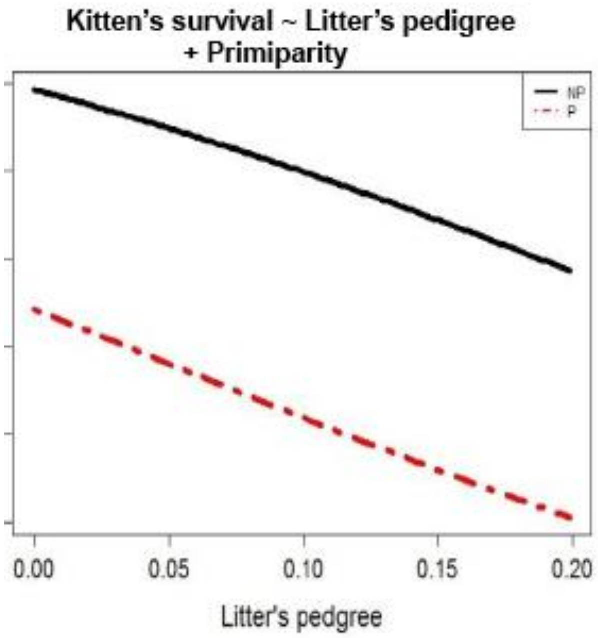
Kleinman-Ruiz, etal.. 2019. Genetic evaluation of the Iberian lynx ex situ conservation programme. Heredity 123 (5):647-661.

- Founders capture most of the genetic variation in the two remnant populations
- Genetic diversity in captivity higher than in remnants and close to maximum posible, due to the mixing of the two stocks and to genetic management

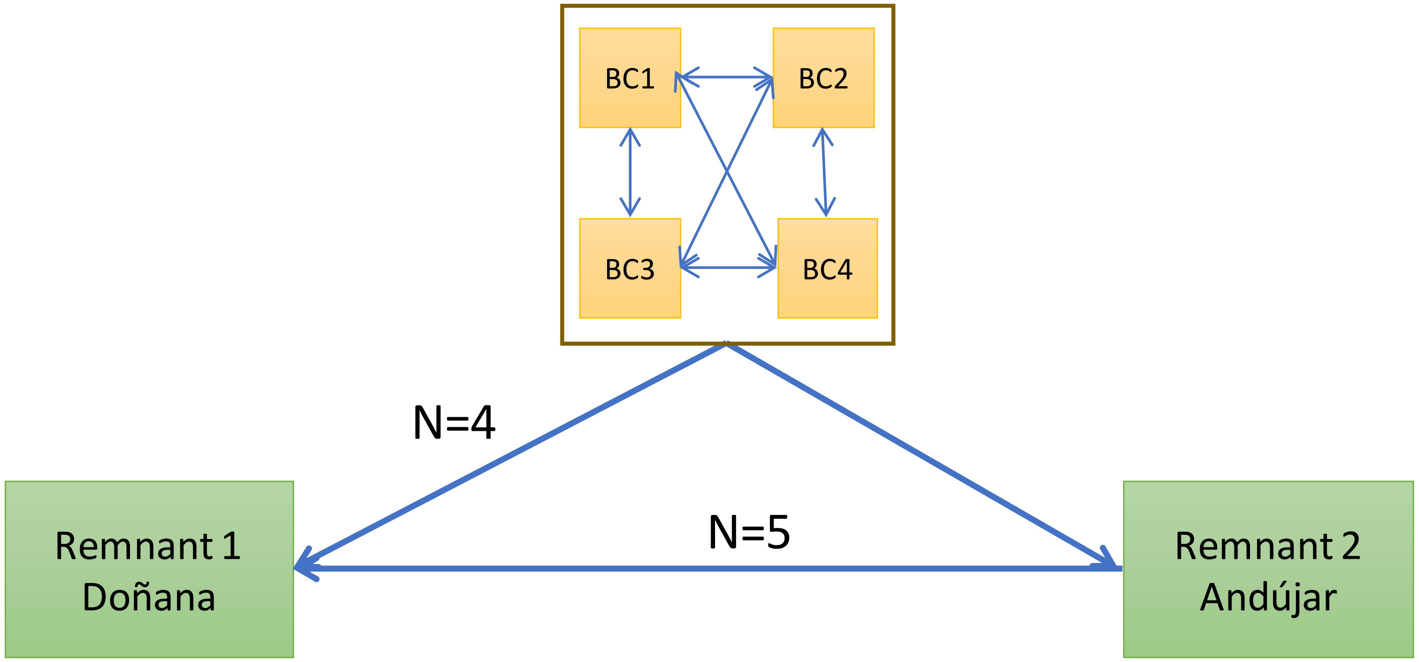
The fitness consequences of genetic management

- Captive-born lynxes are less inbred than wild-born lynxes
- Should result in higher reproduction and survival of released animals
  - Evidence of increased mating success of F1
  - ID for early survial
- Accumulating data from released animals suggests so
  - High survival rate
  - Large litter size


Mating success ~ Father's ancestry + Primaparity

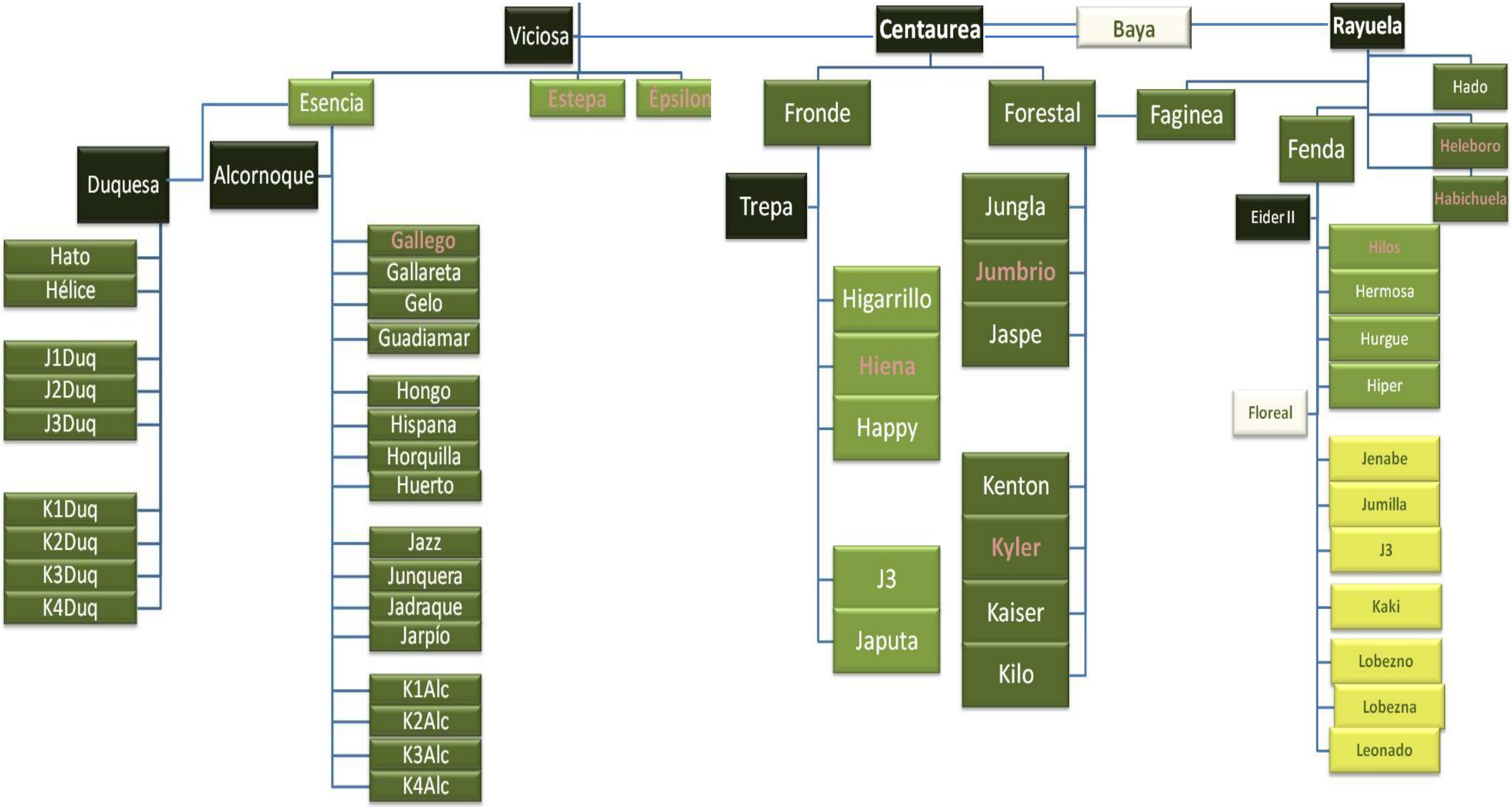

Kitten's survival ~ Litter's pedigree + Primiparity

Translocations for genetic reinforcement



- Nine individuals relased 2007-2019
    - Only three with reproduction confirmed
- Huge impact of first male relased, Baya

The extense progeny of Baya (2008-2014)



Viciosa
Centaurea
Baya
Rayuela

Esencia
Estepa
Épsilon

Duquesa
Alcornoque

Hato
Hélice
J1Duq
J2Duq
J3Duq
K1Duq
K2Duq
K3Duq
K4Duq

Gallego
Gallareta
Gelo
Guadiamar
Hongo
Hispana
Horquilla
Huerto
Jazz
Junquera
Jadraque
Jarpío
K1Alc
K2Alc
K3Alc
K4Alc

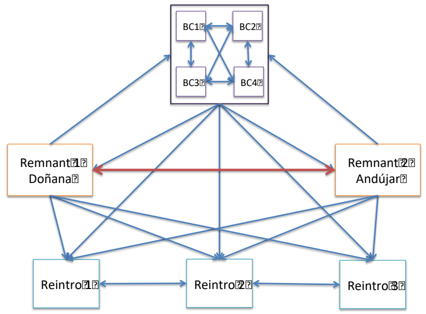
Fronde
Forestal
Trepa
Higarrillo
Hiena
Happy
J3
Japuta

Jungla
Jumbrio
Jaspe
Kenton
Kyler
Kaiser
Kilo

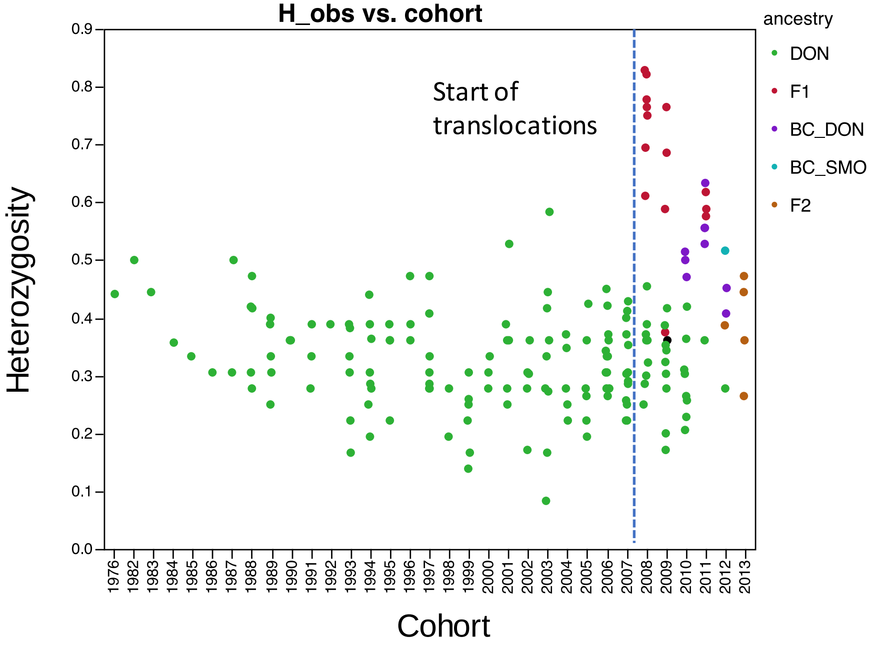
Faginea
Fenda
Hado
Heleboro
Habichuela
Eider II
Floreal

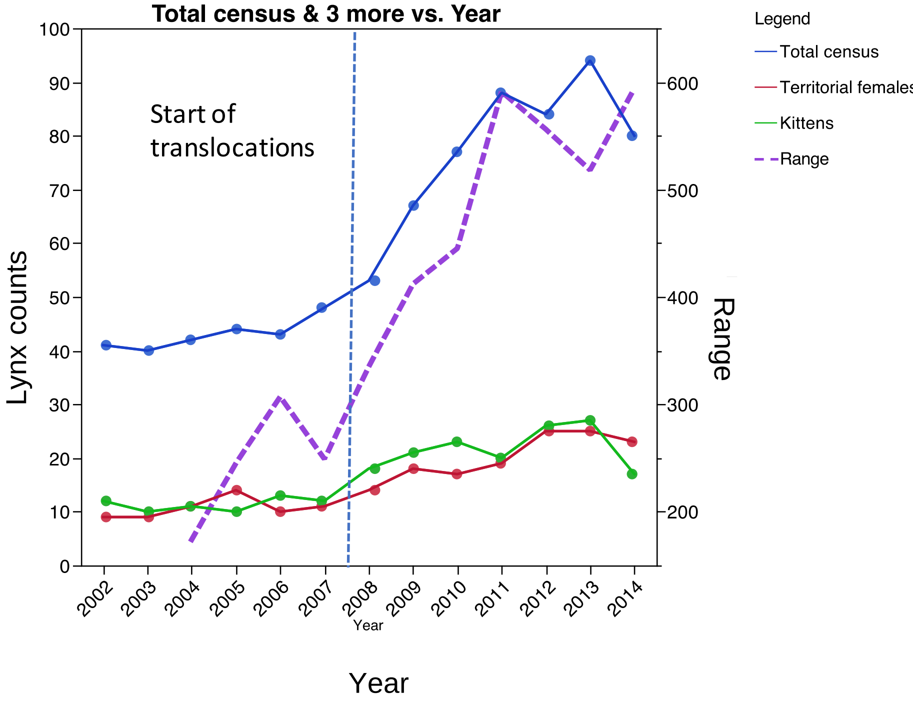
Hilos
Hermosa
Hurgue
Hiper
Jenabe
Jumilla
J3
Kaki
Lobezno
Lobezna
Leonado

Genetic rescue in Doñana



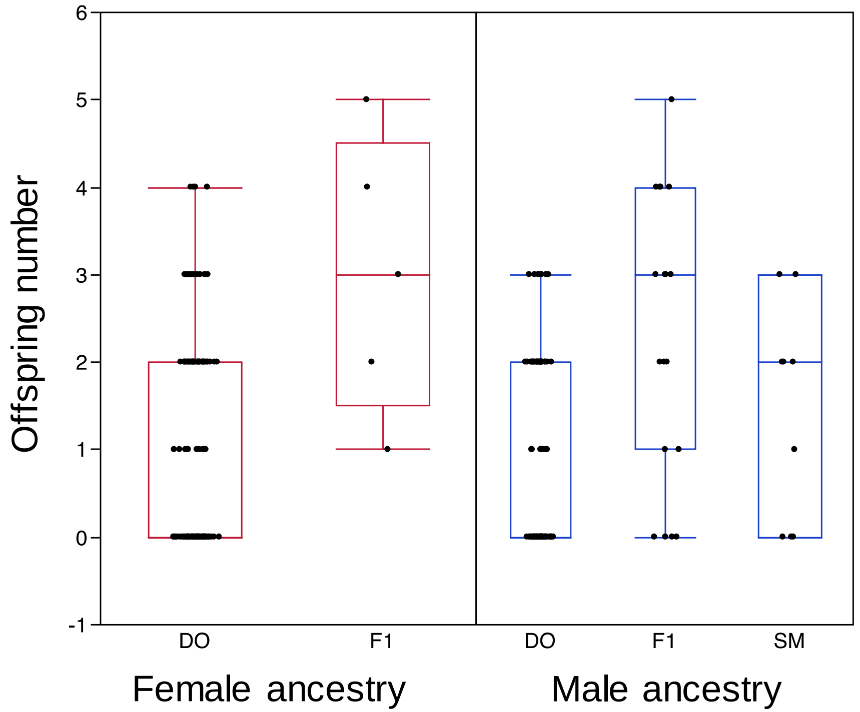
BC1
BC2
BC3
BC4
Remnant 1 Doñana
Remnant 2 Andújar
Reintro 1
Reintro 2
Reintro 3





- Following translocations:
  - Genetic diversity has increased
  - The population has increased

Reproductive output by parent ancestry



Mating success

Source	DF	L-R ChiSquare	Prob>ChiSq
Rabbit abundance	1	17.324142	<.0001*
Male ancestry	2	8.5441719	0.0140*
Female ancestry	1	3.7862033	0.0517

- Males:
  - F1 > SMO ≈ DO
- Females:
  - F1 > DO

Offspring number

No significant results

- Based on kittens observed with females within 9 months following reproduction

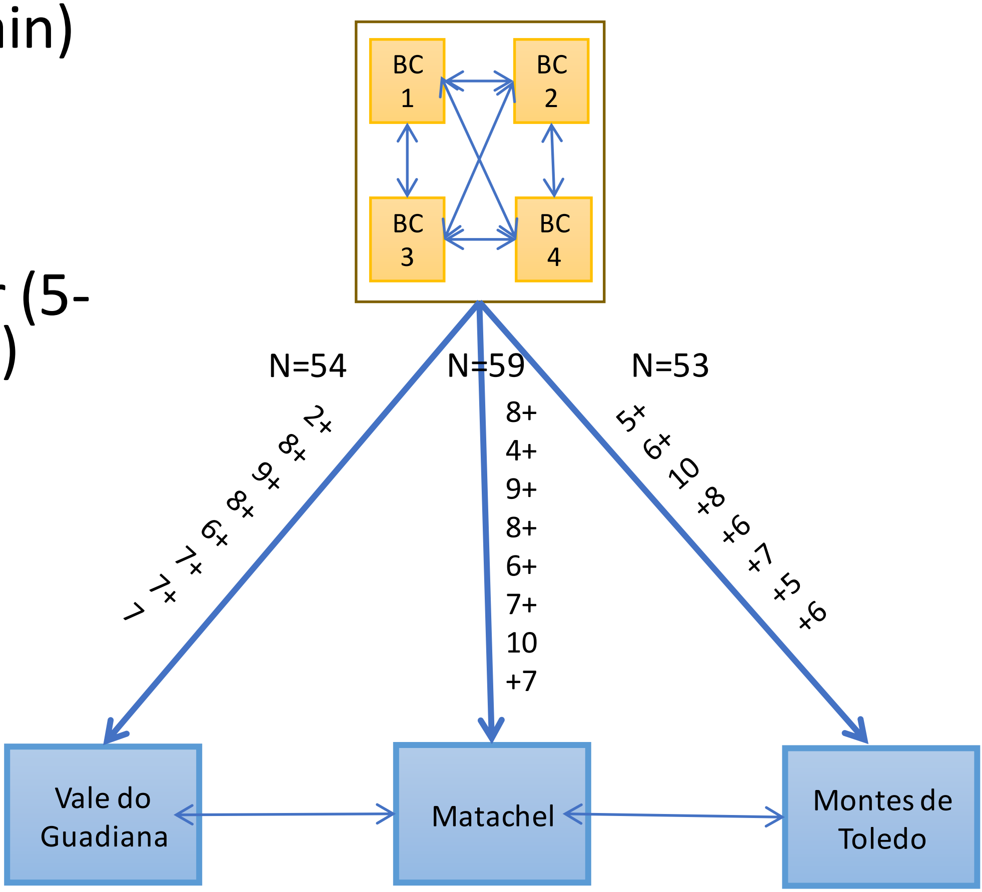
Reintroductions

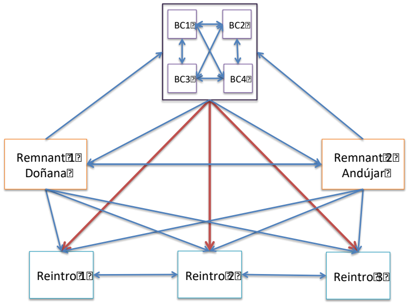
- Areas starting in 2015:
  - Sierra Morena Oriental (C-LM, Spain)
  - Montes de Toledo (C-LM, Spain)
  - Matachel (Extremadura, Spain)
  - Vale do Guadiana (Portugal)

- Captive-born animals
  - 30-45 lynx released per year (5-10 animals per site and year)
  - >300 animals released from captivity by 2021
- Survival >70%
- Reproduction achieved at all sites since year one
  - >300 cubs born in 2021

FUENTE: Programa Life + Iberlince

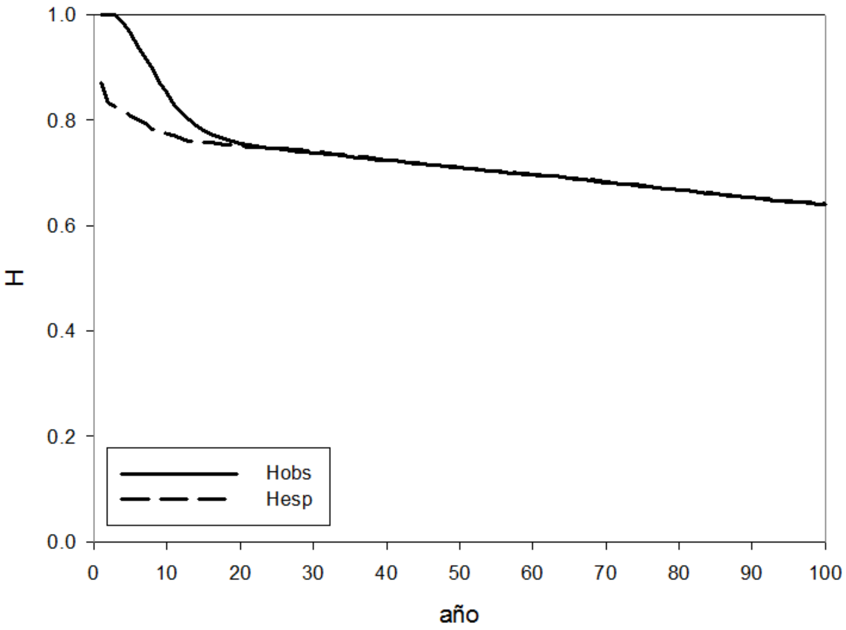
LA VANGUARDIA





Genetic erosion in reintroduced populations

- Reintroduced populations will accumulate inbreeding and lose diversity very fast during the first few generations (founder effect)
- They will continue to do so at lower rates as long as they remain small and isolated



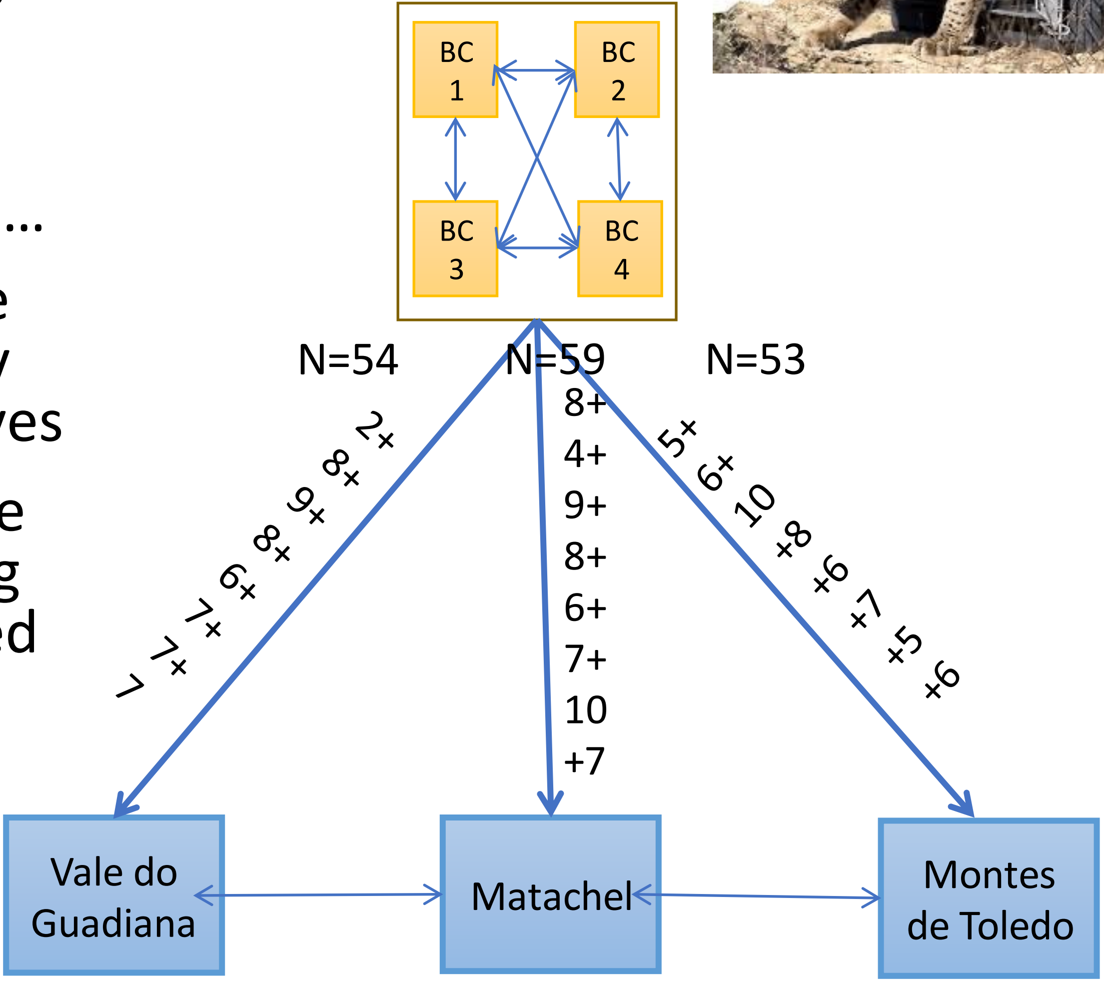
- Favourable scenario
- CC: 30 females
- Census ≈ 300

- ***Δ f* ≈ 0.25 in 20 years**
- ***Ht/Ho* ≈ 70% in 100 years**

Based on spatially-explicit individual-based simulations

Genetic management of reintroductions

- Impossible to select breeders or design matings in the wild, but …
- Released animals can be selected to be minimally related among themselves
- Serial releases create the opportunity for selecting individuals to be released based on MK to existing
- **Requires intensive individual-scale monitoring** to yearly update census and genealogy



BC 1

BC 2

BC 3

BC 4

N=54

7 7+ 7+ 6+ 8+ 9+ 8+ 2+

N=59

8+ 4+ 9+ 8+ 6+ 7+ 10 +7

N=53

5+ 6+ 10 +8 +6 +7 +5 +6

Vale do Guadiana

Matachel

Montes de Toledo

LIFE “Lynxconnect” (2020-2025)

- “Creating a genetically and demographically functional Iberian Lynx (Lynx pardinus) metapopulation)” (LIFE19NAT/ES/001055)
- 18.754.029 euros (60,67% funded by UE)
- 21 partners
  - Public agencies, ONGs, CSIC, …
- It includes an intensive individual-based genetic monitoring and management program assisted by molecular markers

Beneficiario coordinador:

Junta de Andalucía

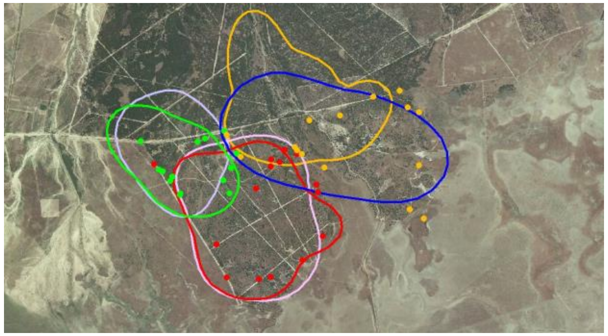
Socios beneficiarios:

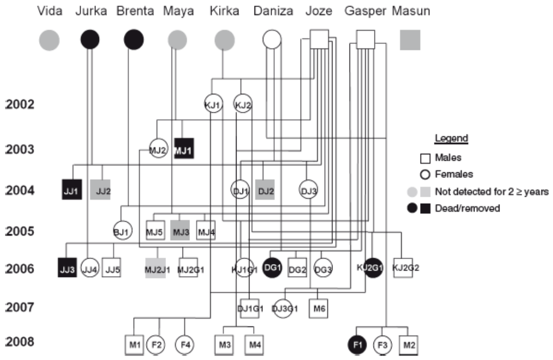
adenex
artemisan
CBD-Hábitat
Castilla-La Mancha
Castilla-La Mancha
cimbal
Estación Biológica Doñana CSIC
Diputación de Badajoz
Junta de Extremadura
Región de Murcia
Junta de Extremadura
FomeCam
FOTEX
ICNF
Infraestruturas de Portugal
WWF

Intensive individual-based genetic monitoring: census and genealogies

- Goals
  - Census of individuals
  - Geo-referenced: territories, movements, immigrants,…
  - **Genealogies**
  - **Genetic parameters**

- Methods
  - Intensive sampling of faeces
  - Individual identification
  - Parentage assignment
  - Kinship estimates





Monitoring: novel SNP markers

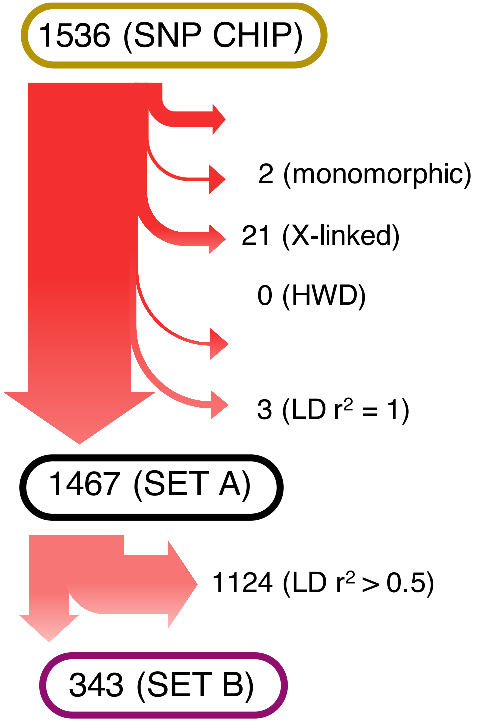
- Novel and more powerful panel of molecular markers for:
    - Population genetics,
    - Non invasive genetic monitoring
    - Genetic management

Marker	Size	PIDs	PnE	PWR FS-HS
SNPs	343	$4.7*10^{-71}$	$2.5*10^{-29}$	> 0.999
	192	$2.5*10^{-43}$	$8.8*10^{-18}$	0.998
	96	$2.4*10^{-22}$	$2.4*10^{-09}$	0.945
	48	$1.4*10^{-11}$	$4.7*10^{-05}$	0.741
	24	$3.7*10^{-06}$	$6.9*10^{-03}$	0.488
	12	$1.9*10^{-03}$	$8.3*10^{-02}$	-
Micros	36	$1.2*10^{-09}$	$5.6*10^{-06}$	0.750
	12	$9.7*10^{-05}$	$1.8*10^{-03}$	0.503

Individual identif.

Parentage assignment

Kinship estimation



1536 (SNP CHIP)

2 (monomorphic)

21 (X-linked)

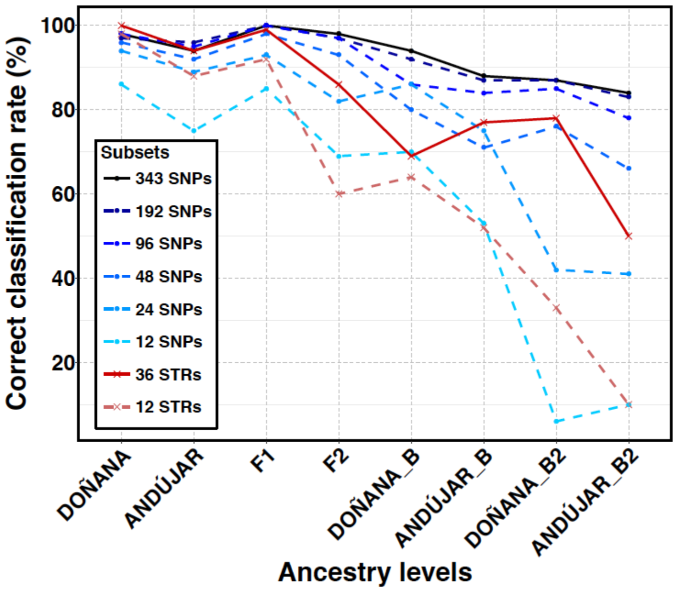
0 (HWD)

3 (LD $r^2 = 1$)

1467 (SET A)

1124 (LD $r^2 > 0.5$)

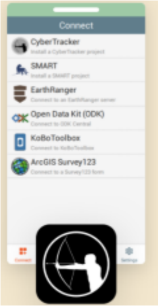
343 (SET B)



Kleinman-Ruiz *et al.* (2017) Novel efficient genome-wide SNP panels for the conservation of the highly endangered Iberian lynx. *BMC Genomics* **18**, 556.

Workflow of genetic monitoring

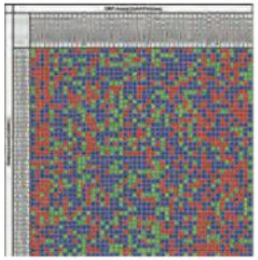
Field

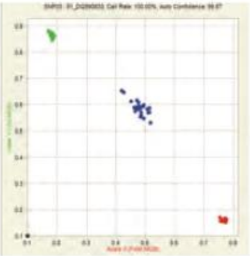


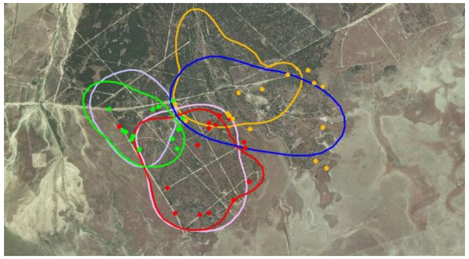
Laboratory

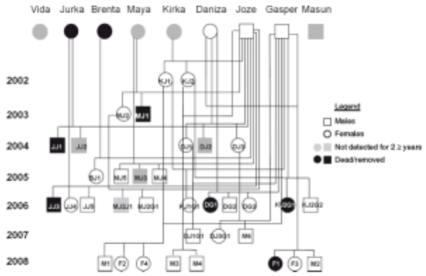
Highly informative SNP panels (Kleinman-Ruiz et al., 2017)

Computer









Iberian lynx conservation

Programs

- Captive breeding
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- Reintroduction

Management

- Selection of founders
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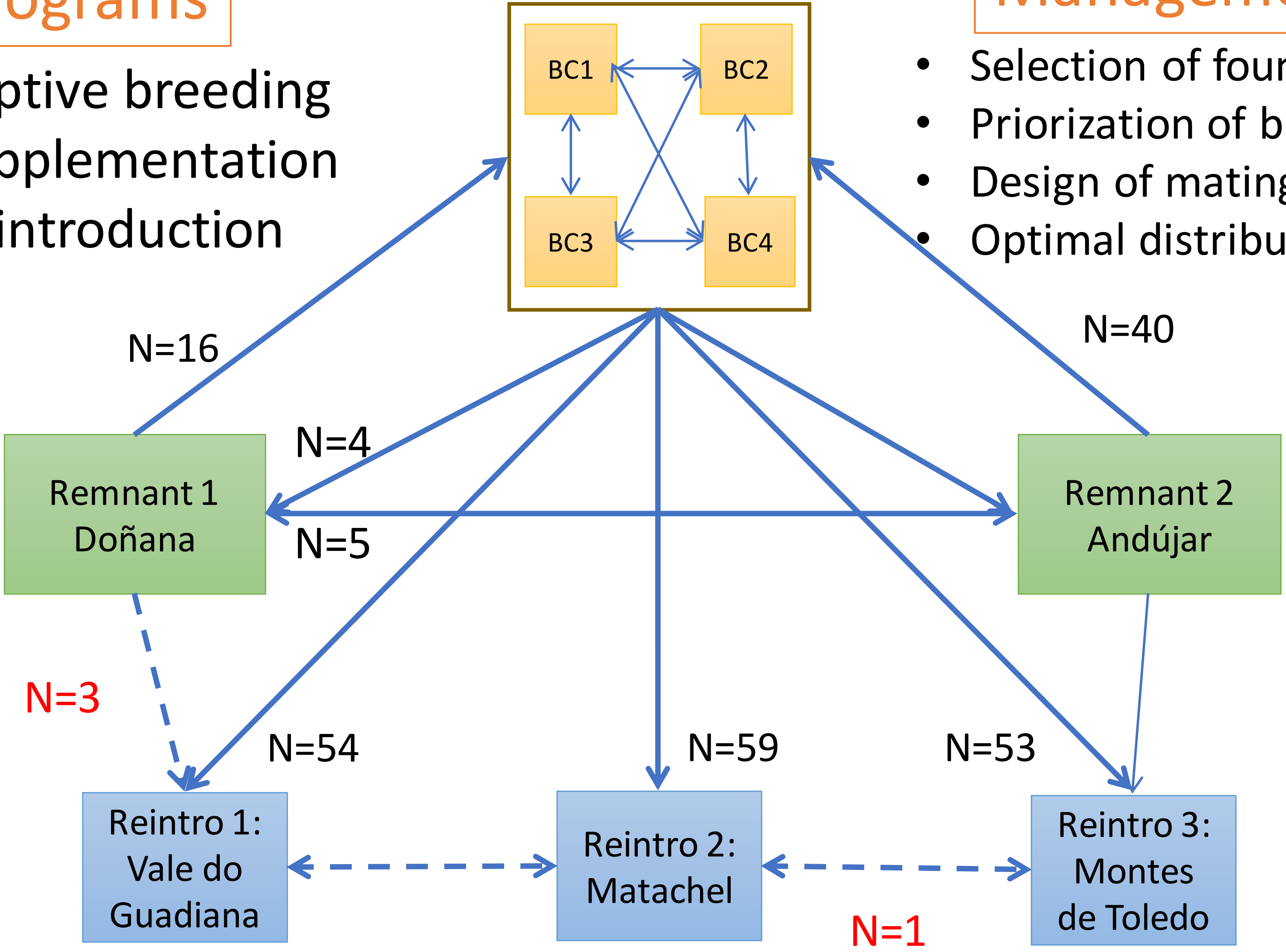
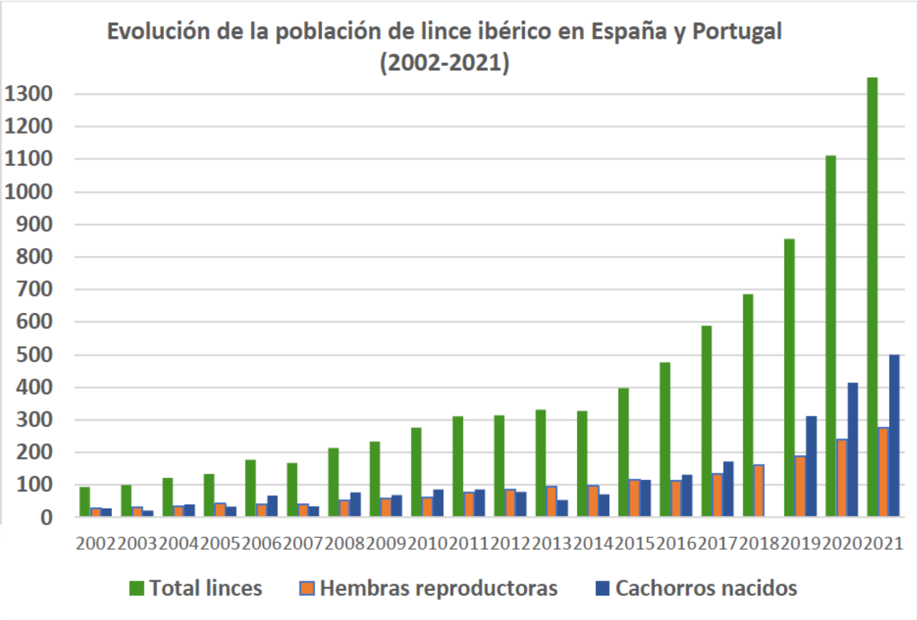
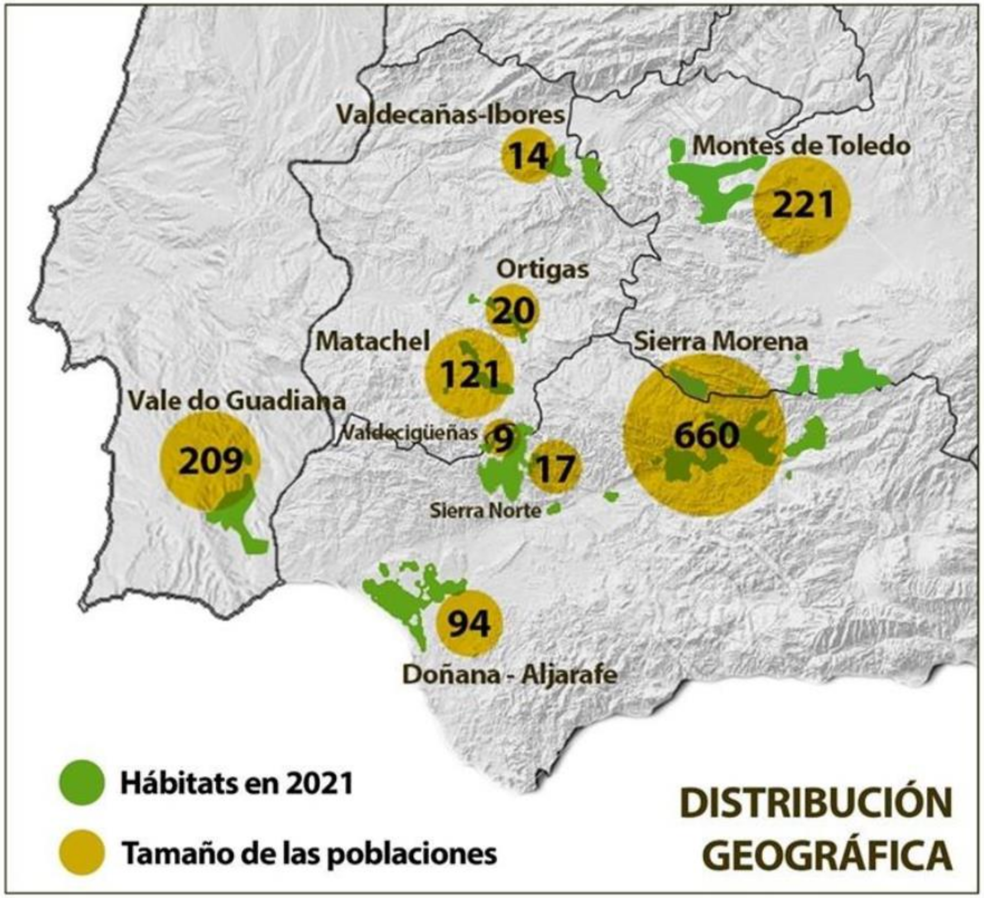


Status 2021





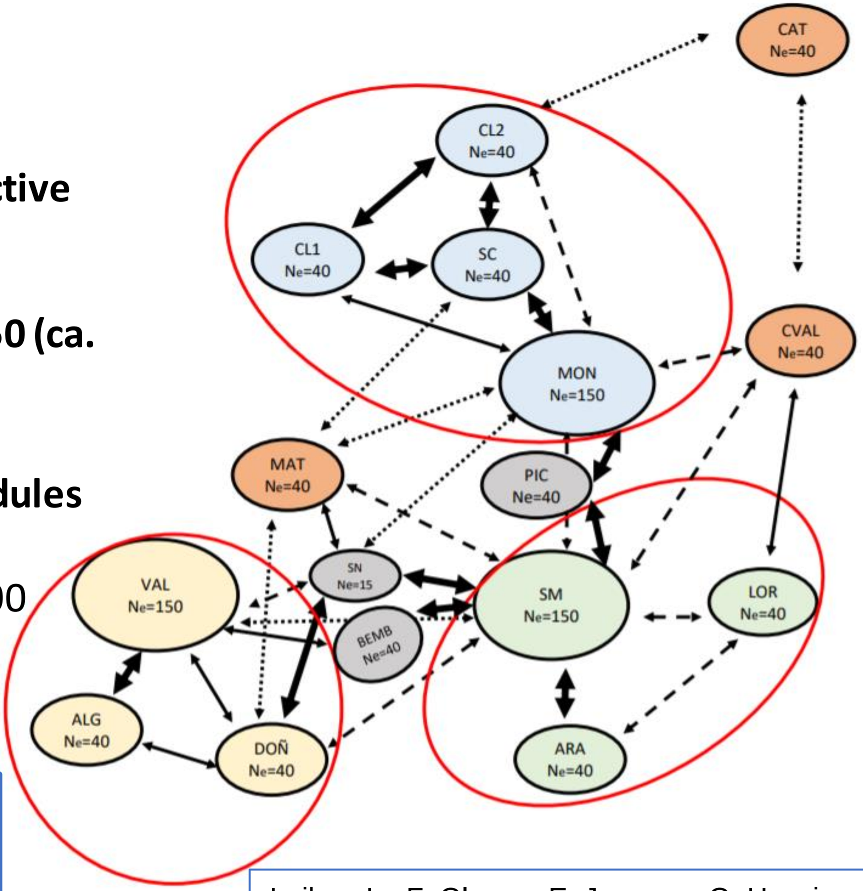
- 1.365 individuals
  - 865 >1-year-old
  - 277 territorial females
  - 500 cubs born
- 5 populations >20 ind
  - 2 remnants
  - 3 reintroduced

lynxconnect

Metapopulation Ne: FCS and genetic viability

- **Migration rates** must be between **1-4 migrants/generation**
- **8 additional populations** must be created.
- All populations must be of **N_{ex}=40 (50 reproductive females**)
- At least **three populations should reach N_{ex}=150 (ca. 190 reproductive females**).
- Viable scenario with **three well connected modules**
- A sum of **N_{ex}≥865** is needed, which implies 2,200 mature individuals ≈ **1,100 breeding females**.

> ➢ Focus reintroduction efforts in well-connected populations.
>
> ➢ Increase the target to 50 reproductive females.
>
> ➢ 1100 breeding females as FRP for demographic plus genetic viability



Laikre, L., F. Olsson, E. Jansson, O. Hossjer and N. Ryman (2016). "Metapopulation effective size and conservation genetic goals for the Fennoscandian wolf (Canis lupus) population." Heredity **117**(4): 279-289.

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  - Lucía Mayor (Master student)
  - Lorena Lorenzo (PhD candidate)
  - Enrico Bazzicalupo (PhD candidate)
  - María Lucena (PhD, 2021)
  - Daniel Kleinman (PhD, 2021)
  - Elena Marmesat (PhD, 2020)
  - Laura Soriano (Technician)

- Funding

Fundación BBVA

Fundación General CSIC

Santander

lynxconnect

Life

GOBIERNO DE ESPAÑA
MINISTERIO DE CIENCIA E INNOVACIÓN

Obra Social "la Caixa"

GOBIERNO DE ESPAÑA
MINISTERIO DE MEDIO AMBIENTE

JUNTA DE ANDALUCIA

- Main collaborators
  - Population genomics
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  - Lynx genomics and evolution
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    - Aurora Ruiz-Herrera (UAB)
    - Oscar Lao (CRG-CNAG)
  - Genetic monitoring and management
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    - Jesús Fernández
    - LIFE Lynxconnect / Lynx ex situ
  - Ancient DNA
    - Michael Hofreiter
    - Love Dalen
    - Axel Barrow

Dank je!