LIFE EURO LARGE CARNIVORES

La comprometida genética del lince ibérico y su gestión

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Outline

- Is genetic erosion limiting the viability of Iberian lynx?

- What can we do about it?

- How to define safe/viable scenarios taking into account genetics?
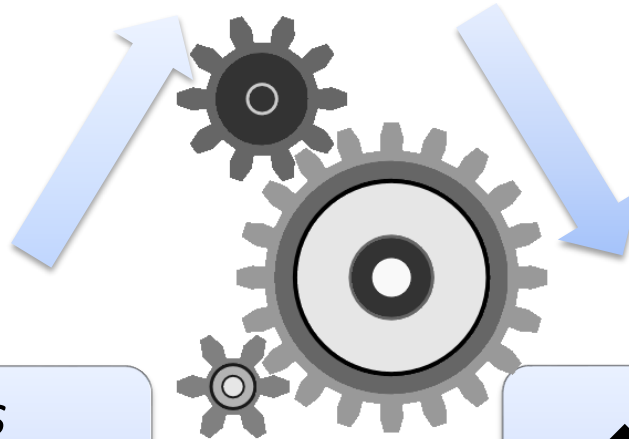
The extinction vortex

Habitat loss
Over-explotiation
Polution
Invasive species
Climate change

→

Small population

→

Extinction

↓ *Fitness*

↓ Adaptive potential

↑ Inbreeding

↓ Diveristy

Genetic risks for population viability

Small populaton

Genetic erosion

Inbreeding, genetic load

Loss of diversity

Lower fitness

Loss of adaptive potential

Short term

Long term (?)

Average genomic diversity

- The Iberian lynx has the lowest genome-wide diversity reported ~~s~~ an ~~s~~ ed

...ruz F, *et al.* ...omic erosion after ...ographic bottlenecks in the ...ndangered Iberian lynx. *Genome* ...ology **17**, 251.

Very low diversity !
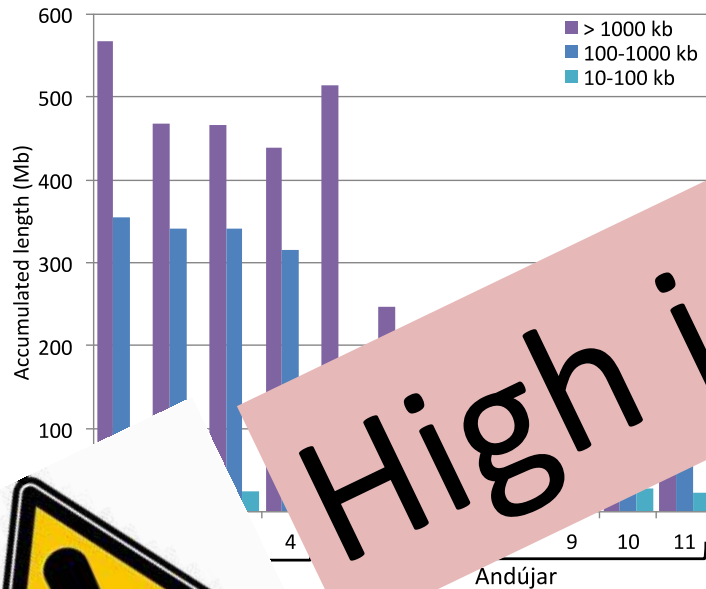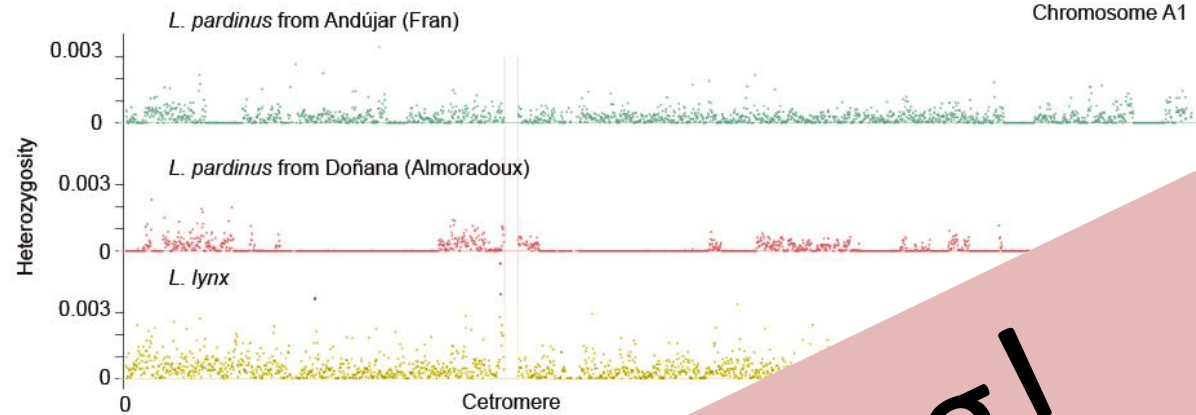
Heterozygous SNP/Mb

Species	Heterozygous SNP/Mb
Western Gorilla	1630
Orang-Utan (Sumatran)	1200
Blind mole rat	1160
Bactrian camel	1158
Giant panda	1120
Mammoth (Wrangel Is.)	1000
Chimpanzee	
Dromedary	
White tiger	
Naked mole rat	
Human	
Orangutan	
	548
	580
	530
Amur tiger	490
White lion	480
Gibbon	370
Tasmanian devil	320
Eurasian Lynx	279
Yangtze River dolphin	262
Snow leopard	230
Altai Neanderthal	170
Domestic cat	120
Lynx pardinus	102

Coding sequences

Species	Species profile	No. of genes	πs (%)	πN/πS	dN/dS
Ciona intestinalis	Highly abundant, invertebrate	1602	5.70	0.046	
Drosophila simulans	Highly abundant, invertebrate	10996			
Oryctolagus cuniculus algirus1	Abundant, mammal				ͻ83
Homo sapiens	Old bottle			ͻ.241	0.229
Chelonoidis nigra2	Fr		0.190	0.310	0.140
Lynx pardinus		14028	0.028	0.286	0.212

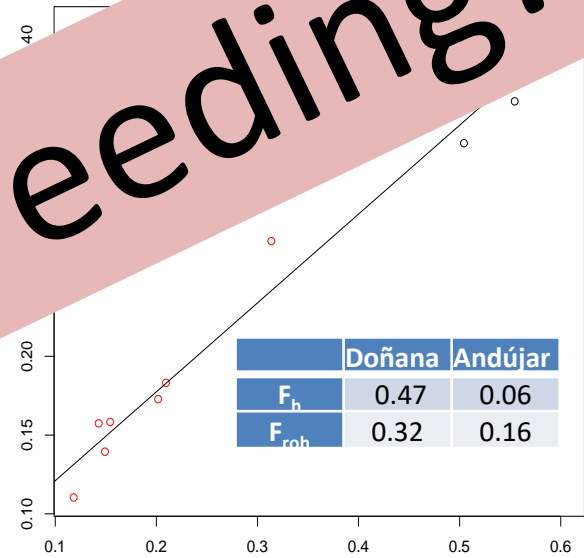High genetic load!

- ...ow ...rsity at coding sequences
- ... non-synonymous to synonymous diversity and ...ons
- high rates of slightly deleterious alleles at moderate or high frequency; some might have become fixed
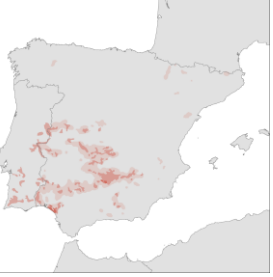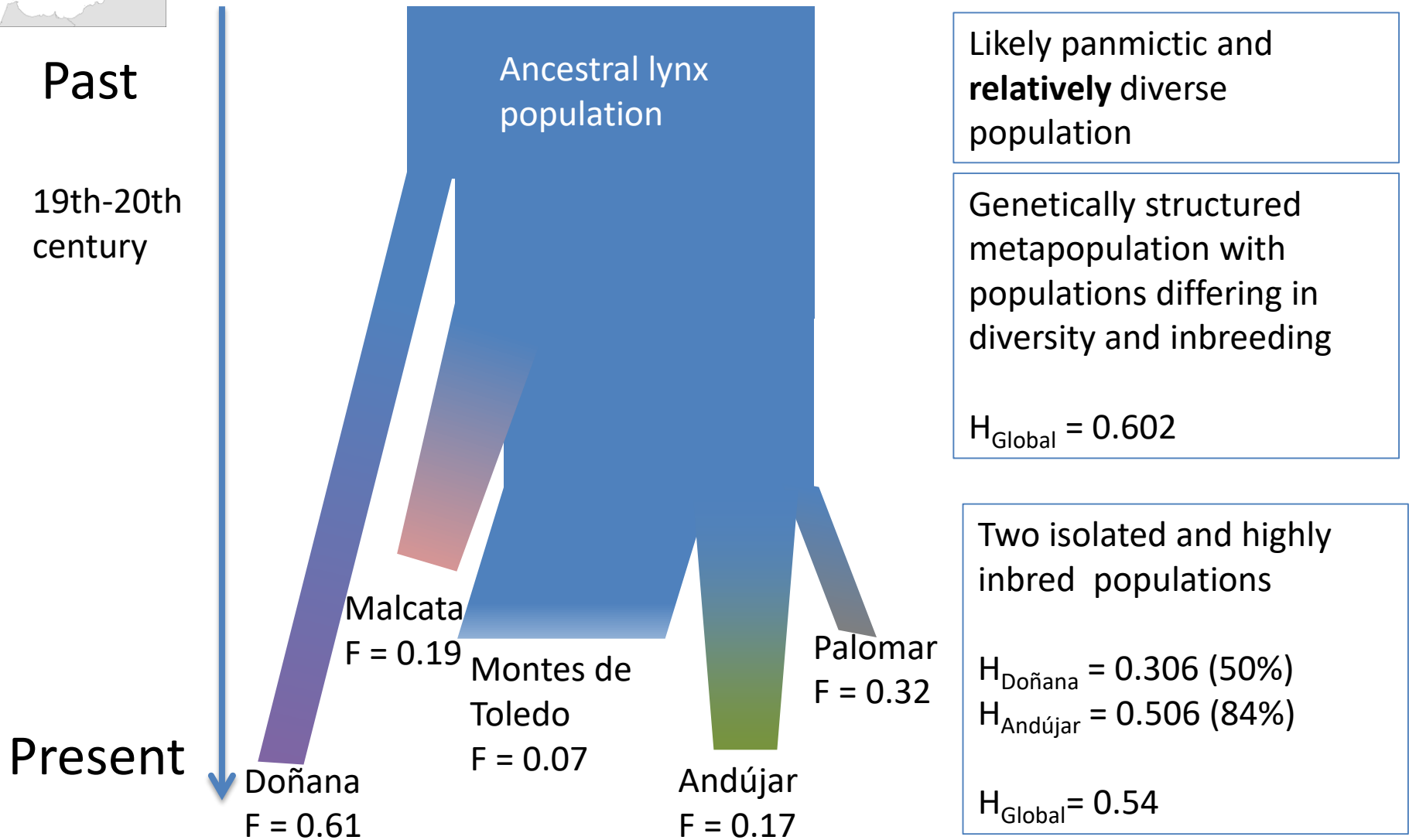
Individual inbreeding: ROHs

L. pardinus from Andújar (Fran)

Chromosome A1

L. pardinus from Doñana (Almoradoux)

L. lynx

Cetromere

- > 1000 kb
- 100-1000 kb
- 10-100 kb

Accumulated length (Mb)

Andújar

	Doñana	Andújar
F_h	0.47	0.06
F_{roh}	0.32	0.16

- ...recent (genealogical)
...in Doñana (except Candiles)
- ...length ROHs, reflect recent low Ne:
...er in Doñana

- High average inbreeding in Doñana, moderate in Andújar
- Inbreeding estimates based on ROHs or allele frequenies are highly correlated

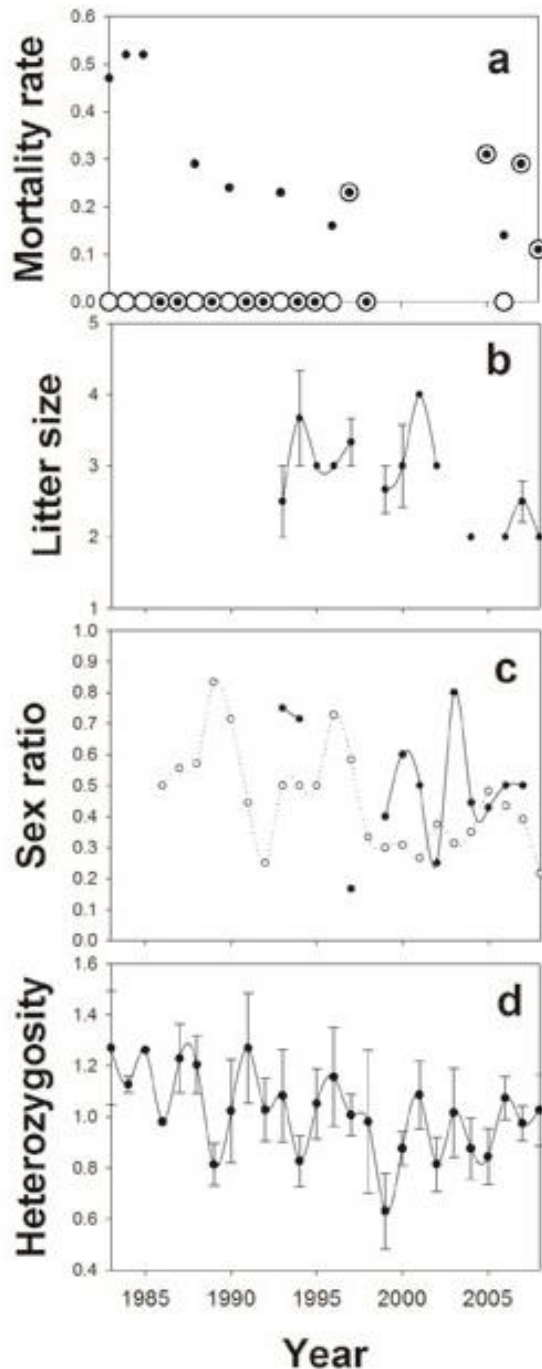**High inbreeding!**

Genetic history: recent erosion

Past

19th-20th century

Ancestral lynx population

Likely panmictic and **relatively** diverse population

Genetically structured metapopulation with populations differing in diversity and inbreeding

H_{Global} = 0.602

Malcata
F = 0.19

Montes de Toleto
F = 0.07

Palomar
F = 0.32

Two isolated and highly inbred populations

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

H_{Global} = 0.54

Present

Doñana
F = 0.61

Andújar
F = 0.17

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.

Extinction vortex in Doñana?

- Concomitant deterioration of genetic and demographic parameters
  - Increased non-traumatic mortality
  - Skewed sex ratios
  - Decreased litter size
    - 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).

Heterozygosity-Fitness Correlations and Inbreeding Depression in Two Critically Endangered Mammals

MARÍA JOSÉ RUIZ-LÓPEZ,*‡‡ NATALIA GAÑAN,* JOSÉ ANTONIO GODOY,† ANA DEL OLMO,*
JULIAN GARDE,‡ GERARDO ESPESO,§ ASTRID VARGAS,** FERNANDO MARTINEZ,**
EDUARDO R. S. ROLDÁN,*§§ AND MONTSERRAT GOMENDIO*††§§

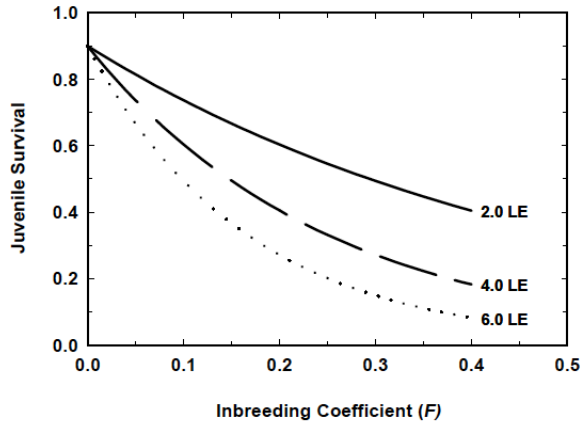- Heterozygosity (inbreeding) correlates with the proportion of normal sperm
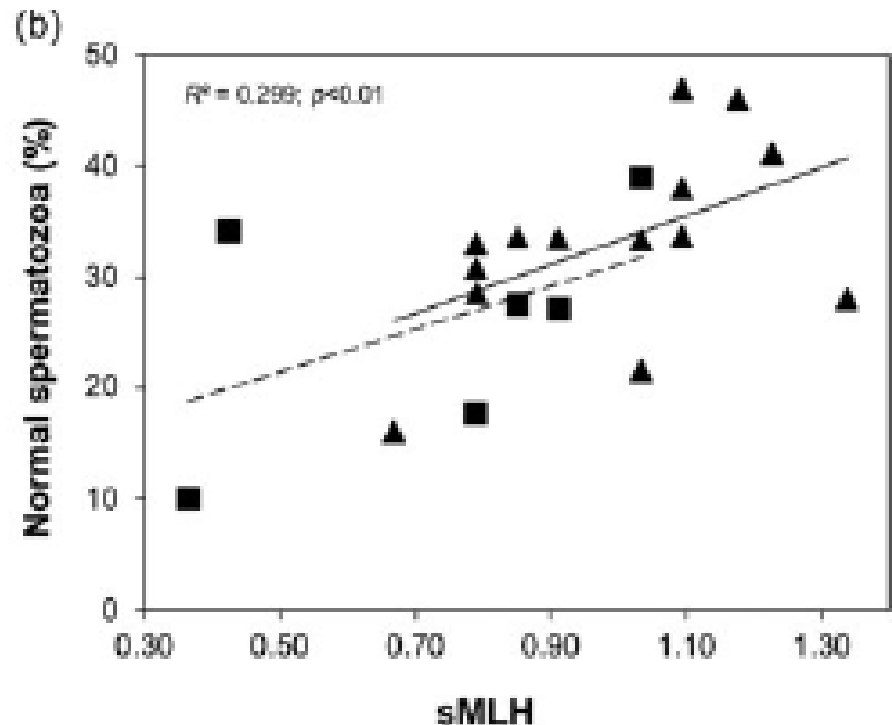
Survival and reproduction in captivity

- Higher mating success of admixed F1 males

- Early survival decreases with inbreeding

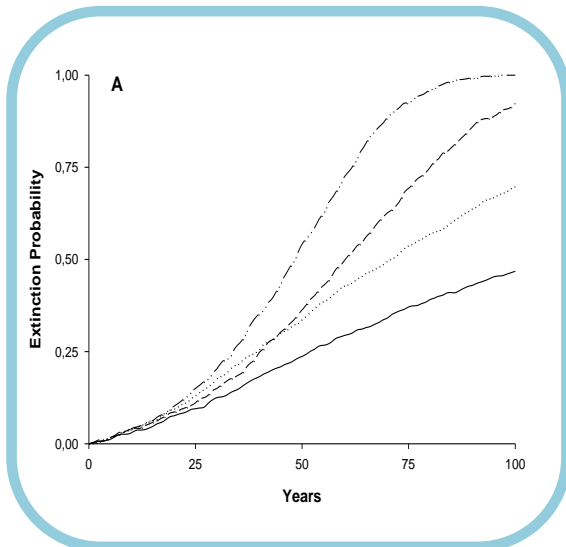- ID expected to be much higher in the wild

Cuenca et al., in preparation

Mating success ~ Father's ancestry + Primaparity

Kitten's survival ~ Litter's pedigree + Primiparity
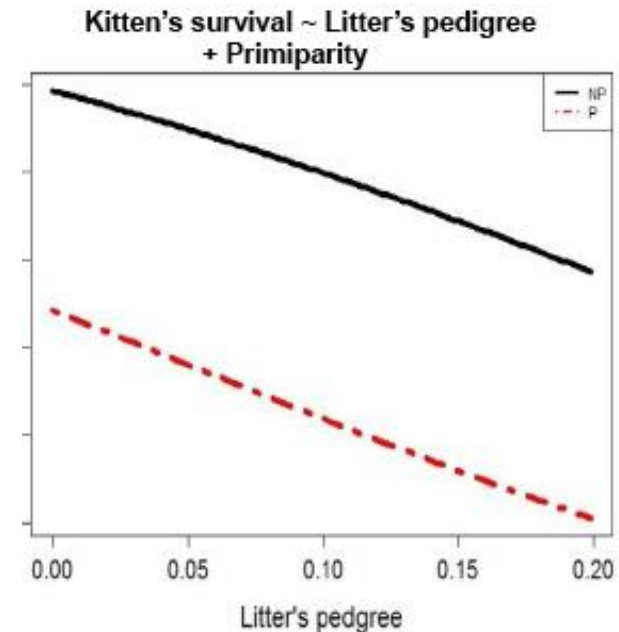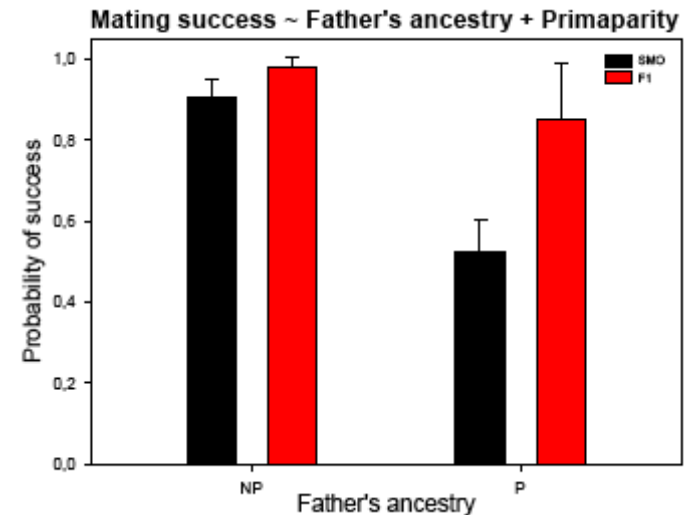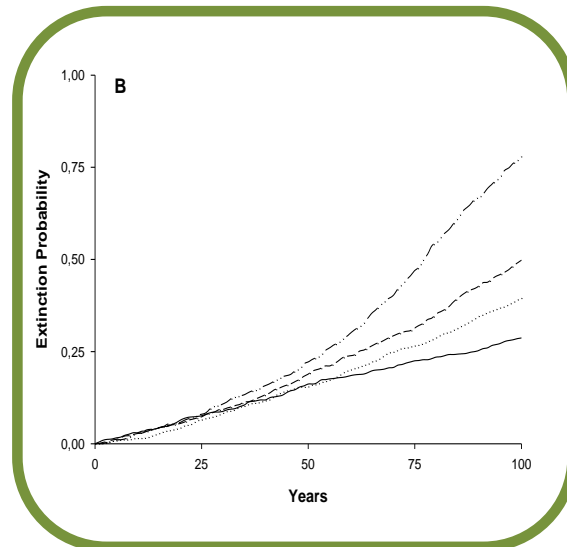
ID and extinction

Decrease in juvenile
survial with inbreeding
$S = S_0 e^{-bF}$

- **PVA with Vortex for realistic lynx scenarios:**
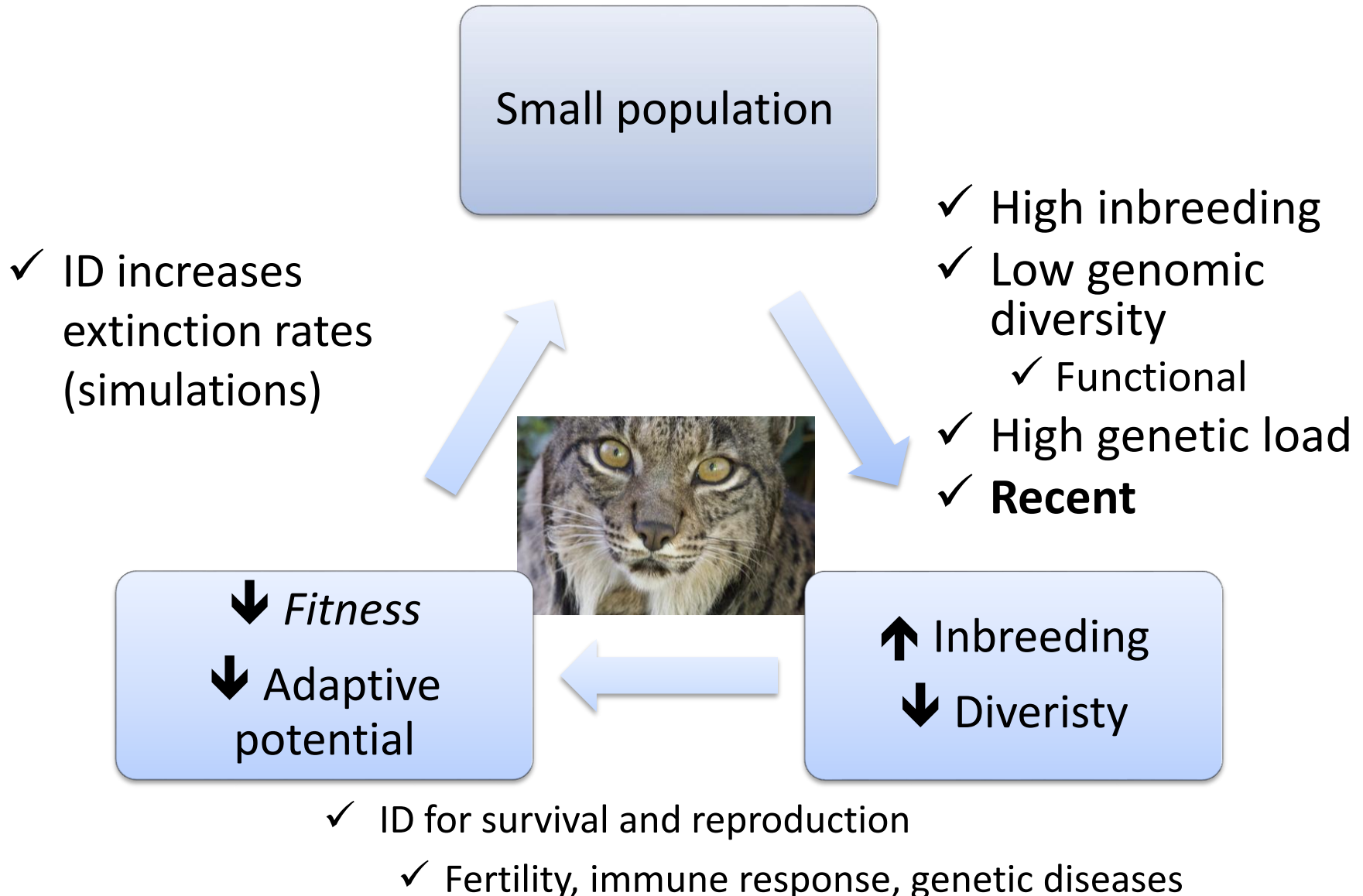 - Even modest amounts of ID can substantially increase extinction probabilities

DOÑANA
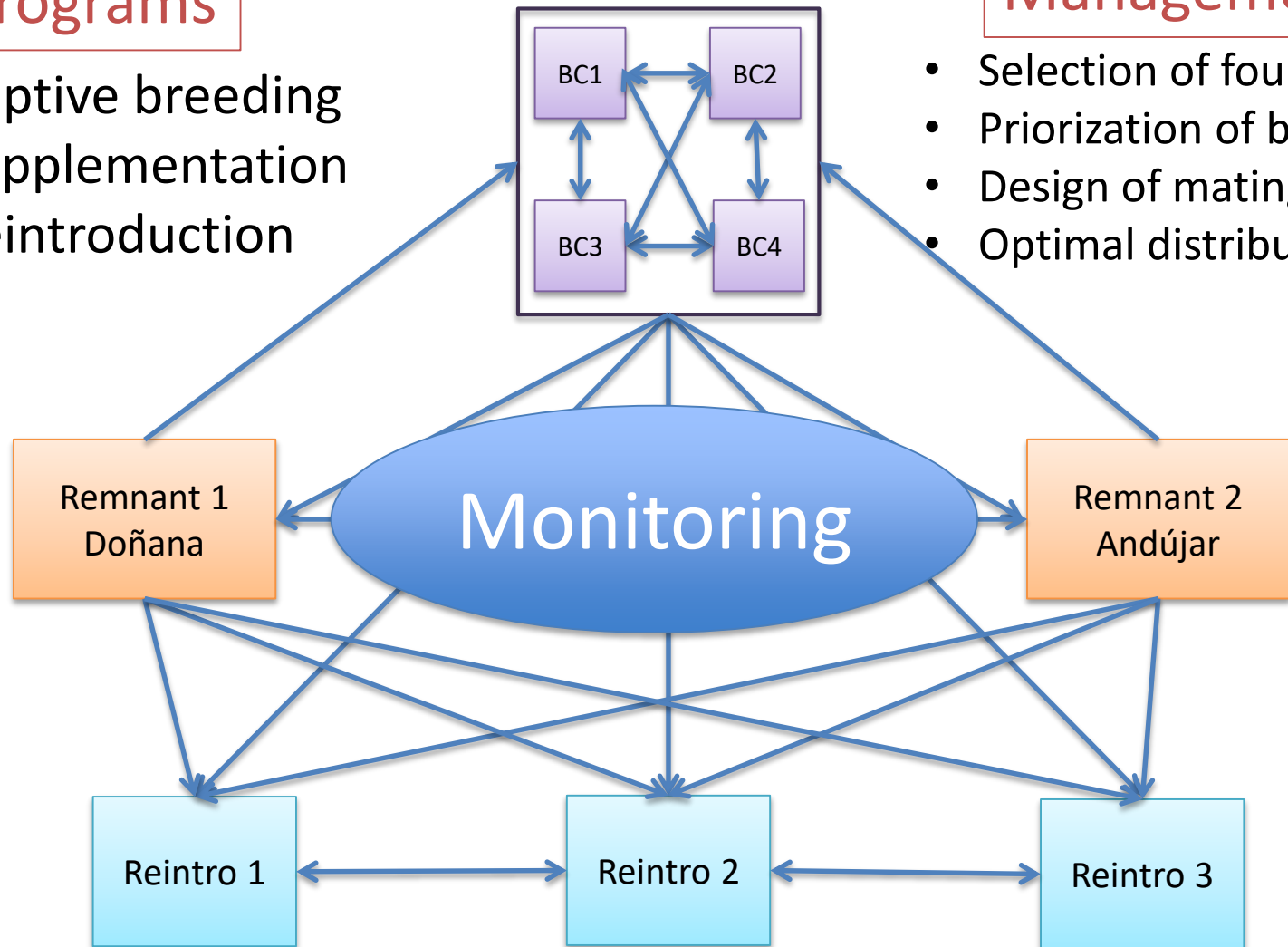

SIERRA MORENA

A lynx extinction vortex?

Small population

✓ High inbreeding
✓ Low genomic diversity
    ✓ Functional
✓ High genetic load
✓ **Recent**

✓ ID increases extinction rates (simulations)

⬇ *Fitness*
⬇ Adaptive potential

⬆ Inbreeding
⬇ Diveristy

✓ ID for survival and reproduction
    ✓ Fertility, immune response, genetic diseases
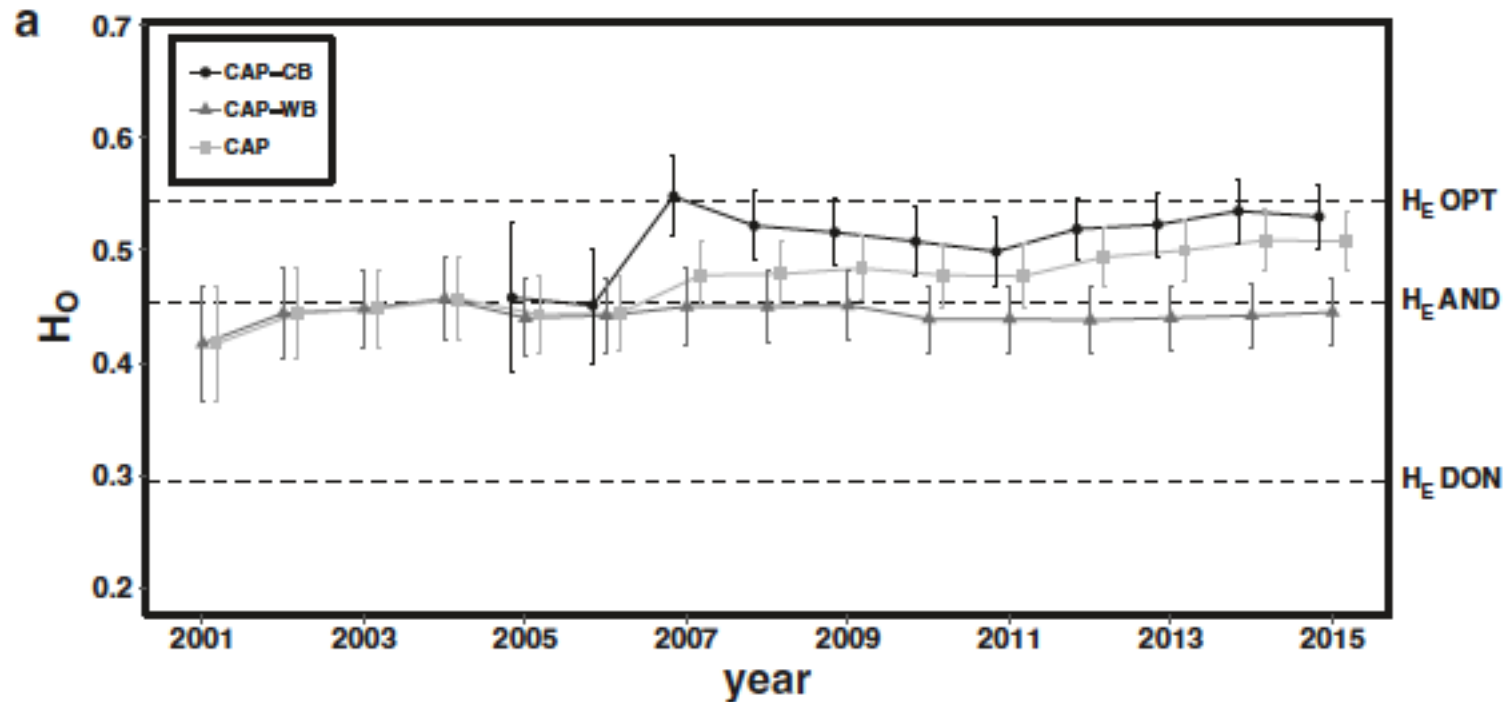
Iberian lynx conservation

Genetic management: principles

- Rate of loss of diversity depends on effective population size (N_e)
- N_e depends on variance in reproductive success
  - $Ne \approx 0.1\text{-}0.2\ N$
- Inbreeding depends on the relatedness between mates
- Genetic management strategy:
  - Equalize founder contribution (maximize Ne)
    - Taking into account relatedness among founders
      - **Minimum kinship strategy**
  - Avoid mating between close relatives
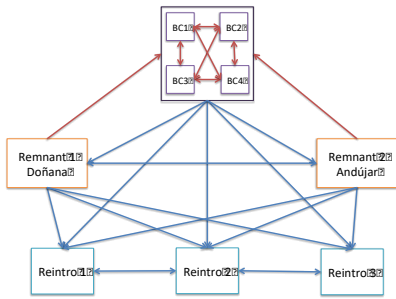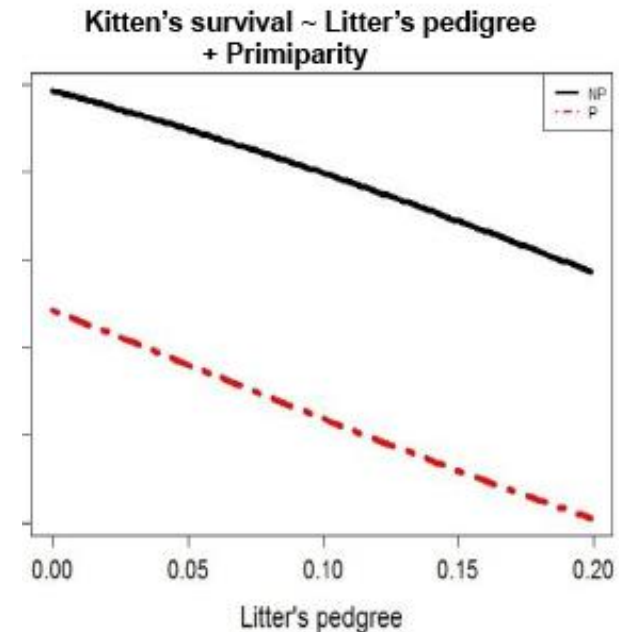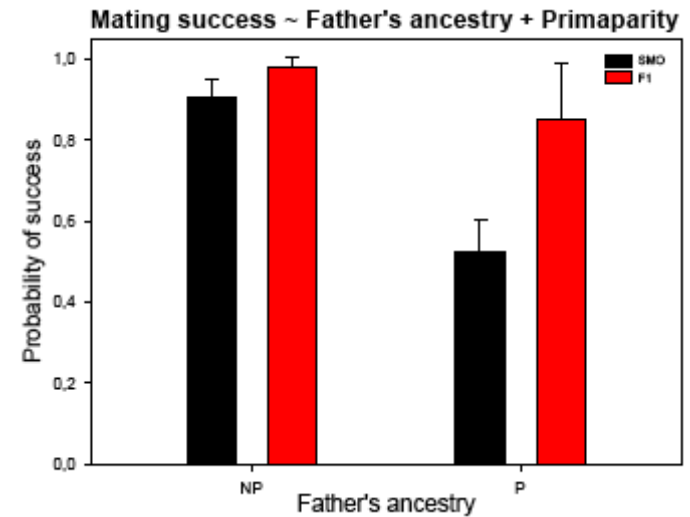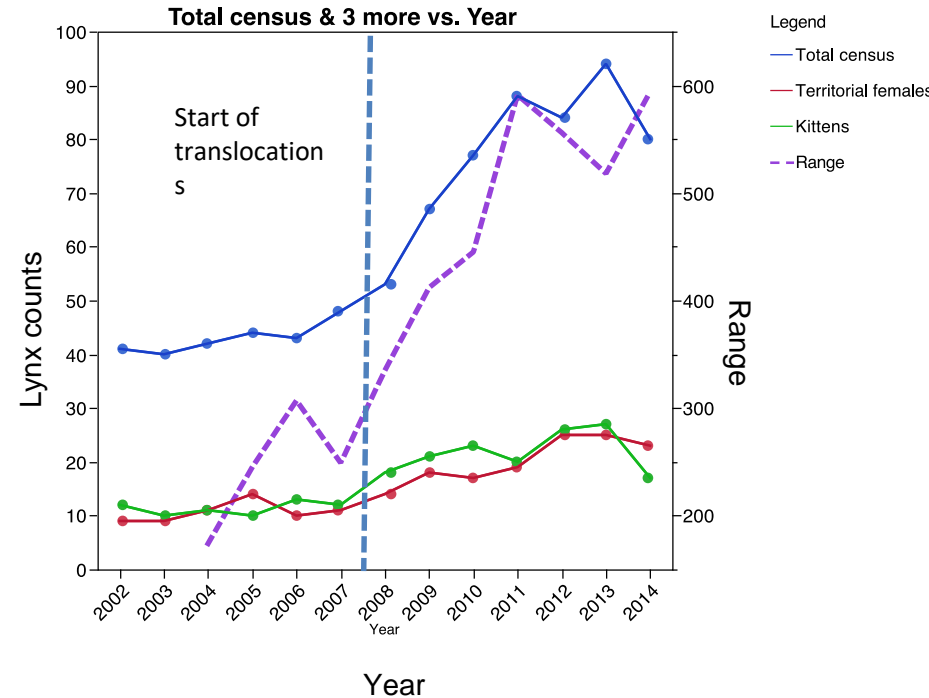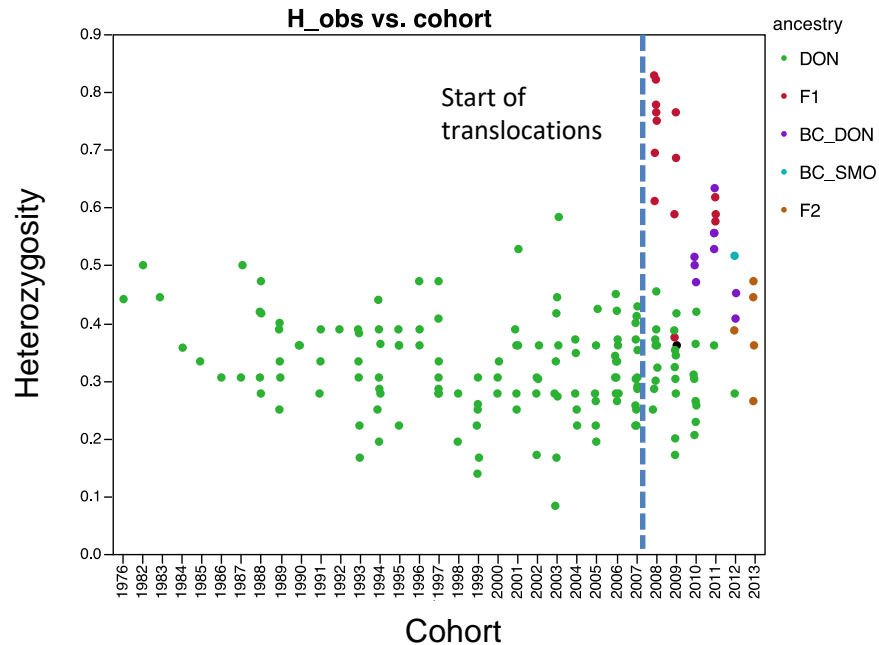
Genetics of captive population

533

Kleinman-Ruiz D, Soriano L, Casas-Marce M, *et al.* (2019) Genetic evaluation of the Iberian lynx ex situ conservation programme. *Heredity*.

The fitness consequences of genetic management

* Captive-born lynxes are less consanguineous than wild-born lynxes

* Should result in higher reproduction and survival of released animals

* 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

Genetic rescue in Doñana
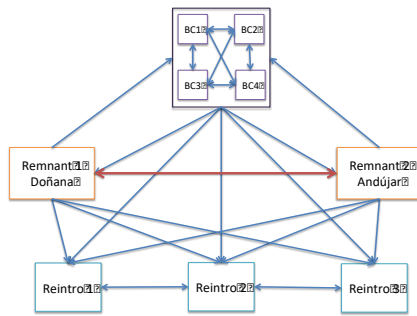
- Following translocations:
  - Genetic diversity has increased
  - The population has increased

Reproductive output by parent ancestry
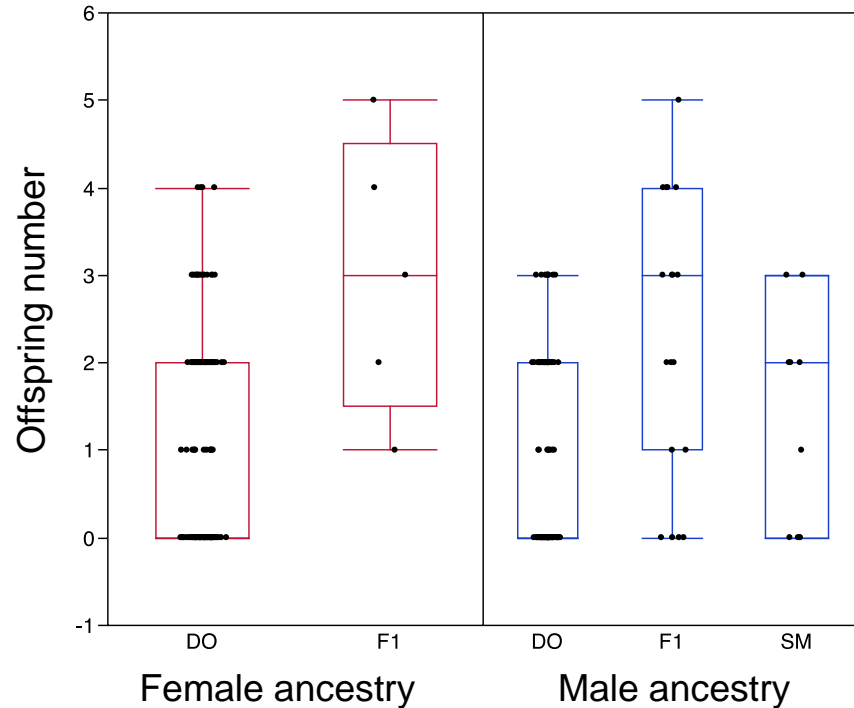
Mating success

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

Genetic management of reintroductions
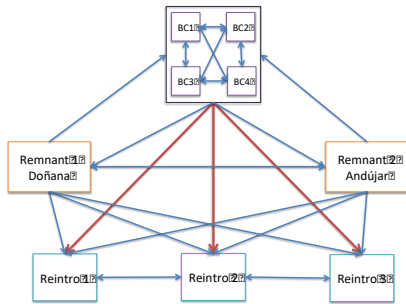
- Reintroduced populations will accumulate inbreeding and lose diversity very fast during the first few generations (founder effect)
- They will continue to do so at lower rates as long as they remain small and isolated
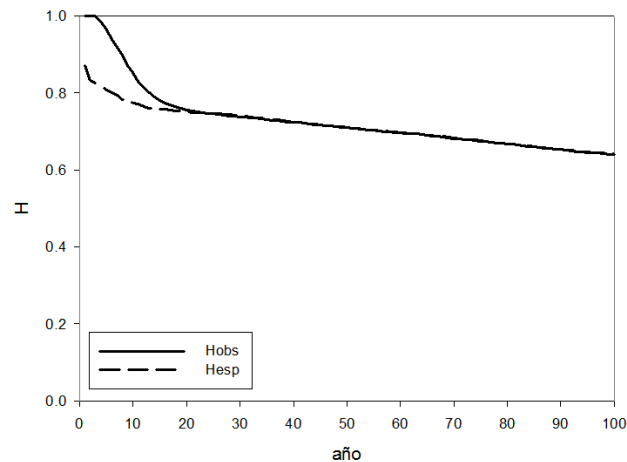
- Favourable scenario
- CC: 30 females
- Census ≈ 300

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

Can be ammeloriated through genetic management

Genetic management of reintroductions

- Impossible to select breeders or design matings in wild populations, but …
- Released animals can be selected to be minimally related among themselves
- Serial releases offer the possibility of selecting animals depending on the actual genetic composition (genealogy) of the recipient population
  - Again, based on minimum mean kinship
- **Requires an intensive individual-based monitoring of reintroduced populations**
- Once consolidated, management and monitoring can transition to population-based, using translocations when/where needed

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
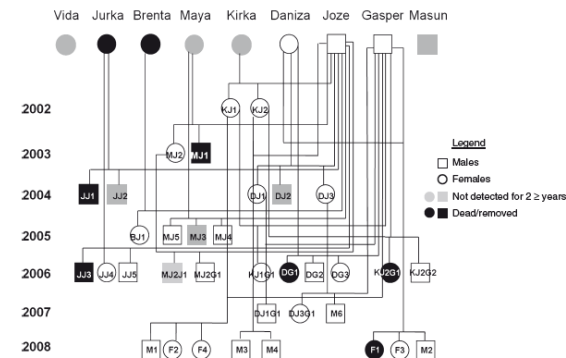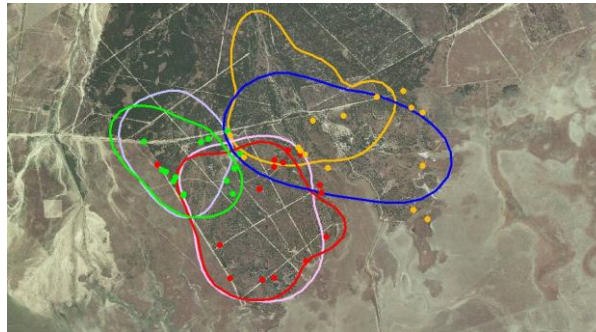
Using faeces to monitor species

Especie	Género	individuo
mtDNA	**Nuclear: X/Y**	**Nuclear: Autosomal SNPs**
Sequencing	SRY	
Diagnostic PCR	ZFX/ZFY	
Others	Amelogenin	

Escarlata
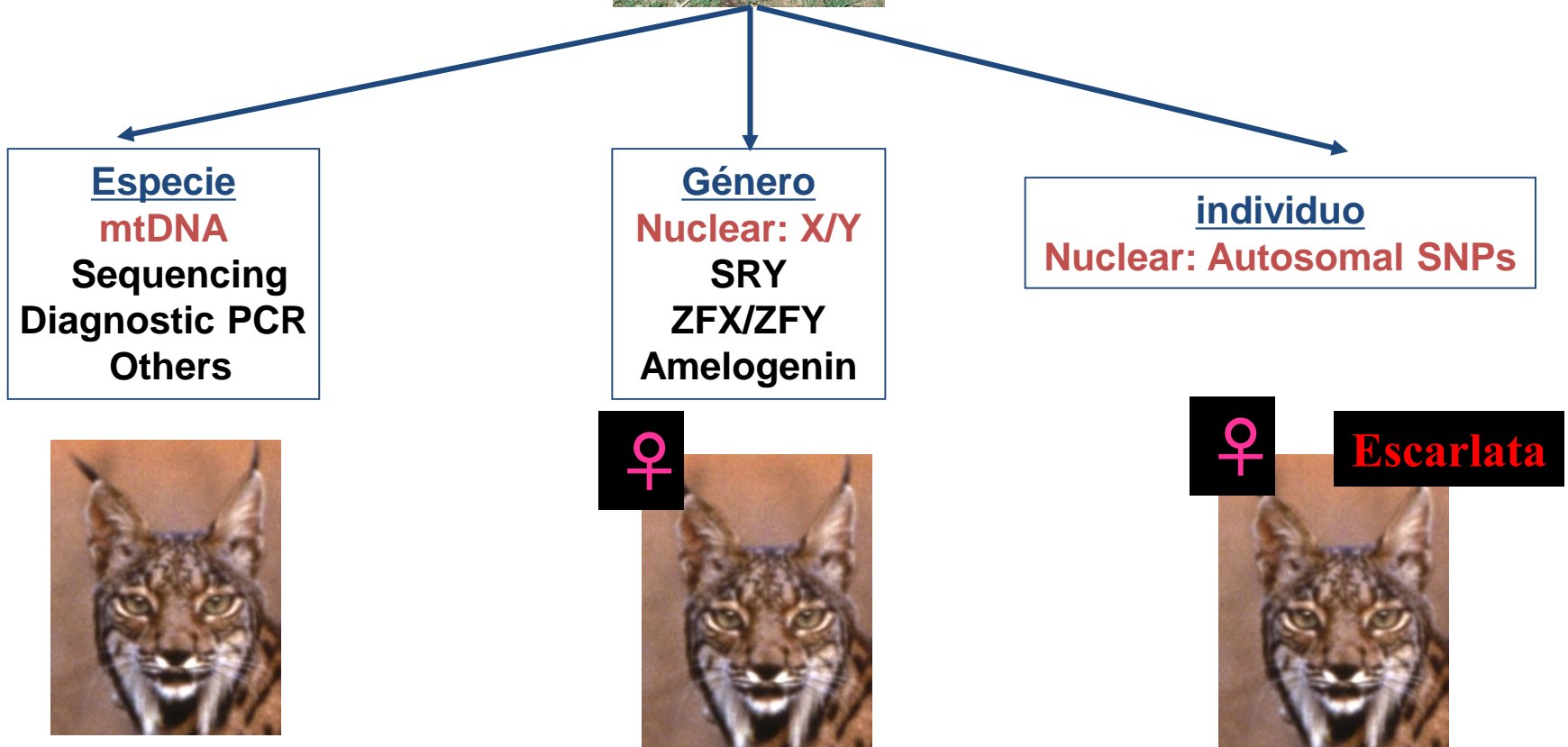
Monitoring: novel SNP markers

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

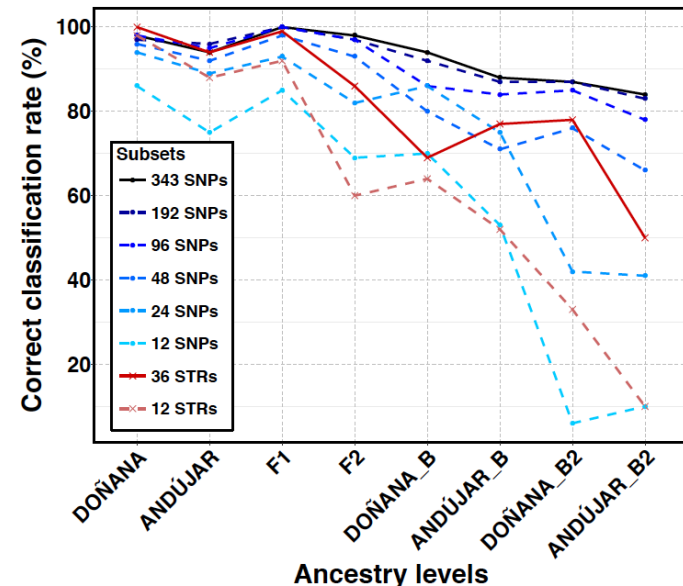| Marker	Size	PIDs	PnE	PWR FS-HS
SNPs	343	$4.7*10^{-71}$	$2.5*10^{-29}$	> 0.999
	192	$2.5*10^{-43}$	$8.8*10^{-18}$	0.998
	96	$2.4*10^{-22}$	$2.4*10^{-09}$	0.945
	48	$1.4*10^{-11}$	$4.7*10^{-05}$	0.741
	24	$3.7*10^{-06}$	$6.9*10^{-03}$	0.488
	12	$1.9*10^{-03}$	$8.3*10^{-02}$	-
Micros	36	$1.2*10^{-09}$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

Individual identif. — Parentage assignment — Kinship estimation

Kleinman-Ruiz D, Martinez-Cruz B, Soriano L, *et al.* (2017) Novel efficient genome-wide SNP panels for the conservation of the highly endangered Iberian lynx. *BMC Genomics* **18**, 556.
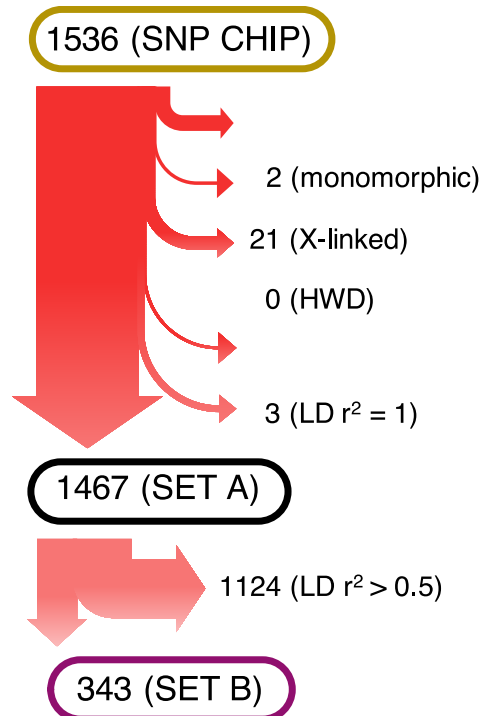
1536 (SNP CHIP)

2 (monomorphic)
21 (X-linked)
0 (HWD)
3 (LD r^2 = 1)

1467 (SET A)

1124 (LD r^2 > 0.5)

343 (SET B)

Genetic risks for population viaiblity

Small populaton

Genetic erosion

Inbreeding, genetic load

Loss of diversity

Lower fitness

Loss of adaptive potential

Short term

Long term (?)

Genetics in the definition of FRVs

- Since 2012 genetic diversity is explicitly included as a criterion for the favourable reference range in the Habitats Directive (Evans & Arvela 2011).
  - Page 16: "The following factors should be considered when estimating Favourable Reference Range (FRR) for both species and habitat types:
    - Current range;
    - Potential extent of range taking into account physical and ecological conditions (such as climate, geology, soil, altitude);
    - Historic range and causes of change;
    - Area required for viability of habitat type/species, including consideration of connectivity and migration issues.
    - **Variability including genetics** (Laikre te al., 2009) "
  - Page 18: "Favourable Reference Populations should be based on the ecology **and genetics** of the species."
- Developed and updated in Bijlsma et al. (2018)

50/500 rule for genetic viability

- Widely used "rule of thumb"
  - Ne > 50 to avoid short term ID
    - Based on observation in zoos and animal breeding
    - Δf = 0.01 por generación
  - Ne > 500 to assure evolutionary potential
    - Derived from theoretical expectations for mutation-drift equilibrium for quantitative traits
    - Δf = 0.001 or generación
- Ne typically 1/10 or 1/5 of adult numbers
- Recently updated to 100/1000 (Frankham et al., 2014)

Incorporating genetics to PVA (Frankham et al. 2014)

- PVA typically used to define MVP based on extinction probabilities (demographic viability)
  - Can also be used to define MVP based on genetic objectives (genetic viability)
    - MVP for genetic viability >>> MVP for demographic viability
- PVA in *Vortex* can consider ID and thus model short term fitness effects
  - Applied to juvenile survival
  - Parameterized by the number of lethal equivalents
    - 3.14 (default) often used; 12 more appropriate for total fitness
- Often results in Ne/N ratios of 0.3 (higher than observed 0.2)
  - Lower variance in reproductive success (Poisson)
- It does not explicitly model long term adaptive potential
- Call for standardization in MVP definition
  - 99% probability of persistence in 40 **generations**

Beyond one single closed panmictic population

- Species typically occur as metapopulations
- The effective size of the metapopulation is not necessarily the sum over subpopulations
  - Only if panmicitic
  - The lower the migration rate the higher the global the effective size
- Even low levels of migration can hamper genetic erosion
  - "One-migrant-per-generation" rule of thumb is unrealistic
  - Depend on the number of subpopulations, their effective sizes, and the pattern of gene flow among them.
- Need to explicitly consider the combined effects of size and migration in modelling
- Migration is dependent on distance and landscape matrix

Spatially-explicit individual-based modelling (Revilla et al., 2015)

Asistencia técnica para el análisis de viabilidad espacialmente explícito de posibles escenarios de reintroducción de lince ibérico en España (REF.: TEC0003132)

Conclusions: lessons learned

- The Iberian lynx is one of the most genetically eroded species
- Accumulating evidence suggest that inbreeding is limiting reproduction and survival (ID)
- Simulations suggest observed levels of ID are enough to reduce population viability
- Genetic management in captivity and translocations ameliorates inbreeding and ID
- Incipient reintroduced populations are expected to accumulate inbreeding fast
- This can be compensated by genetic management consisting in releasing animals minimally related to existing ones
- Critical to know the census and genealogy of the reintroduced population
- This can be achieved with an intensive individual-based non-invasive genetic monitoring program
- Long-term viability assessments need to explicitly consider genetic issues and genetic goals
- Existing spatially-explicit individual -based models can address both demographic genetic viability efficiently and realistically

Acknowledgments

- ## The ConGen group
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  - Daniel Kleinman (PhD)
  - Mireia Casas Marcé (PhD)
  - Begoña Martínez-Cruz (Posdoctoral Researcher)
  - Laura Soriano (Technician)

- ## Collaborators
  - EBD (Lynx demography and ecology)
    - Eloy Revilla
    - Alejandro Rodríguez
    - Francisco Palomares
    - Miguel Delibes
  - The Iberian lynx genome consortium (genomics)
  - MNCN (Reproductive physiology)
    - Eduardo Roldán
  - **INIA (Genetic management)**
    - **Jesús Fernández**
  - LIFE Projects
  - Ex situ conservation Program

¡Gracias!