

GENETIC CONSEQUENCES OF LONG-TERM ISOLATION FOR THE LAST FRENCH POPULATION OF *ERYNGIUM VIVIPARUM* AND IMPLICATIONS FOR ITS CONSERVATION

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INTRODUCTION



- Endemic of the Atlantic region of Europe (France, Spain, Portugal), *Eryngium viviparum* is an endangered pioneer specie (directive habitat 97/43/CEE), growing in seasonally flooded grasslands.
- In eighties, about 30 populations remained in France (Morbihan). They all disappeared except one small population (>1000m²), totally isolated since 1987. A **national action plan** (2013-2018) as been implemented to improve its conservation statut.

- The small size and isolated situation generally leads to a decrease in genetic diversity and an increase in the **risk of inbreeding depression**. To control the health of the population we studied the genetic diversity with **microsatellites** among *Eryngium viviparum* french population and some other populations from Iberian peninsula.

- We want to answer two main questions:
 - How is the genetic diversity among the last french population ?
 - Is the French population genetically distant from the others ?

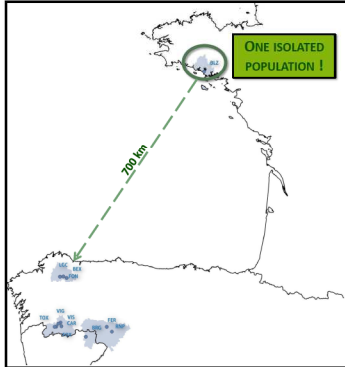


Fig.1 – Geographic location of the 12 studied populations of *Eryngium viviparum*

METHODS

- Molecular analysis were performed on 258 samples among the 12 populations studied [Fig.1].
- Genomic DNA was extracted using a modified CTAB method (Doyle & Doyle, 1990).
- Samples were genotyped at 7 microsatellites loci (Genoscreen consortium) [Tab.1].
- PCR were processed performing multiplex reactions with fluorescent labeling of the forward primers (Applied Biosystems).
- Analysis of genotypes were realized on Genemapper v.4.0. and data analysis were realized on GENEPOP (Raymond et Rousset, 1995) and GENETIX (Belkhir et al., 2000).

Locus	Number of repetition	Type	Number of alleles	Fis
pmEv01	22	aca	5	-0,0517
pmEv02	21	gt	9	0,0707
pmEv04	21	ga	19	0,0206
pmEv05	20	gt	11	0,1195
pmEv09	17	atgt	15	0,0455
pmEv10	17	ca	11	-0,0132
pmEv17	16	ac	7	-0,0526

Tab.1 – Characteristics and diversity of the 7 primers used in this study. Only one locus (pmEV05) show a higher homozygote excess.

RESULTS & DISCUSSION

Within-population genetic diversity

- We found 77 alleles in total with the 7 microsatellites used.

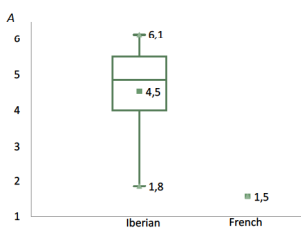


Fig.2.a – Mean number of allele per locus (A)

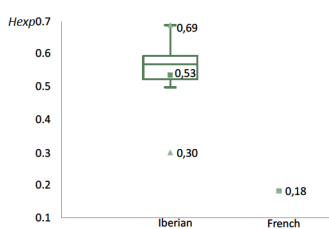


Fig.2.b – Expected heterozygosity (Hexp)

- Within populations number of alleles per locus vary from 1.5 to 6.14 [Fig 2a] and with the mean gene diversity ranging from 0.18 to 0.69 [Fig 2b]. **The french population clearly shows the lower genetic diversity.**
- No private allele was found for Belz, suggesting that there is **no specificity in France** within this 7 loci.

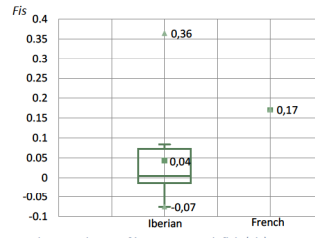


Fig.3 – Estimate of heterozygote deficit (Fis)

- Estimate of the inbreeding coefficient range from $F_{is} = -0.76$ to 0.364 [Fig.5]. Two population showed an inbreeding value significantly different from zero : Belz and a small size Spain population. That suggests a small but significant **deficit of heterozygotes**.
- This results assume a predominantly clonal reproduction for these two populations.

Among-population differentiation

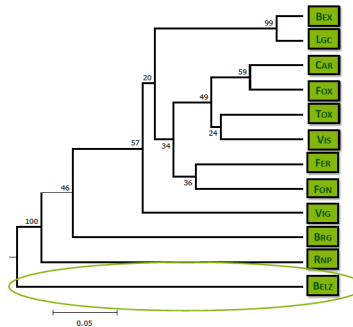


Fig.4 – UPGMA dendrogram based on estimated Fst

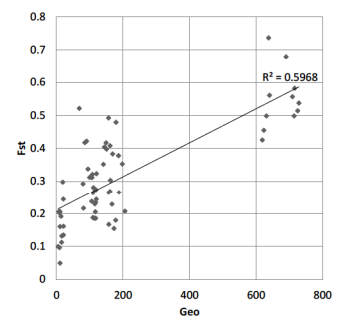


Fig.5 – Correlations between Fst and geographic distances

- Fst values indicated a **strong differentiation** between French population and all others, explaining its isolation on the tree.
- Genetic differentiation appear positively correlated with geographical distance. This results show that **genetic Belz is highly distant from all other populations**, probably du to isolation and distance.

IMPLICATIONS FOR CONSERVATION ?

- Genetic diversity within the latest french population is weak, probably related to geographic isolation. This loss of diversity could ultimately lead to inbreeding depression leading to the extinction of that population.
- To restore a good level of diversity réintroductions and reinforcement are envisaged as part of the National Action Plan.
- However genetic differences of the french population with others eliminates the possibility of using iberian individuals for reintroduction that would be maladaptive to environmental conditions.



What solutions ?

- Compare ecology among french and iberian populations
- Maintain the french population with appropriate management
- Reintroduce historic stations where the species never reappeared even after restoration
- Establish connections between reintroduced populations and Belz

ACKNOWLEDGMENT

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