

# Preliminary Genetic Analysis of some Western Palaearctic Populations of Bonelli's Eagle, *Hieraaetus fasciatus*.

Pedro Cardia, Bárbara Fráguas, Miguel Pais, Thomas Guillemaud, Luís Palma, M. Leonor Cancela, Nuno Ferrand and Michael Wink

## ABSTRACT

Twelve allozyme loci were analysed from Bonelli's Eagles of Portugal; PEPD2, MPI, NP and PX proved to be polymorphic. All the remaining loci were found to be monomorphic. Two alleles were detected for PEPD2, MPI and NP loci and the PX locus exhibited three alleles. DNA sequences of the mitochondrial cytochrome b gene from birds originating from Saudi Arabia to Portugal, show a low degree of haplotypic differentiation. However, two clades comprising birds from the western and eastern Mediterranean are apparent. A single primer PCR analysis revealed 4 groups. DNA analysis shows some phylogeographic differentiation but also an obvious mixing of haplotypes in the Mediterranean region.

## INTRODUCTION

Bonelli's Eagle, *Hieraaetus fasciatus*, is a medium-sized eagle whose numbers have strongly declined in west Palearctic, where it is restricted to the Mediterranean zone (Snow & Perrins, 1998). Of the 800 pairs estimated to exist in Western Europe, 10% (75-90 pairs) are found in Portugal (Palma *et al.*, 1996) and considered to be endangered (Cabral *et al.*, 1990). It is distributed along two main areas of high density, northeast and southwest regions (Figure 1), separated by a diffuse distribution area of low density.

The genetic analysis of natural populations provides information on its genetic variability, their genetic relationships and the gene flow that may exist between them, allowing the development of sound conservation strategies. This work provides a preliminary genetic analysis of some Portuguese Bonelli's Eagle populations and their relationships with other Western Palaearctic populations. Allozyme studies were done in Portugal and DNA studies in Germany.

## MATERIAL AND METHODS

Blood samples were collected from the brachial vein of Bonelli's Eagle nestlings, as well as from birds found "freshly" dead, and stored in EDTA buffer for preservation. Samples were divided

in two fractions - serum and red cells - and both were stored at  $-20^{\circ}\text{C}$ . Samples were obtained from individuals of southwest and northeast populations and from one individual caught in central Portugal.

*Allozyme variation*

The study of genetic variability at the protein level was based on a set of 12 loci (Table 1). It was performed by means of conventional electrophoretic techniques and isoelectric focusing in carrier ampholyte pH gradients.

Gene frequencies were obtained by direct count, and were used to calculate the average expected heterozygosity for each population. However, it was impossible to perform statistical test on the data because of the small sample size (23 individuals).

**Figure 1. Portuguese Bonelli's Eagle populations studied in the present work by allozyme analysis**



**Table 1. Protein markers and separation methods used in this work.**

| <i>Protein markers</i>           | <i>Separation methods</i> | <i>Protein markers</i>               | <i>Separation methods</i> |
|----------------------------------|---------------------------|--------------------------------------|---------------------------|
| Adenine deaminase (ADA)          | CE                        | Peptidase B (PEPB)                   | CE                        |
| Albumin (ALB)                    | IEF                       | Peptidase C (PEPC)                   | CE                        |
| Glucosephosphate isomerase (GPI) | CE                        | Peptidase D1 (PEPD1)                 | CE                        |
| Isocitrate dehydrogenase (IDH)   | CE                        | Peptidase D2 (PEPD2)                 | CE                        |
| Manosephosphate isomerase (MPI)  | CE                        | Phosphogluconate dehydrogenase (PGD) | CE                        |
| Nucleoside phosphorylase (NP)    | CE                        | unknown plasmatic protein (PX)       | IEF                       |

Abbreviations: IEF - isoelectric focusing; CE - conventional electrophoresis.

### DNA variation

DNA was extracted from blood and feather samples of *H. fasciatus*, that derived from Europe: Spain, Portugal, Corsica and Greece; the Near East: Israel and Saudi Arabia; and North Africa: Tunisia and Morocco.

A PCR was performed with a single PCR primer (GACA)<sub>4</sub> which produced several DNA products (usually of nuclear DNA origin) in each individual (details in Wink *et al.*, 1998). In a second set of experiments we have amplified the mitochondrial cytochrome b gene by PCR and have sequenced it (1200 bp) with an ALFexpress DNA sequencer (for methods see Wink & Sauer-Gürth, this volume). The aligned sequences were analysed by the character state method Maximum parsimony (Swofford, 1993).

## RESULTS AND DISCUSSION

### Allozyme study

Of the 12 loci analysed, PEPD2, MPI, NP and PX proved to be polymorphic. All the remaining loci were found to be monomorphic. Two alleles were detected for PEPD2, MPI and NP loci and the PX locus exhibited three alleles. Allelic frequencies for PEPD2, MPI, NP and PX loci, and observed and expected average heterozygosities data are presented in Figures 2-6, respectively.

Figure 2. PEPD2 allele frequencies in Southwest (SW), Northeast (NE) and total populations

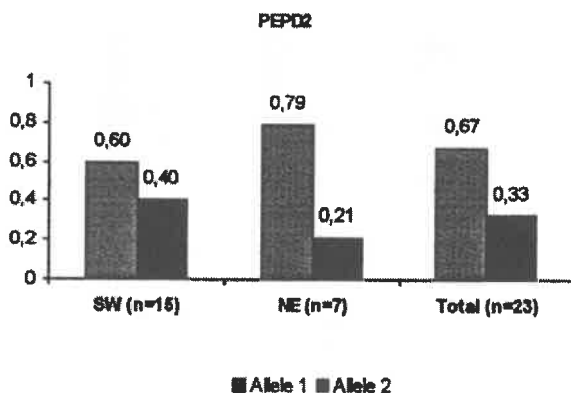


Figure 3. MPI allele frequencies in SW, NE and total populations.

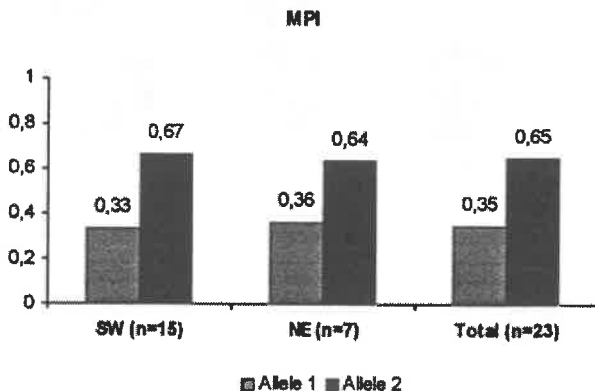


Figure 4. NP allele frequencies in SW, NE and total populations.

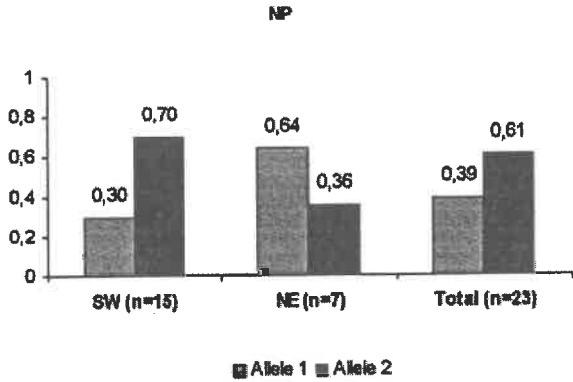


Figure 5. Px allele frequencies in SW, NE and total populations

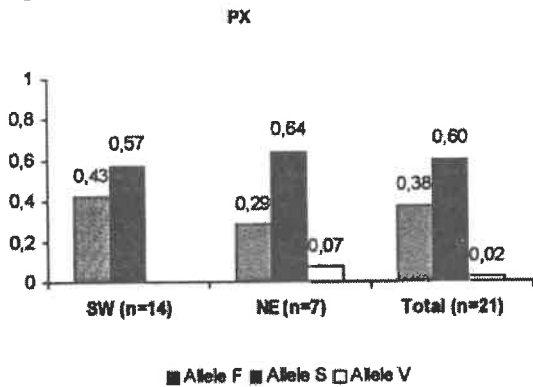
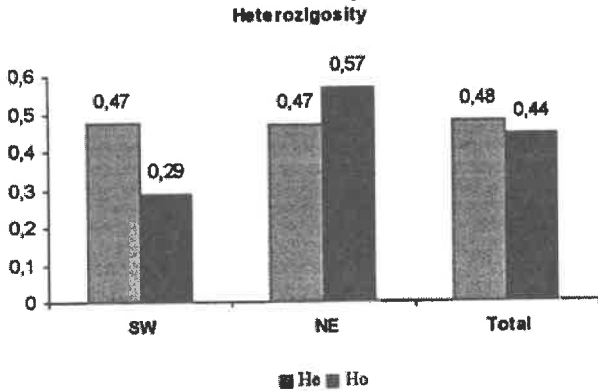


Figure 6. Observed and expected heterozygosities ( $H_o$  and  $H_e$ ) in NE, SW and total populations.



Fifty-three individuals were sampled and screened for the above stated *loci* (Table 1). However, as many of those individuals were closely related (*i. e.* brothers) only one individual per breeding pair was used for the calculation of gene frequencies. This resulted in an effective sample size of only 23 individuals (15 individuals from Southwest, 7 individuals from Northeast and 1 individual from central Portugal).

Gene frequencies at *PEPD2*, *MPI* and *PX* loci do not appear to differ significantly among the studied populations. However, the *NP* locus shows an asymmetric distribution of allele frequencies in Southwest and Northeast populations, *NP\*1* being the commonest in Northeast (64.3%) and *NP\*2* in Southwest (70%). This might be due to the existence of a North-South gradient in allele frequencies at this locus. Another difference between NE and SW is found when comparing the expected and observed average values of heterozygosity ( $H_e$  and  $H_o$ , respectively). NE population has a higher  $H_o$  than  $H_e$  while SW population has a higher  $H_e$  than  $H_o$ .

An interesting feature is the existence of the variant *PX\*V* at the *PX* locus, identified in a single individual from the Northeast.

### DNA study

DNA sequences of the mitochondrial cytochrome b gene show a low degree (< 1% sequence divergence) of haplotypic differentiation. As can be seen from the resulting MP consensus cladogram (Figure 7), two main clusters are apparent. Cluster 1 which corresponds to some degree to Cluster I in the GACA-analysis (Figure 8), contains birds from both Europe, North Africa and the Near East, whereas Cluster 2 has predominantly birds from the western Mediterranean region (group II-IV in Figure 8).

**Figure 7. Phylogeography of *H. fasciatus* inferred from nucleotide sequences of the cytochrome b gene (>1000 nucleotides)**

**Analysis by Maximum Parsimony; illustration as a 50% consensus cladogram.**

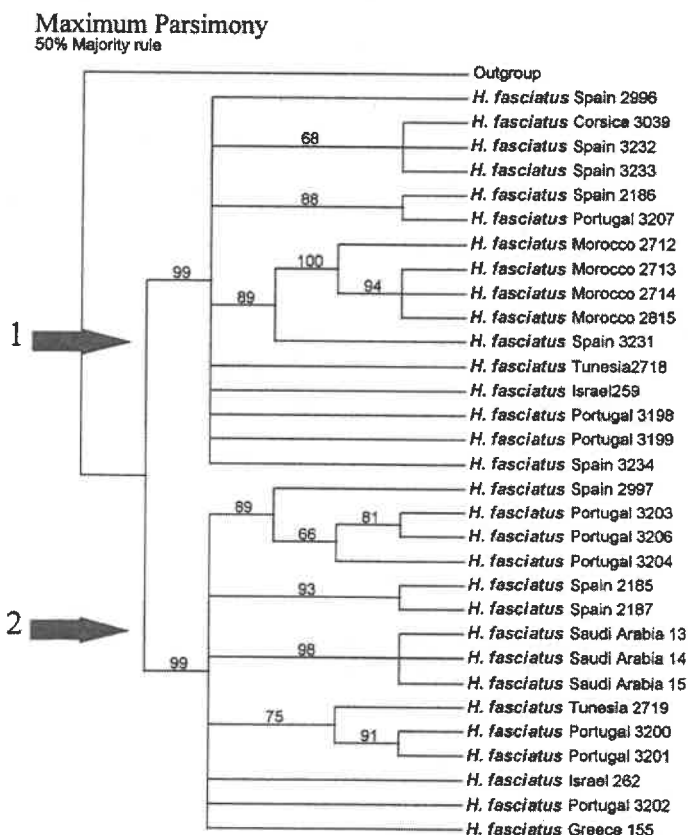
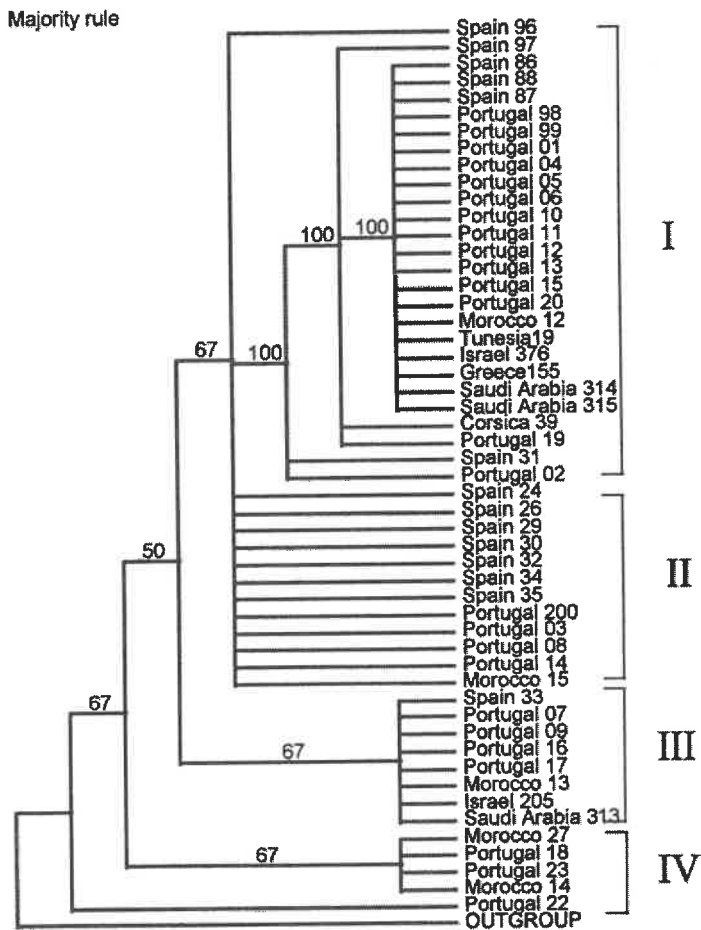


**Figure 8. Phylogeographic relationships of *H. fasciatus*.**  
Dendrogram based on the presence or absence of 4 polymorphic alleles detected by GACA-PCR.



Using the single-primer PCR approach, complex DNA profiles can be generated. Most of the PCR products were invariant. However, 4 polymorphic “alleles” were discovered. A corresponding dendrogram is illustrated in Figure 8, in which 4 main groups can be recognised. Birds from the Eastern Mediterranean are mainly found in group I, but two birds from this origin also turned up in group III, indicating a certain degree of gene flow between east and western Mediterranean.

This first and preliminary DNA analysis suggests some phylogeographic differentiation but also an obvious mixing of haplotypes in the Mediterranean region.

### PERSPECTIVES.

Work is currently underway to detect new enzyme polymorphisms, as well as to include new individuals from unsampled Portuguese populations. At the DNA level, microsatellites have already been cloned and will be used to further characterise Bonelli’s Eagle populations.

## ACKNOWLEDGEMENTS

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Pedro Cardia<sup>1,2</sup>, Nuno Ferrand<sup>1,2</sup> Bárbara Fráguas<sup>2</sup>  
1-Centro de Estudos de Ciência Animal  
Campus Agrário de Vairão  
4480 Vila do Conde  
Portugal  
2-Departamento de Zoologia e Antropologia  
Faculdade de Ciências da Universidade do Porto  
4050 Porto, Portugal

Miguel Pais  
Universidade de Évora  
7000 Évora  
Portugal

Thomas Guillemaud, Luís Palma and M. Leonor Cancela  
Universidade do Algarve-UCTRA  
Campus de Gambelas  
8000 Faro, Portugal

Michael Wink  
Institut für Pharmazeutische Biologie  
Universität Heidelberg  
Germany.

