RESOLVING THE POPULATION STRUCTURE OF *Rhinolophus hipposideros* (BECHSTEIN, 1800) IN GALICIA (NW SPAIN).

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INTRODUCTION

*The efforts directed towards the conservation of biodiversity should be focused on genetically distinct populations, and less on the species as a whole (Hughes et al., 1997; Hobbs and Mooney, 1998). Then, the identification of natural populations can provide us with an excellent tool for the management and conservation of the biological diversity (Moritz, 1995).

*One of the most used methods to infer the population structure of a sample is the Analysis of Molecular Variance (AMOVA) (Excoffier et al. 1992), an analysis of the variation of allele frequencies. Despite AMOVA has been a widely used tool (Fitzpatrick, 2009) and has shown its utility either to test the possible presence of gene flow barriers (Carstens et. al., 2004; Chen et. al., 2006; Vasconcelos et. al., 2006) or to establish conservation units (Vila et. al., 2006), the before-hand setting of the analysis groups would not offer the best answer to what is the best possible population structure model.

*To find out the most accurate population structure, we should obtain the highest FSC values (differentiation within populations) and the lowest FCT values (differentiation among populations) as possible (Weyeneth et al., 2011).

*In this study we want to compare four different grouping strategies, tested with a hierarchical AMOVA with an a-posterity method, the sequential SAMOVA (Dupanloup et. al., 2002), to check which can offer better results to unravel the population structure of *R. hipposideros* in Galicia.

METHODS

1-Sampling and genetic analysis.

We captured 480 *Rhinolophus hipposideros* (Bechstein, 1800) in 41 colonies and obtained two tissue samples from the wings of every individual.

DNA was extracted through the ethanol-isopropanol method and HVII, a portion of the Control Region of the mtDNA, was amplified for all the individuals.

We made a hierarchical AMOVA analysis, which takes account of genetic distance among haplotypes: FCT, the degree of differentiation within populations FSC, the degree of differentiation among populations and FST, the degree of differentiation among all the colonies.The analysis was run with Arlequin 3.5.1.3, with 10.000 permutations. As a comparison we ran a sequential SAMOVA. The software SAMOVA 1.0 requires an initial number of K groups and offers the results in terms of genetic distance (FST, FCT and FSC). We ran SAMOVA with 1000 simulated annealing processes for K-values ranging from 2 to 21, using all 41 colonies.

2-Geographical cluster.

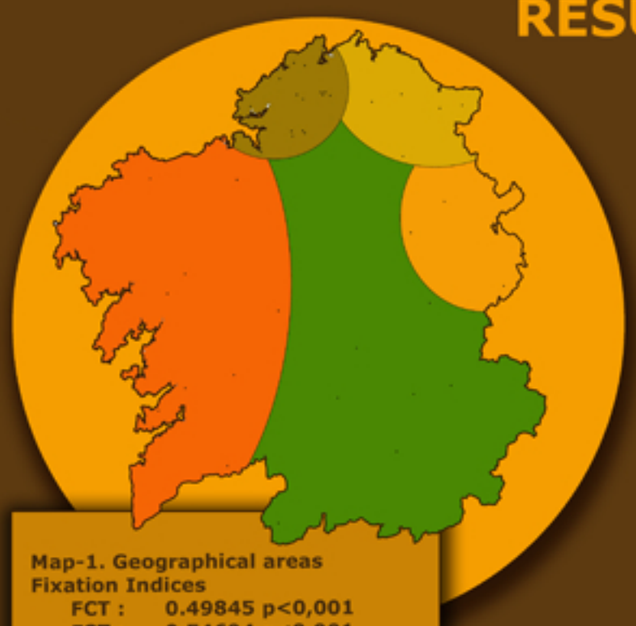
*Geographical areas: Populations were established based on the geographical position of the colonies and clustered in five subjective groups (see Chen et al., 2006).

*Macrobioclimates: Galicia has three main macroclimate areas: Temperate, Temperate-submediterranean and Mediterranean (Rivas-Martínez, 2007).

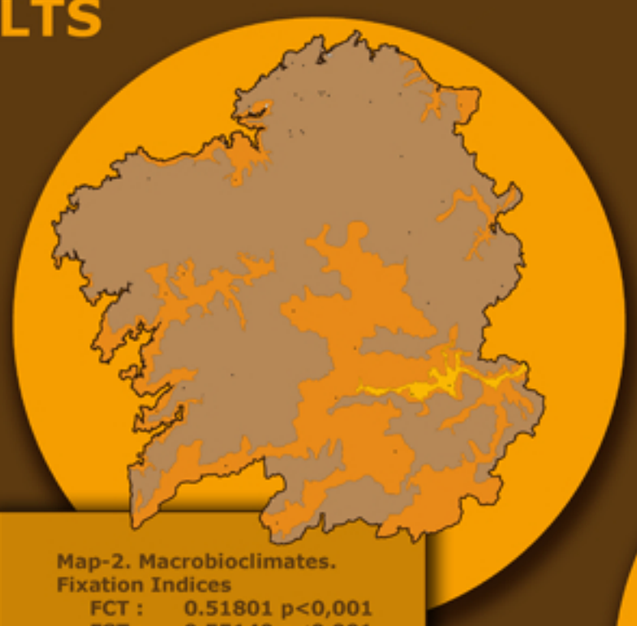
*Phytogeography: In Galicia we can find nine main diffferent phytogeographical areas (Izco, 1987).

*River basins: To test if rivers act as corridors for gene flow, we set the populations based on river basins.

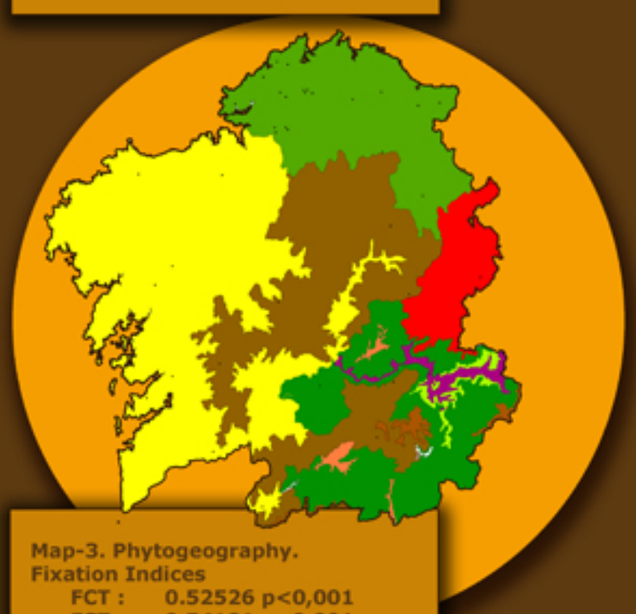
RESULTS



Map-1. Geographical areas
Fixation Indices
FCT : 0.49845 p<0,001
FST : 0.54694 p<0,001
FSC : 0.09668 p<0,001

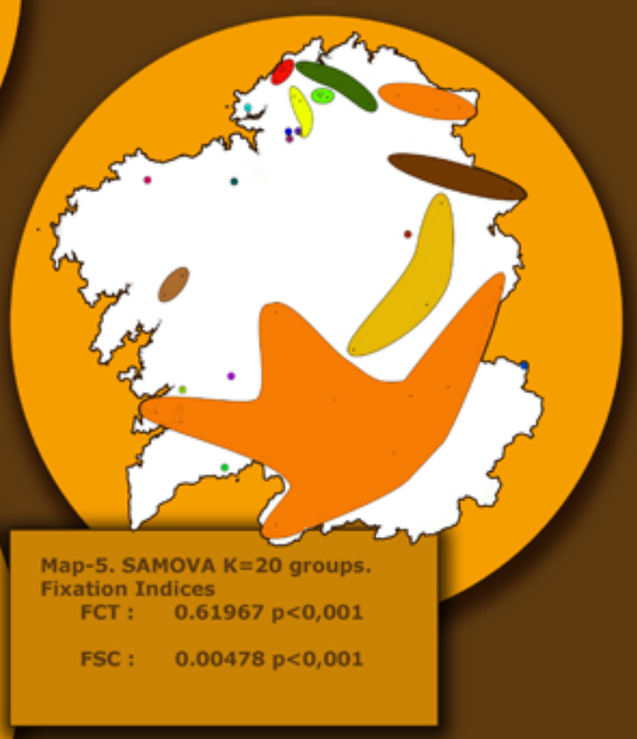


Map-2. Macrobioclimates.
Fixation Indices
FCT : 0.51801 p<0,001
FST : 0.55149 p<0,001
FSC : 0.06946 p<0,001



Map-3. Phytogeography.
Fixation Indices
FCT : 0.52526 p<0,001
FST : 0.54181 p<0,001
FSC : 0.03487 p<0,001

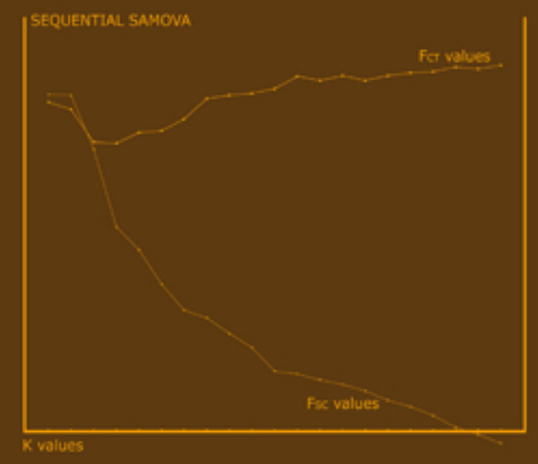
Map-4. River basins
Fixation Indices
FCT : 0.42713 p<0,001
FST : 0.54328 p<0,001
FSC : 0.20276 p<0,001

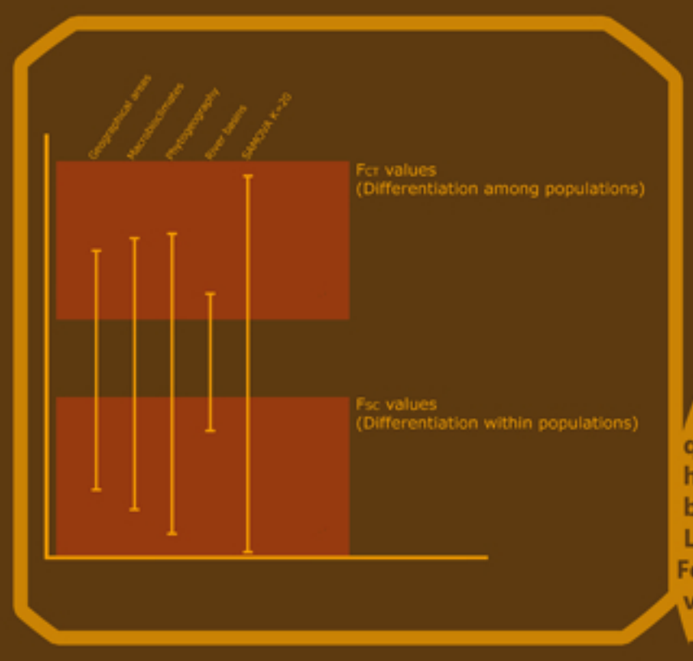


Map-5. SAMOVA K=20 groups.
Fixation Indices
FCT : 0.61967 p<0,001
FSC : 0.00478 p<0,001

Values of sequential SAMOVA

N of groups (K)	Fsc (Differentiation within populations)	Fct (Differentiation among populations)
2	0.57262	0.54761
3	0.57229	0.49183
4	0.48045	0.49030
5	0.34663	0.50805
6	0.30818	0.51128
7	0.24914	0.53094
8	0.20485	0.56623
9	0.19158	0.57226
10	0.16487	0.57534
11	0.14085	0.58310
12	0.10154	0.60414
13	0.09576	0.59671
14	0.08635	0.60575
15	0.07741	0.59730
16	0.06731	0.60165
17	0.05084	0.60520
18	0.04005	0.60968
19	0.02509	0.61208
20	0.00478 p<0,001	0.61967 p<0,001
21	<0	





DISCUSSION

R. hipposideros shows a strong philopatric behavior, such as other species of the genus Rhinolophus (Rossiter et al., 2002) or even many other bats (Castella et al 2001; Kerth et al. 2000), so the expected gene flow between populations has to be low and, thus, the differentiation among populations, should be high.

Because of that, almost any kind of cluster to be analyzed in order to obtain a population structure, or to test any geographical hypothesis, will show that they are, in fact, structured.

The results prove that every cluster made before-hand is valid and very significative (p<0,001), which means in every case we can reject the null hypothesis of no structure. Nevertheless, it does not mean that every population model has the same validity to resolve our question. Low values of genetic differentiation within populations indicate the similarity of the individuals belonging to it. Meanwhile, high values of genetic differentiation among populations indicate that the ones are very different from the others. A combination of both ensures the identification of genetic diversity without overlapping and help us to establish Management Units (see Allendorf and Luikart, 2007).

Following that argument, and placing together the results of the five grouping strategies, we can observe how the most desirable values are provided by sequential SAMOVA, with a final value of K=20 groups.

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