

Colliard C.<sup>1</sup>, Sicilia A.<sup>2</sup>, Turrisi G. F.<sup>3</sup>, Arculeo M.<sup>2</sup>, Perrin N.<sup>1</sup>, Stöck M.<sup>1</sup>

1. Department of Ecology and Evolution, Biophore Building, University of Lausanne, 1015 Lausanne, Switzerland

2. Dipartimento di Biologia Animale, University of Palermo, Via Archirafi 18, 90123 Palermo, Italy

3. Dipartimento di Biologia Animale "Marcello La Greca", University of Catania, Via Androne 81, 95124 Catania, Italy

## INTRODUCTION

One of the key questions in evolutionary biology is the amount and speed of reproductive isolation accumulating in allopatric speciation (Coyne & Orr 2004). After phylogeographical analyses, a potential secondary contact zone in Sicily between endemic *Bufo siculus* and Italian Peninsular-origin *B. balearicus* was predicted (Stöck *et al.* 2006, 2008). *B. siculus* is phylogenetically closer to North African *B. boulengeri* than to neighbouring *B. balearicus* (Stöck *et al.* 2006), and *B. balearicus* has been separated ca. 2.75 (1.19-4.9) My from the *boulengeri-siculus* clade (Stöck *et al.* 2008). The aim of the study was to analyse whether both taxa meet each other in Sicily, and, if so whether they would hybridize, and what structure does have the potential hybrid zone.

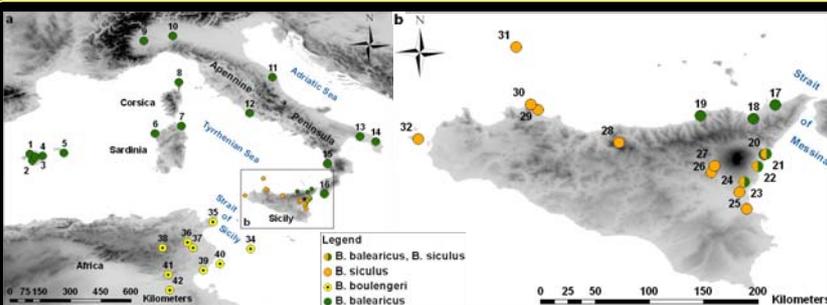


Fig. 1: Map of the distribution of the three different mtDNA haplotype groups in green toad populations (a) across the entire study area and (b) in Sicily and two off-coast islands.

## DO BOTH TAXA MEET EACH OTHER IN SICILY?

### mtDNA

- Mitotyping and sequencing of *B. balearicus* and *B. siculus* (Colliard *et al.*, In press)

Three populations (pop. 21 to 23, Fig. 1b) containing *B. balearicus* and *B. siculus* mtDNA

➔ *B. balearicus* and *B. siculus* have overlapping ranges in Sicily

## Bayesian clustering analyses

- Included all individuals from Sicily, from two off-coasts islands and ten F1-hybrids from a laboratory cross (Fig. 2)

- Seven microsatellite markers

- Individuals were considered as hybrids when assigned to less than 90% to a cluster

- Laboratory F1-hybrids were correctly assigned (50%-50% assignment) but no natural F1-hybrids were found (Fig. 2)

- Two of the three populations having both mtDNAs (pop. 21 and 22) were assigned to *B. balearicus* and the other one (pop. 23) to *B. siculus* (Fig. 2).

- Few individuals in populations 21 and 26 were considered as **hybrids** (backcrosses, see arrows Fig. 2)

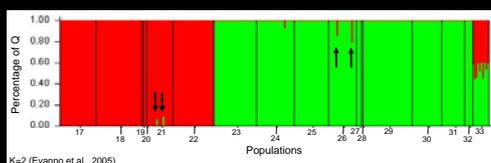


Fig. 2: Bar plot from STRUCTURE included all populations from Sicily, two off-coasts islands and ten F1-hybrids from a laboratory cross (pop. 33).

## DO BOTH TAXA HYBRIDIZE?

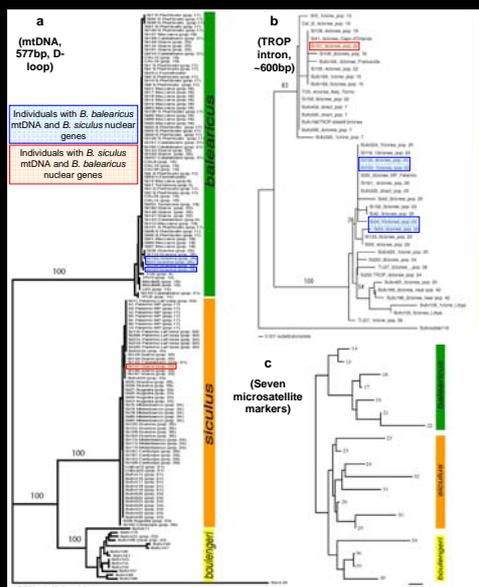


Fig. 3: (a) NJ tree built on the mitochondrial control region (577 bp mtDNA, D-loop), (b) NJ tree built using a nuclear intron (~600bp, Tropomyosin, intron) and (c) NJ tree built using a pairwise  $F_{ST}$  matrix based on seven microsatellite loci

## Phylogenetic analyses

- Congruence of major topologies through the three types of markers (Fig. 3)

- Few individuals show incongruence and present cytonuclear disequilibria since they own mtDNA from one species and nuclear genes from the other species (see red- and blue-framed Fig. 3a and 3b)

➔ *B. balearicus* and *B. siculus* hybridize

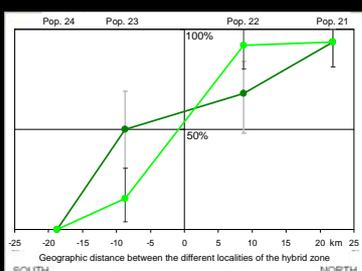


Fig. 4: Preliminary graphical chart of a south-north transect through the hybrid zone. Vertical lines represent 95% CI.

## STRUCTURE OF THE HYBRID ZONE

- The shift in nuclear genome occurs over a shorter distance than that of mitochondrial genome (17 km, Fig. 4)

➔ The **cline of the nuclear genome appears steeper** than that of the mtDNA

## DISCUSSION – CONCLUSION

- F1-hybridization is relatively rare in the wild

Potential causes:

➔ Potential occurrence of **pre-mating isolation** (e.g. advertisement call) ?

- **Differential nuclear and mtDNA introgression** into the other species' gene pool, creating a **strong cytonuclear disequilibria**

Potential causes:

➔ **Selection against nuclear hybrids** ?

➔ **Sex-specific differences in dispersal or effective size** ?