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Evolution of *Bombina bombina* and *Bombina variegata* (Anura: Discoglossidae) in the Carpathian Basin: A history of repeated mt-DNA introgression across species

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Abstract

The structure and geographic location of hybrid zones change through time. Current patterns result from present and historical population–environment interactions that act on each of the hybridizing taxa. This is particularly evident for species involved in complex hybrid zones, such as that formed by the toad species *Bombina bombina* and *Bombina variegata* (Anura: Discoglossidae), which interact along extensive areas in Central Europe. We used data on external morphology and partial sequences of the cytochrome oxidase I (*cox1*) and nicotinamide adenine dinucleotid dehydrogenase subunit 4 (*nad4*) mitochondrial DNA (mt-DNA) genes to analyze the current patterns of genetic structure shown by both species of *Bombina* along their contact zone in Hungary. Phylogenetic, phylogeographic, and historical demography analyses were applied to 1.5 kb mt-DNA obtained from 119 individuals representing 24 populations from Hungary and additional specimens from Slovakia, Albania, and Bosnia-Herzegovina. We use these data to infer the evolutionary history of the isolated populations of *B. variegata* in Hungary and to discriminate between competing biogeographic scenarios accounting for the historical interactions between species in this region. Results from the inferred phylogenetic branching pattern and sequence divergence among species and populations support the following: (i) recent population expansion has occurred in Hungarian populations of *B. bombina*, which are genetically very homogeneous; (ii) the Hungarian populations of *B. variegata* correspond to two distinct mitochondrial lineages (Carpathian and Alpine, respectively); average maximum-likelihood-corrected sequence divergence between these lineages is 8.96% for *cox1* and 10.85% for *nad4*; (iii) mt-DNA divergence among the three isolated western populations of *B. variegata* from Transdanubia is low, with four closely related haplotypes, which suggests that the isolation between these populations is the result of a recent process, possibly mediated by the invasion of *B. bombina*; and (iv) we have detected discordances between morphology and mt-DNA data in the Transdanubia region (Bakony Mountains, Mecsek Mountains, Örség area), suggesting mt-DNA introgression across species in this regions. These results are discussed with reference to previous biogeographic hypotheses.

Keywords: *Bombina bombina*; *Bombina variegata*; Mitochondrial-DNA; Phylogeography; Evolution; Interspecific hybridization; Historical demography; Hungary

1. Introduction

The prevalent speciation mechanism in amphibians from temperate regions is isolation and subsequent divergence in allopatry (Riddle et al., 2000; Wake, 1997). If the time elapsed since the vicariant event took place is long enough, random genetic differentiation between the sister lineages would render them reproductively isolated, even in the absence of prezygotic isolation mechanisms (although other processes, such as natural selection and epistasis, may also play an important role, see for example Coyne and Orr, 2004). But in some instances, differentiation is insufficient for complete reproductive isolation to evolve, and a

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zone of introgression or hybridization is formed upon secondary contact (Barton and Hewitt, 1985; Harrison, 1993; Hewitt, 1988). Field identification of contact zones is greatly facilitated when morphological differentiation has been attained by each lineage prior to the establishment of the contact zone. However, recent studies have shown that in amphibians, the location of a hybrid zone based on morphological traits or nuclear markers can be discordant with its location based on mt-DNA, which is sometimes displaced hundreds of kilometers away (García-París et al., 2003; Jackman and Wake, 1994; Moore, 1995; Moritz et al., 1992).

In Europe three species of *Bombina* occur. *Bombina bombina* (Linnaeus, 1761) in Eastern and Northern Europe, *Bombina variegata* (Linnaeus, 1758) in Western and Southern Europe, and *Bombina pachypus* (Bonaparte, 1838) in the Apennine Peninsula and Sicily (Fig. 1). A relatively narrow hybrid zone in which morphological, nuclear and mtDNA markers change concurrently is formed by two of these species, *B. bombina* and *B. variegata*, in Central Europe (Fig. 1). The geographic ranges of the two species meet along several thousand kilometers, from Poland through Slovakia, Hungary, Austria, Romania, and Bulgaria, to Croatia (Szymura, 1993). Transects within the contact zones have been studied in detail in Poland (Szymura, 1983; Szymura and Barton, 1986, 1991), Austria (Gollmann, 1984), Hungary (Gollmann, 1987; Gollmann et al., 1986), Slovakia (Gollmann et al., 1986), Croatia (MacCallum et al., 1998), and Romania (Vines et al., 2003). Regional sections of this hybrid zone show different genetic structures. For example, in Poland and Croatia the hybrid zones are narrow, 6–9 km wide, and the edges are defined by the presence of genetically pure specimens. But in the Romanian transition zone (east of Apahida) hybrid populations are completely isolated from both pure *B. variegata* (20 km away) and pure *B. bombina* populations (100 km away) (Vines et al., 2003).

The Carpathian Basin is a complex transitional zoogeographic and climatic area located between the Carpathian Mountains and the western Alpine domain. The Northern Middle Range (Mátra Mountains, Bükk Mountains, Aggteleki Karst area and Zemplén Hills) shows climatic and zoogeographic influences from the Carpathians, in contrast to the mountainous areas of western Transdanubia (Kõszegi Mountains, Soproni Mountains, and Örség region), and the Transdanubian Middle Range (Bakony Mountains), which have more influences from Alpine areas. Finally, the island-like hilly ranges in southern Hungary (Villányi and Mecsek Hills), isolated at the lower course of the Danube, show Mediterranean influences (Varga, 1995).

Bombina bombina and *B. variegata* are present throughout the Carpathian Basin, including most of Hungary and nearby Slovakia, Ukraine, and Romania. *B. variegata* is patchily distributed in this region, with populations restricted to four mountainous regions: the Northern Middle Range, the Transdanubian Middle Range, the hilly ranges in southeastern Transdanubia, and the Western Transdanubian Alpine foothills (Fig. 1).

Within the Carpathian Basin, the Northern Middle Range, the Transdanubian Middle Range, and the Southern Transdanubia play a key role for analyzing evolutionary interactions between both toad species, because in these areas *B. variegata* populations are completely surrounded by *B. bombina* (Szymura, 1993). In the Alpine foothills *B. variegata* populations are not isolated, but the ranges of the two species meet in this region. These four hilly areas surround the Pannonian lowland where *B. bombina* is a common, widely distributed species (Méhely, 1891). Hybridization between *B. variegata* and *B. bombina* in or around the islands occupied by *B. variegata* in Hungary has

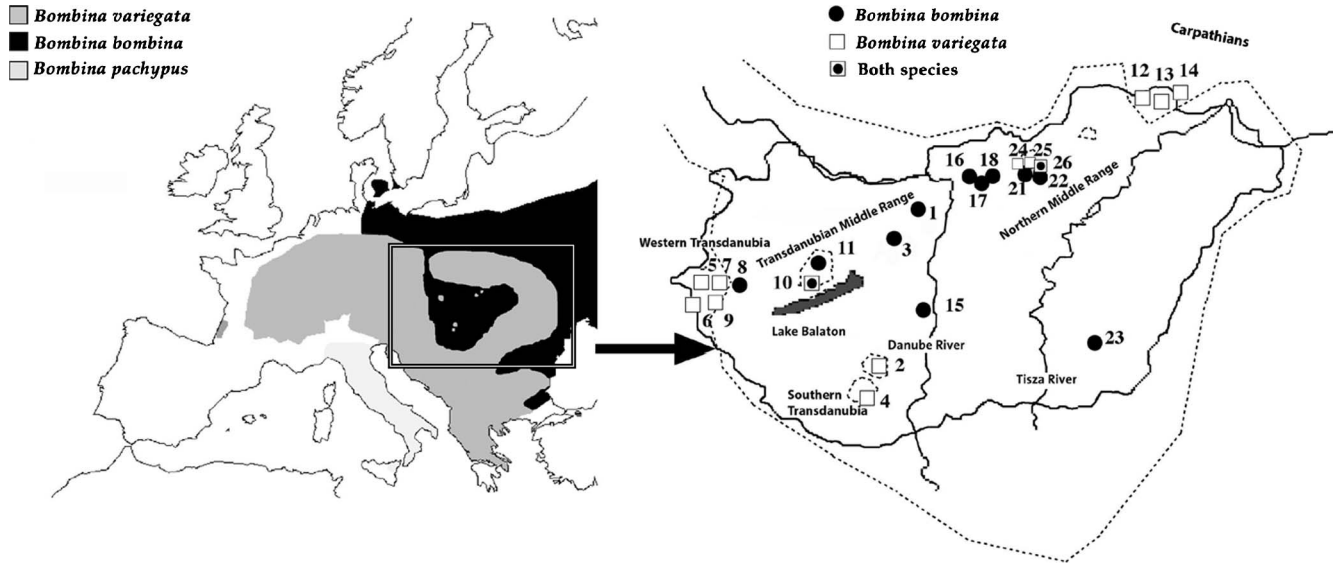

Fig. 1. Distribution of *Bombina bombina*, *B. variegata*, and *B. pachypus* in Europe (left) and in Hungary (right). Numbers indicate sampling localities (see also Table 1). Only localities in Hungary are shown in the map. Continuous line shows the border of Hungary, dashed line represents the border of the distribution of *B. variegata*.