

Phylogeography and historical demography of the Italian treefrog, *Hyla intermedia*, reveals multiple refugia, population expansions and secondary contacts within peninsular Italy

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Abstract

We investigated the geographical patterns of genetic diversity in the Italian treefrog through sequence analysis of a mitochondrial cytochrome *b* gene fragment. Three main mitochondrial lineages were identified, distributed in northern, central and southern Italy, respectively. Their divergence appears indicative of a split time largely predating Late Pleistocene climatic oscillations, and syntopy between them was only observed in the geographically intermediate populations. The historical demographic reconstructions suggest that in both northern and central Italy, an expansion occurred during the last major glacial phase, when a vast widening of the lowland habitats followed the glaciation-induced fall of the sea level. Instead, in southern Italy an expansion event likely followed the end of the last glaciation, although the inference of expansion appears less reliable for the southern clade than for the others. Within this geographical area, a sharp phylogeographic discontinuity separated peninsular from Sicilian populations, and the overall pattern of diversity suggests that the latter derived from a recent colonization of the island, probably through a Late Pleistocene land bridge. Phylogenetic, phylogeographic and historical demographic analyses thus concur in delineating a scenario of multiple refugia, with four groups of populations which survived the last glacial–interglacial cycles in at least three distinct refugia arranged along peninsular Italy, and have recently come into contact following range expansions. Therefore, these results support the hypothesis that a plethora of microevolutionary processes, rather than the prolonged stability of populations, were mainly responsible for shaping the patterns of diversity within this major biodiversity hotspot.

Keywords: *Hyla intermedia*, Italy, mitochondrial DNA, multiple refugia, phylogeography, secondary contacts

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Introduction

Quaternary climatic oscillations have played a major role in shaping the present geographical distribution of both species and their genetic diversity (recent reviews in Hewitt 2004a, b). Following these oscillations, most temperate European taxa were forced into repeated cycles of retreat within refugial ranges during pleniglacials and of expansion

during subsequent interglacial phases. A huge amount of literature based on both palaeoecological and genetic data indicate the three Mediterranean peninsulas of Iberia, Italy and the Balkans as important southern Quaternary glacial refugia (see Weiss & Ferrand 2006 and the many references therein). Populations from these refugial ranges have often been observed to harbour a high genetic diversity. Also, widely distributed species often show in these areas the largest portion of this diversity, leading to the pattern of so-called 'southern richness, northern purity' (e.g. Hewitt 1996, 1999, 2000; Taberlet *et al.* 1998; but see also Stewart & Lister 2001; Petit *et al.* 2003; Deffontaine *et al.* 2005; and

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