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Conservation of the endangered Mediterranean tortoise *Testudo hermanni hermanni*: The contribution of population genetics and historical demography

Saliha Zenboudji [a,*], Marc Cheylan [a], Véronique Arnal [a], Albert Bertolero [c], Raphael Leblois [d], Guillelme Astruc [a], Giorgio Bertorelle [e], Joan Ll. Pretus [f], Mario Lo Valvo [g], Giuseppe Sotgiu [h], Claudine Montgelard [a,b]

[a] CEFE UMR 5175, CNRS, Université de Montpellier, Université Paul-Valéry Montpellier, EPHE, 1919 route de Mende, 34293 Montpellier Cedex 5, France
[b] Department of Zoology, University of Johannesburg, P.O. Box 524, Auckland Park 2006, South Africa
[c] Associació Ornitològica Picampall de les Terres de l'Ebre, C/La Galera 53, 43870 Amposta, Spain
[d] INRA, UMR1062 CBGP, F-34988 Montferrier-sur-Lez, France
[e] Department of Life Sciences and Biotechnology, University of Ferrara, via L. Borsari 46, 44100 Ferrara, Italy
[f] Department of Ecology, University of Barcelona, Spain
[g] Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo, via Archirafi 18, 90123 Palermo, Italy
[h] Association Zirichiltaggi-Sardinia Wildlife Conservation, s.v. Filigheddu 62/C, 07100 Sassari, Italy

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ABSTRACT

Estimating the genetic variation and demographic trends of species in decline is of major concern in conservation genetics. This study contributes to understanding how historical and anthropogenic factors shape the distribution of current genetic diversity in one of the most endangered reptiles in Western Europe, the Hermann's tortoise *Testudo hermanni hermanni*. We used 17 microsatellite loci, chosen from a pyrosequencing library specifically developed for the subspecies to genotype eight populations distributed over about 30 sample localities covering almost the entire geographic distribution of the sub-species. The population genetic results reflect a very strong genetic structure and identify three major clusters among the Hermann's tortoise in the occidental Mediterranean basin: a continental cluster (Albera in Spain, Var in France and continental Italy), an insular cluster (Corsica, Sardinia and Sicily) and a cluster of mixed lineage (Minorca). Each of the eight studied populations is characterized by only one lineage except for Minorca, in which two lineages were identified. In contrast to what most empirical studies predict, the genetic diversity of the Hermann's tortoise is greater on islands than on the continent. Islands can therefore be considered as genetic sanctuaries with high conservation potential for this species, particularly in Corsica. Historical demographic patterns inferred with a generalized stepwise-mutation model (GSM) using maximum likelihood showed significant past demographic changes in only two of the eight sampled populations: a demographic bottleneck was detected in the Albera population (Spain) and a demographic expansion in Corsica (France). In contrast to what was argued in previous studies, these results indicate that the Hermann's tortoise lineage found in Corsica is autochthonous. The origin of both lineages found in Minorca remains speculative. Lastly, our study identifies the six most relevant management units (sensu Moritz, 1994) for conservation purposes on the basis that they represent a significant part of the evolutionary legacy of the species. Some conservation recommendations were proposed, in particular for the most threatened population in Albera.

1. Introduction

Conservation biology is an applied science that includes diverse disciplines (Soulé, 1985). Of these, genetics contributes fundamental knowledge concerning the genetic state of populations (both genetic structure and diversity), as well as demographic trends, inbreeding/outbreeding and introgression (Frankham et al., 2009). Consequently, a variety of roles have been identified for the implications of genetics in conservation (see DeSalle and Amato, 2004). According to Shaffer et al. (2015), one of the most direct roles of genetics in informing amphibian and reptile conservation actions involves identifying and managing the human activities that endanger wild populations. They identify three main topics that we feel are particularly relevant: the trade in threatened species, captive breeding, and the design of natural reserves and parks. For several key species, genetics has helped to identify the source of traded species, the reduction in genetic diversity associated with declining populations, and the patterns of movements and reintroductions (Velo-Antón et al., 2011; Welton et al., 2013). Moreover, genetics has contributed to successfully preventing inbreeding and outbreeding depression by creating ex-situ captive breeding programs that mimic natural mating systems, and by managing levels

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* Corresponding author at: 1919 route de Mende, 34293 Cedex 5 Montpellier, France.
E-mail address: saliha.zenboudji@cefe.cnrs.fr (S. Zenboudji).

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of genetic variation to maximize the probability of successful reintroductions into the wild (Austin et al., 2011; Witzenberger and Hochkirch, 2011). At a larger scale, genetics can and should play a critical role in reserve design (Vasconcelos et al., 2012; von der Heyden et al., 2014). Understanding the ecological and evolutionary processes that maintain and generate biodiversity is essential for designing effective natural reserves (Smith and Grether, 2008). Genetics has been used to define many types of conservation units, such as evolutionarily significant units (ESUs) and management units (MUs, Moritz, 1994), and measures such as phylogenetic diversity (PD, Winter et al., 2013). These have all been proposed as critical tools in identifying the most important landscapes for protection as they can help to capture evolutionary processes (Faith, 2008; Smith and Grether, 2008).

The contraction of a species' geographic range as a consequence of habitat fragmentation constitutes a major threat to its diversity (Young and Clarke, 2000; Freeland et al., 2011). Fragmentation increases the isolation of populations in sparse remnant patches, causing a decrease in population size often referred to as a population bottleneck. Its main consequence is the loss of alleles through genetic drift leading to a reduction in genetic diversity (Bouzat, 2010; Frankham et al., 2009). In the long term, completely isolated population fragments will suffer elevated rates of inbreeding due to reduced gene flow between populations. This negative spiral, leading ultimately to the disappearance of a population, has been named the “extinction vortex” (Frankham et al., 2009). For the management of populations, effective population size (*Ne*) is a critical piece of information (Frankham, 2007). Because reptiles and amphibians are often difficult to count in the wild, direct estimates of the number of breeding individuals are rare (Shaffer et al., 2015). The application of genetic methods can not only help to estimate the effective breeding size of a population, but for several species also offers a valuable alternative that allows the historical patterns of population expansion or decline to be inferred (Funk et al., 1999; Phillipsen et al., 2011; Casas-Marce et al., 2013; Kamath et al., 2015). From a conservation perspective, the identification of demographic bottlenecks is crucial because these lessen the chance of survival of populations that have undergone significant declines (Peery et al., 2012). The quantification of genetic variation and demographic trends in declining and/or fragmented species is a major concern in conservation genetics studies (Lozier, 2014) since the evolutionary potential and, ultimately, the persistence of a species depend, among other things, on its ability to maintain genetic diversity (Frankham et al., 2009; Lozier, 2014).

Dramatic declines in the abundance of numerous species, particularly reptile species, have been driven by historical climatic events as well as several anthropogenic and ecological factors. These include landscape transformation, environmental fluctuation and demographic stochasticity (Collins and Storfer, 2003; Araújo et al., 2006; Bouzat, 2010; Böhm et al., 2013). This study focuses on the genetic diversity and demographic history of one of the most endangered reptiles in Europe, the western Hermann's tortoise *Testudo hermanni hermanni* Gmelin, 1789. The Hermann's tortoise is distributed in the Mediterranean region (Fig. 1). Two subspecies have been described by Wermuth (1952) on the basis of morphology and coloration patterns: *Testudo hermanni boettgeri* mainly occupies the Balkans, where it has a large and more or less continuous distribution that extends to north-eastern Italy; whereas *T. h. hermanni* occupies the western part of Europe in isolated populations (Cheylan, 2001; Bertolero et al., 2011). These two subspecies are separated by the plain of the Po River in northeastern Italy. The fragmented distribution of *T. h. hermanni* is mainly due to anthropic pressures resulting from habitat destruction and overharvesting, as well as to climate fluctuations of the Quaternary period that have reduced population ranges in refuges located on the northern fringe of the Mediterranean Sea (Cheylan, 1981; Morales Pérez and Sanchis Serra, 2009). Due to its marked decline throughout most of its distribution range (Cheylan, 1984; Bertolero et al., 2011; Cheylan et al., 2011), *T. h. hermanni* was classed as ‘endangered’ on the IUCN Red List (European Reptile and Amphibian Specialist Group, 1996) 20 years ago. Subsequently, the Var (France) and Albera (Spain) populations were also classified as ‘endangered’ on the French and Spanish Red Lists (Llorente et al., 2002; UICN France et al., 2015). In contrast, the populations of *T. h. hermanni* in Corsica and the Balearic Islands seem to show good demographic dynamism (Cheylan, 1995; Bertolero, 2014a).

Practical conservation measures to protect *T. h. hermanni* have mostly been undertaken where the species is identified as vulnerable

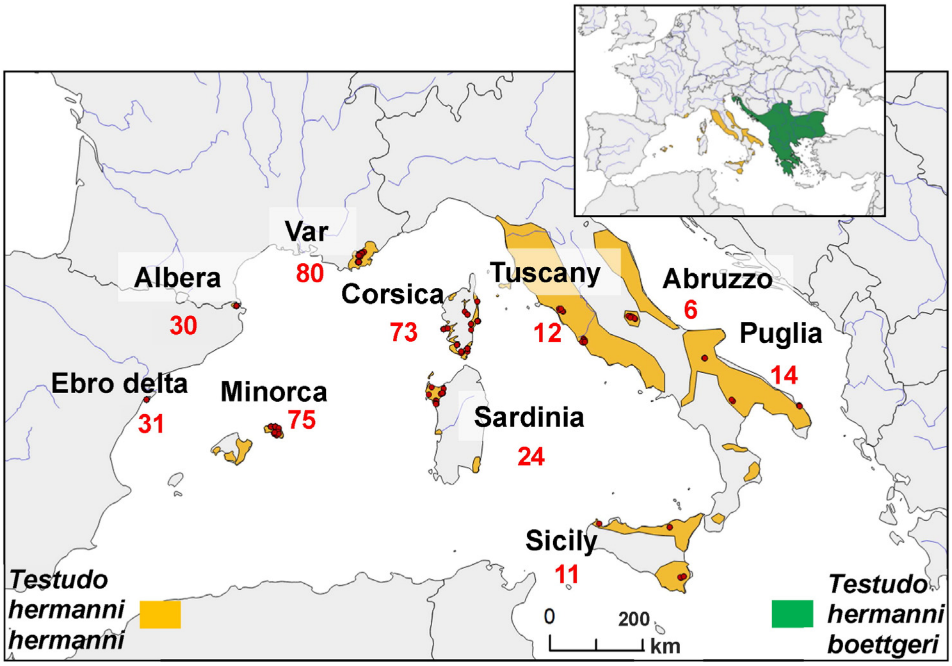

Fig. 1. Sampling areas and sample sizes of *Testudo hermanni hermanni* (see Table 1 for details). Sampled localities are indicated by red dots. The inset map shows the current distribution of the two *Testudo hermanni* subspecies: *T. h. hermanni* in orange and *T. h. boettgeri* in green (from Bertolero et al., 2011). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)