Phylogeographic link between Sicilian and Corso-Sardinian Testudo h. hermanni confirmed

GABRIELE GIACALONE1, MARIO LO VALVO1, UWE FRITZ3

1 Laboratorio di Zoologia applicata, Dipartimento di Biologia Animale, Università degli Studi di Palermo, Via Archirafi 18, I-90123 Palermo, Italy.
2 Present address: Dipartimento di Botanica, Università degli Studi di Catania, Via Antonino Longo 19, I-95125 Catania, Italy.
3 Museum of Zoology (Museum für Tierkunde), Senckenberg Dresden, A.B. Meyer Building, Königsbrücker Landstr. 159, D-01109 Dresden, Germany. Corresponding author. E-mail: uwe.fritz@senckenberg.de.

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Abstract. Our study confirms that Sicilian and Corso-Sardinian Testudo h. hermanni share a certain mtDNA haplotype, while tortoises from peninsular Italy harbour slightly different haplotypes. When the fossil record is considered, this striking pattern agrees well with the idea of local extinction in Corsica and Sardinia and later replacement by tortoises originating elsewhere, either by natural oversea dispersal or translocation by man.

Keywords. Testudo hermanni, phylogeography, Corsica, Sardinia, Sicily.

Traditionally, the fauna of the islands Corsica, Sardinia and Sicily is considered to constitute the Tyrrhenian faunistic province (La Greca, 1995). However, while Corsica and Sardinia are known to share many endemic or closely related taxa, Sicily represents a distinct entity (Lanza and Vanni, 1987; Lanza, 1988; Cheylan, 1992). Most of Sicily’s zoogeographic affinities point at the Italian peninsula (Cheylan, 1992), although some endemic taxa are known (Stöck et al., 2008), one of which (Bufo siculus) suggests a link to North Africa. Also other taxa support this relationship between Sicily and North Africa (La Greca, 1990, 1995; Cheylan, 1992).

Fritz et al. (2006) demonstrated that two Sicilian Testudo hermanni hermanni harboured a mitochondrial haplotype also occurring on Corsica and Sardinia, an unexpected finding when it is considered that tortoises from the Italian peninsula possess distinct haplotypes. In order to find out whether the two individuals studied by Fritz et al. (2006) might represent introductions from Corsica or Sardinia, we obtained blood samples of T. h. hermanni from five other Sicilian populations (Fig. 1) and sequenced most of the mitochondrial cytochrome b gene (cyt b), the marker used by Fritz et al. (2006). In addition, we sequenced part of the faster evolving mitochondrial control region (CR; Table 1) from two Sicilian, three Corsican, and two Sardinian tortoises.
Fig. 1. Collection sites of Sicilian *Testudo hermanni hermanni* samples used in this study and in Fritz et al. (2006). Site numbers refer to Table 1.

Table 1. Geographic origin of *Testudo hermanni* samples used in this study, Sicilian samples from Fritz et al. (2006) and the studied mtDNA fragments. Sample numbers refer to the Tissue Collection of the Museum of Zoology Dresden (MTD T); site numbers, Fig. 1.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Site</th>
<th>Cyt b</th>
<th>CR</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>2140</td>
<td>1 – Castellammare del Golfo (Palermo), Sicily</td>
<td>+</td>
<td>-</td>
<td>Fritz et al. (2006)</td>
</tr>
<tr>
<td>2141</td>
<td>2 – Balestrate (Palermo), Sicily</td>
<td>+</td>
<td>-</td>
<td>Fritz et al. (2006)</td>
</tr>
<tr>
<td>4078</td>
<td>3 – M. M. Belsito (Madonie), Sicily</td>
<td>+</td>
<td>-</td>
<td>This study</td>
</tr>
<tr>
<td>4075</td>
<td>4 – Collesano-Campofelice (Madonie), Sicily</td>
<td>+</td>
<td>-</td>
<td>This study</td>
</tr>
<tr>
<td>4077</td>
<td>4 – Collesano-Campofelice (Madonie), Sicily</td>
<td>+</td>
<td>-</td>
<td>This study</td>
</tr>
<tr>
<td>4082</td>
<td>4 – Collesano-Campofelice (Madonie), Sicily</td>
<td>+</td>
<td>-</td>
<td>This study</td>
</tr>
<tr>
<td>4074</td>
<td>5 – Caronia (Nebrodi), Sicily</td>
<td>+</td>
<td>-</td>
<td>This study</td>
</tr>
<tr>
<td>4079</td>
<td>6 – S. Agata di Militello (Nebrodi), Sicily</td>
<td>+</td>
<td>+</td>
<td>This study</td>
</tr>
<tr>
<td>4083</td>
<td>7 – Augusta (Siracusa), Sicily</td>
<td>+</td>
<td>-</td>
<td>This study</td>
</tr>
<tr>
<td>4084</td>
<td>7 – Augusta (Siracusa), Sicily</td>
<td>+</td>
<td>+</td>
<td>This study</td>
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<td>+</td>
<td>-</td>
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<tr>
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<td>+</td>
<td>-</td>
<td>This study</td>
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<tr>
<td>1921</td>
<td>Porto Vecchio, Corsica</td>
<td>-</td>
<td>+</td>
<td>This study</td>
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<tr>
<td>1927</td>
<td>Casabianda, Corsica</td>
<td>-</td>
<td>+</td>
<td>This study</td>
</tr>
<tr>
<td>1936</td>
<td>Fontane du Salario (Ajaccio), Corsica</td>
<td>-</td>
<td>+</td>
<td>This study</td>
</tr>
<tr>
<td>1114</td>
<td>Porto Palmas (Sassari), Sardinia</td>
<td>+</td>
<td>+</td>
<td>Cyt b: Fritz et al. (2006), CR: this study</td>
</tr>
<tr>
<td>1115</td>
<td>Bay of Porto Ferro (Sassari), Sardinia</td>
<td>+</td>
<td>+</td>
<td>Cyt b: Fritz et al. (2006), CR: this study</td>
</tr>
</tbody>
</table>
Laboratory procedures followed Fritz et al. (2006). For amplification and sequencing of an approximately 1000 bp long fragment of cyt b the primers CytbG (Spinks et al., 2004), mt-f-na, mt-c-For2, and mt-E-Rev (Fritz et al., 2006) were used; for an approximately 850 bp long CR fragment, the primers Thr-L15569 and Phe-H26 (Palkovacs et al., 2003). An ABI 3130 Genetic Analyzer was used for automatic sequencing in both directions. Obtained sequences were checked by eye and aligned. Cyt b sequences were compared with published data (Fritz et al., 2006). Using TCS 1.21 (Clement et al., 2000), a parsimony network was constructed from a 1008 bp long alignment including all 31 previously published Western Mediterranean T. h. hermanni sequences (Fritz et al., 2006) plus our 10 new sequences from Sicily (Fig. 2). The alignment of the CR sequences comprised 855 bp.

All of our 10 Sicilian T. h. hermanni harboured the same cyt b haplotype (H5) as the two Sicilian tortoises studied by Fritz et al. (2006). With respect to the CR, all Corsican and Sardinian tortoises shared the same haplotype; this haplotype was also found in one Sicilian tortoise. The second Sicilian individual (MTD T 4079) possessed a similar haplotype differing in only one mutational step (position 815 of our alignment: T instead of A). The two CR haplotypes are deposited under accession numbers FN298446-FN298447 in GenBank.

Compared to the eastern subspecies T. h. boettgeri, the phylogeographic structure of Western Mediterranean T. h. hermanni is strikingly shallow (Fritz et al., 2006). This is unexpected because the fossil record provides evidence for its long presence and wide distribution in the Western Mediterranean (Delfino, 2002; Morales Pérez and Sanchis Serra, 2009). Amongst others, fossils are known from the Middle Pleistocene of Corsica (Hervet, 2000,

Fig. 2. Parsimony network of mtDNA haplotypes (cyt b fragment, 1008 bp) of Western Mediterranean Testudo hermanni hermanni, using the dataset of Fritz et al. (2006) and 10 new Sicilian sequences. Haplotype nomenclature follows Fritz et al. (2006). Symbol size corresponds to haplotype frequency; missing haplotypes, dots. Each line between symbols represents one mutation step. The arrow indicates the connection to Eastern Mediterranean T. h. boettgeri haplotypes. Black symbols, peninsular Italy and southern France (Var); dark grey, Sicily; light grey, Corsica and Sardinia; white, Spain (i: introduced populations or populations known to comprise also allochthonous individuals, n: native population). H1: n = 12, H2: n = 1, H3: 7, H4: n = 1, H5: n = 18, H6: n = 1, H7: n = 1. When only Western Mediterranean haplotypes are considered, H1 is under coalescent theory ancestral to H2-H7 (outgroup probability of H1: 0.3175).
2001; Hervet and Salotti, 2000) and from the Plio-Pleistocene boundary of Sardinia (Abbazzi et al., 2004). On the Italian peninsula, the oldest findings date back to the Pliocene; the oldest fossils from Sicily are from the Middle Pleistocene (Delfino, 2002), suggesting that Sicily was colonized later than Corsica and Sardinia. Consequently, some extent of genetic differentiation of the island tortoises and a closer relationship of the Sicilian and the peninsular Italian populations should be expected, but not between Sicily and Corso-Sardinia.

The weak genetic differentiation of Western Mediterranean tortoises might be related to a major climatically caused extinction event some 38000-39500 years ago, wiping out many populations (Morales Pérez and Sanchis Serra, 2009). However, while our study confirms that Sicilian and Corso-Sardinian *T. h. hermanni* are not clearly differentiated in the studied genetic markers, they are slightly distinct from peninsular Italian tortoises (and from native Spanish tortoises; Fig. 2). This pattern supports the survival of several local populations in the Western Mediterranean, in agreement with fossil evidence (Delfino, 2002; Morales Pérez and Sanchis Serra, 2009) for instance on the Italian peninsula and Sicily. Furthermore, the genetic identity of Corsican, Sardinian, and Sicilian tortoises agrees also well with the idea of local extinction in Corsica and Sardinia and later replacement by tortoises originating elsewhere, either by natural oversea dispersal or translocation by man.

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REFERENCES


