The study of the genetic pattern of *Testudo graeca graeca* in the north of Africa helps to identify the origin of south-eastern Spanish populations

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INTRODUCTION

The Moorish Tortoise (*Testudo graeca;* Fig. 1) includes in its Western Mediterranean distribution five parapatric subspecies that have their main range in the North of Africa (Morocco, Algeria, Tunisia and Lybia), but also occurs in some isolated locations in Europe (Fig. 2), where the origin of populations has been identified recent (Álvarez et al., 2000; Fritz et al., 2009; Salinas et al., in press; Graciá et al., in press). Although this species is one of the most charismatic of the circum-Mediterranean fauna and is endangered throughout its range by habitat loss and its collection as a pet, the genetic variability of North African populations in a regional context has not been studied. This knowledge is relevant not only for the species' conservation and management, but also could help us to gain a deeper understanding of its biogeography in the north of Africa and identify the possible origin of European populations.

In this work we undertook the study of genetic patterns within the subspecies *Testudo graeca graeca* across its entire range in North Africa (Morocco and Algeria). Furthermore, we assessed the origin of some south-eastern Spanish tortoises in this context.

Figure 1.- A juvenile individual of *Testudo graeca graeca* sampled in the Saharian Atlas (Djelfa, Algeria).

MATERIALS AND METHODS

We sampled 87 individuals from 9 natural populations of *T. g. graeca* located in different localities in the Saharan and Tellian Atlas in Algeria and Morocco (Fig. 3). Individuals were genotyped at seven microsatellite markers: Test 76, Test 71, Test 10, Gp96, GmuB08, GmuD16 and GmuD51 (previously used with the same subspecies by Salinas et al., in press). Different genetic units were detected (k) using TESS 2.3 software (Chen et al., 2007; Durand et al., 2009).

A 666-bp-long mt-DNA fragment comprising part of the cytochrome *b* was sequenced for 10 individuals from the Algerian Saharan Atlas, sampled for the first time. For PCR amplifications we used GLUDG-5 and Cytb3-3 primers (Palumbi, 1996). The resulting haplotypes were collapsed in the TCS 1.21 program (Clement et al., 2000) together with available *T.g. graeca* sequences in GenBank. We considered in this analysis only samples with a known geographical origin in the north of Africa (Fritz et al., 2009).

Finally and just as a preliminary analysis to locate the ancient origin of south-eastern Spanish tortoises in North Africa, we performed an assignment test in GENECLASS2.0 (Piry et al., 2004). We genotyped 30 tortoises for the same seven microsatellites and we used the Algerian genetic groups detected during this study as reference populations.

Figure 2.- Subspecies' distribution in the Western Mediterranean range of *T. graeca* (green - *T. g. cyrenaica*; yellow - *T. g. nabeulensis*; red - *T. g. graeca*; orange - *T. g. marokkensis*; blue - *Tg. soussensis*).

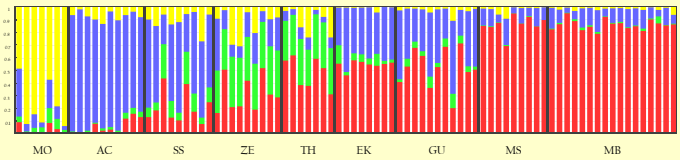

Figure 3.- Bar plots for k = 4 generated using an admixture model in TESS 2.3. Bars represent the probability of each individual belonging to each cluster. Geographical locations of these Moroccan and Algerian populations are shown in Fig. 3.

RESULTS AND DISCUSSION

While the Test 76 and Gp 96 microsatellite markers were monomorphic, the rest of the loci provided us with a total of 79 different alleles. k = 4 was the most supported value for the cluster analysis (Fig.4). Bar plots showed spatial congruence of the assignment of individuals to different clusters revealing two main clines of differentiation, one parallel to coast and another toward the interior. Six new haplotypes (B1.8-B1.13) were found at the two populations in the Saharan Atlas. In these populations we only found one shared haplotype with the Tellian Atlas in a single sample (Fig. 4).

Our results showed that the Tellian and Saharan Atlas mountain ranges harbour different genetic units with more levels of admixture to the east and south of the Tellian Atlas. The valley of the Moulouya River also constitutes one of the most important barriers shaping this pattern. These results are congruent with the previously described biogeography of the species.

In the assignment test for south-eastern Spanish tortoises, only 9 out of the 30 analyzed individuals were assigned with a high level of membership to the Algerian genetic units we had detected. Five were assigned to the AC+SS+GU group, 1 to the TH+ZE group and 3 to the MO population. The majority of the south-eastern Spanish samples showed low levels of membership to the different Algerian units, highlighting the high level of differentiation found among these two populations, which is in line with previous studies (Fritz et al., 2009; Salinas et al., in press; Graciá et al., in press). In addition, our results revealed the west of Algeria and the east of Morocco coastal areas contained the higher proportion of the shared genetic pool between south-eastern Spanish and North African tortoises, suggesting this area as the possible geographic origin of south-eastern Spanish populations. These results support both human-mediated introduction or ancient range expansion as possibilities for its arrival. In any case, we consider these lasts as preliminary results. To better clarify this point further studies comparing the genetic patterns of both populations in a regional context are needed.

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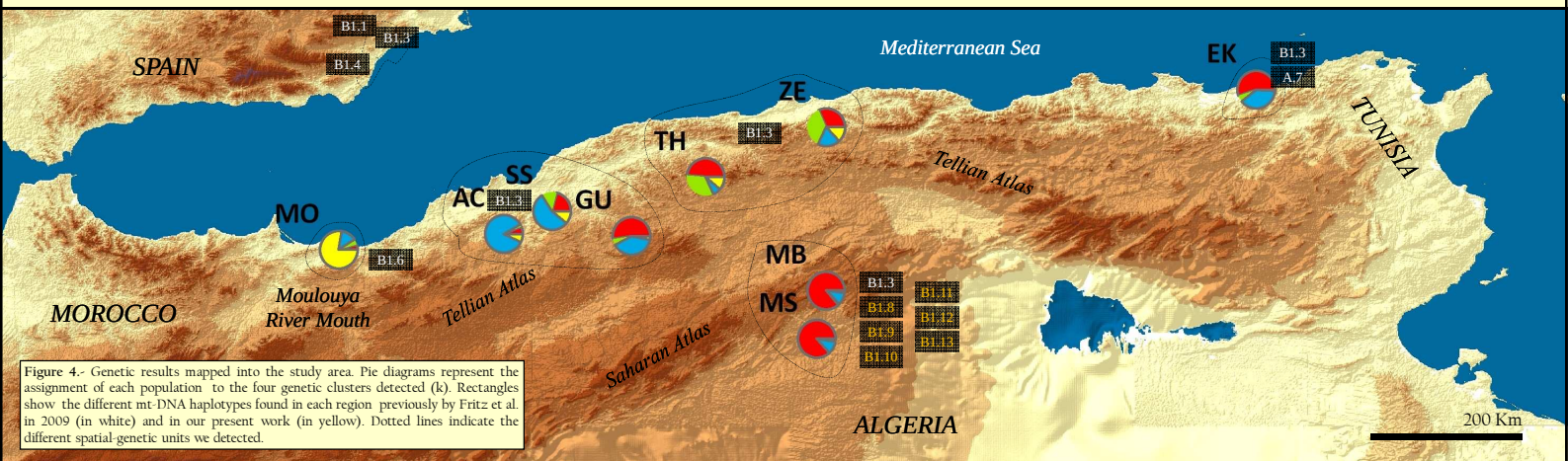

Figure 4.- Genetic results mapped into the study area. Pie diagrams represent the assignment of each population to the four genetic clusters detected (k). Rectangles show the different mt-DNA haplotypes found in each region previously by Fritz et al. in 2009 (in white) and in our present work (in yellow). Dotted lines indicate the different spatial-genetic units we detected.