Assessing genetic variability in *T. aestivum* populations from Turkey

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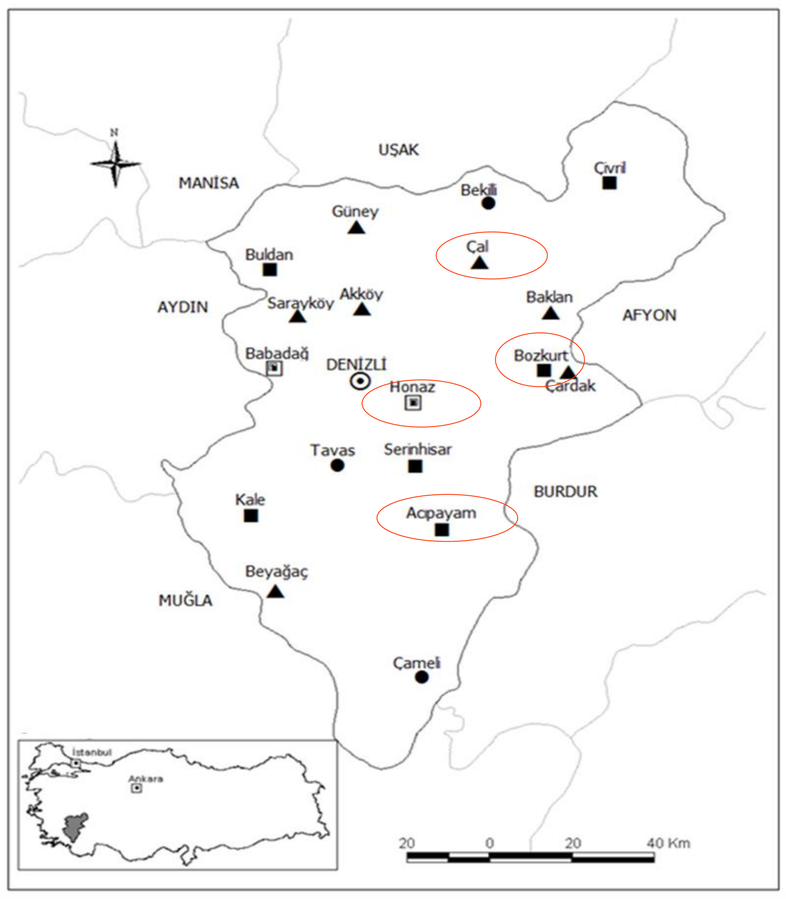
Introduction

Previous studies have shown that within the *T. aestiuvum* species-complex, specimen can show a *continuum* of morphological traits between the *T. uncinatum* and the *T. aestivum* morphotypes and different ITS/RFLP patterns (Paolocci *et al*. 2004). *T aestivum*, differently from *T. melanosporum* and *T. magnatum* whose geographical ranges are confined around the Mediterranean area and surroundings, is distributed all over Europe. Natural *T. melanosporum* and *T. magnatum* populations from the southernmost areas of their ranges show higher levels of genetic diversity than those from northern sites (Murat *et al*. 2004; Rubini *et al*. 2005; Riccioni *et al*. 2008). As it is likely that these species experienced a population bottleneck during the last ice age, these data have been interpreted to mean that refugia for *T. magnatum* and *T. melanosporum* were located in the southernmost areas of their original ranges to spread northward as the ice receded.
Whether *T. aestivum* also experienced a population bottleneck during the last ice age is currently unknown. A detailed analysis of distribution and extent of genetic variability within and among its southernmost populations would certainly help to address this crucial point and likely mark these truffles according to their origin.
To this end, we started collecting truffles from different natural sites located in the Anatolian peninsula (Turkey). Here we present preliminary results on biodiversity of *T. aestivum* samples from this region by means of ITS sequencing analysis.

Materials and Methods

The collected truffles were identified on the basis of morphological traits according to Montecchi e Sarasini (2000). Genomic DNA was isolated as previously reported (Paolocci *et al*. 1999) and amplified using the ITS1-ITS4 primer pair and PCR condition reported in Paolocci *et al*. (2004). ITS sequence were aligned using ClustalX software (Thompson *et al*. 1997). The phylogenetic analysis was performed with MEGA version 5.05 (Tamura *et al*. 2011) by using the neighbor-joining method. Bootstrap test was performed using 1000 replicates.

Fig.1 Map of the Anatolian areas where truffles were collected



Results

More than 150 truffles were collected in the Denizli province, Anatolian peninsula, during the period May-August 2010. They were from four main areas (Fig.1) and different sites within each area (Table1). The most of them were ascribed to the *T. aestivum-uncinatum* species-complex on the basis of their morphology, whereas only a few samples displayed the morphotype typical of *T. mesentericum* (2) and *T. rufum* (1).

Table 1. Provenance and number and truffles sampled

Area	N° of samples	N° of collection sites/area
Honaz	36	6
Bozkurt	82	6
Cal	12	5
Acipayam	23	3

DNA was isolated from a subsample (30) of truffles exhibiting the *T. aestivum-uncinatum* morphotype harvested in Honaz, Bozkurt and Cal areas. The ITS sequence analysis confirmed the identity of all the specimen and highlighted polymorphism between truffles from different areas but also from the same collection site. Interestingly, some truffles (t8, t13, t34, t36, t50, t56, t120, and t129) exhibited the G:C rich region in their ITS2 region reported to interfere with nucleotide calling when sequencing reactions are resolved by capillary electrophoresis (Paolocci *et al*. 2004). Because of this, to get insight into the extent and distribution of the genetic variability among *T. aestivum* samples from Anatolia and between these samples and those collected outside Turkey, only the ITS1 and 5.8S regions were considered. In Fig.2 is given the dendrogram resulting from the alignment of ITS1-5.8S amplicons from Turkish samples with those from *T. aestivum-uncinatum* truffles collected in Italy. Italian samples were selected as a representative group of truffles from one of the southernmost area of the geographical range of this species. Notably, regardless of their specific collection sites, most of the truffles (8 out of 10) from the Cal area grouped apart from all the others. In addition to this, the vast majority of samples from Bozkurt and Honaz tended to cluster apart from those from Italy.

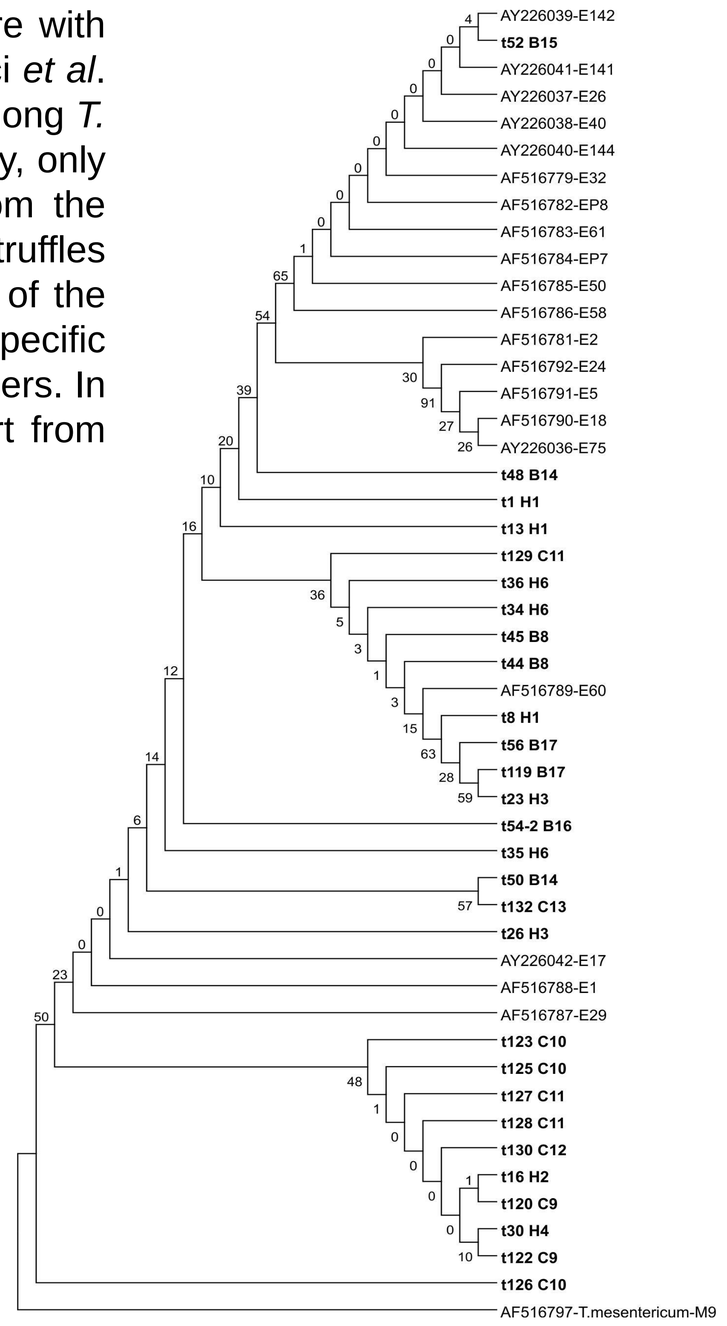


Fig.2 Dendrogram showing the similarity between *T.aestivum* truffles collected in Turkey and Italy. The samples from Turkey are indicated in bold type, letters indicate the areas (B= Bozkurt; C =Cal; H=Honaz) and the number next to the letter the collection site.

Discussion and perspectives

These preliminary analyses let us to argue that *T. aestivum-T. uncinatum* truffles from Anatolian peninsula could be typed according to their provenance and that they likely display specific signatures to be differentiated from European populations. The ongoing sequencing of ITS from additional Anatolian samples and alignment with sequences of *T. aestivum* truffles from all over Europe will provide us with more data to verify these hypotheses.

References
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