SOD sequences in Ceratitis capitata (Diptera: Tephritidae) samples from different geographic origins

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The Mediterranean fruit fly, Ceratitis capitata (Wiedemann), is one of the world’s most economically important pest species. Owing to its own dispersal ability and human-mediated movement, this pest has rapidly spread from its putative source area in Central Africa to many areas with tropical and temperate climates. The origin and colonization pattern of this pest is still debated. Our work – as a part of a worldwide genetic study of Ceratitis capitata – presents the results of sequencing the Cu, Zn Sod gene in eleven different samples of Ceratitis capitata from widely distributed areas around the world: three from Spain (Menorca Island, Málaga, and Madrid); two from the North Mediterranean (Italy and Greece); one from Israel; two from Africa (Tunisia and South Africa); one from Réunion Island; and two from America (California, U.S.A. and Guatemala). Among the 1739 bases analysed, 19 informative polymorphic sites were detected: two deletions – including three and five nucleotides – 9 transversions, and 2 transitions. Three heterozygotes were detected (Menorca, Málaga and California). A phylogenetic tree was constructed and two main clusters were observed, one of which included three of the sequences from the three heterozygotes (i.e. sequences from Menorca, Málaga and California). The second cluster includes the other 11 sequences. Results are discussed in relation to the putative origin and possible colonization pathways of this pest.

INTRODUCTION

The Mediterranean fruit fly, Ceratitis capitata (Wiedemann), is one of the world’s most economically important pest species, attacking over 200 different vegetables, fruits and nuts. This species is responsible both for direct economic losses in fruit production and for the considerable efforts implemented for the detection and eradication programmes in those countries where this pest is found. Owing to its own dispersal ability and human-mediated movement, this pest has rapidly spread from its putative source area in Central Africa to many areas with tropical and temperate climates, such as the Mediterranean region, South Africa, Central and South America, and Australia. It is suspected that the Iberian Peninsula has played an important role in the spread of C. capitata through the Mediterranean region and possibly elsewhere.

The origin and colonization pattern of this pest is debated. Since the early 1990s, molecular methods have been applied trying to solve this issue. Our work – as a part of a worldwide genetic study of C. capitata – presents the first results of sequencing the Cu, Zn Sod gene in 11 worldwide samples of C. capitata, in order to provide insights about the putative origin and colonization pathways of this pest.

MATERIALS AND METHODS

Specimens representing 11 different geographical populations of C. capitata, were used in this study (Table 1): three from Spain (Menorca Island, Málaga and Madrid); two from the northern Mediterranean (Italy and Greece); two from Africa (Tunisia and South Africa); and one each from Israel, Réunion Island, the U.S.A. (California) and Guatemala.

Genomic DNA was extracted from individual flies, conserved at –80°C or ethanol preserved, following the protocol of Reyes et al. (1997). The primers for amplification were designed from DNA sequence of the Medfly Cu, Zn Sod gene (GenBank, Accession no. M76975) using the DNASTAR program (Lasergene 1997):

5’-CTATGTGATTCAGTTTTGTGCA TCCGC-3’ and
5’-CAGCTTCCTGGTTTTGGAGAAGGCT AGTGGG-3’.

PCR reactions were carried out in a 100 µl volume

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Table 1. Populations of Ceratitis capitata sampled in this study.

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with 0.2 mM of each dNTP, 10 µl of 10× *Taq* buffer, 1 µM of each primer, 2.5 units of polymerase Takara Ex*Taq*™ (Takara Shuzo Co.) and 25 ng of genomic DNA. Parameters for amplification were an initial denaturation at 94°C for 5 min followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 67°C for 1 min and extension at 72°C for 1 min; reactions were additionally kept at 72°C for 7 min to complete extension.

Owing to diploidy and the potential for heterozygosity, PCR products were cloned, using the *TA* cloning kit (Invitrogen), to isolate each allele before sequencing. DNA sequencing was done with an ABI Model 3700 automatic sequencer (Applied Biosystems) using the Ready Mix Big Dye Terminator v. 3.0 according to the manufacturer's protocol (Genetix). Besides the two amplification primers, three internal primers were used for sequencing: 5'-GTAAATAATATGCCTCCAAA-3'; 5'-TGAGTTATT TGTATTGCTTA-3'; 5'-ACATGCTGATC CTGATGATTTG-3'.

To distinguish real alleles from *Taq* errors, we sequenced at least three clones from each PCR reaction and then we identified and ignored singletons (nucleotides that vary in only one allele). *Sod* sequences were aligned using the DNASTAR program (Lasergene). A tree was constructed using the maximum parsimony (MP) method and *C. capitata* (M76975) as the outgroup. The reliability of the tree was evaluated employing 1000 bootstrap replicates. These analyses were carried out using the PAUP 4.0b8 software package (Swofford 2002).

**RESULTS**

A DNA fragment of approximately 1.75 Kb resulted from PCR amplifications. In these samples, after a correction for errors associated with cloning PCR products, a total of 33 sequences revealed three heterozygotes (from Málaga, Menorca and California samples) and six different alleles based on 19 polymorphic sites (1% of the whole sequence). Two of these 19 polymorphic sites involve deletions three and five nucleotides long respectively, nine are transversions and two are transitions. Out of the 19 polymorphic sites, 17 are in the first intron of *Cu, Zn Sod* and the other two in the second intron of the gene. Table 2 shows the six alleles. Allele 1 was present in all samples, except in the outgroup and on Réunion (REU). A2 was present only in the California (CAL) sample. A3 was found in the individual from Málaga (MAL). Allele 4 was also rare, being found only in the heterozygous individual from Menorca (MEN). Allele 5 was only present in the individual from Réunion (REU). And finally, A6 was present in the outgroup.

The phylogenetic relationships of the alleles are shown in Fig. 1. The dendrogram shows two main clusters, with high bootstrap values, one includes alleles A2, A3 and A4 present in the three heterozy-
gotes (i.e. sequences from California, Málaga and Menorca). The other cluster includes the remaining 11 sequences, belonging to alleles A5 (in REU), A6 (in outgroup) and A1 (present in all samples except REU and outgroup). Alleles A1 and A5 differ by only one nucleotide, while A1 and A6 differ by two (Table 2).

**DISCUSSION**

These are preliminary results of a worldwide genetic study of *C. capitata*, seeking to analyse the relationships of Medfly populations and to detect putative sources of new infestations. Owing to its economical importance as a major agricultural pest species, several studies are being carried out, using different genetic tools, to assess the genetic variability and structure of populations, as well as the possible colonization pathways of this pest (e.g. Gasperi et al. 1991; Kourt et al. 1992; Malacrida et al. 1992; Gasparich et al. 1995; Baruffi et al. 1997; Gasparich et al. 1997; Haymer et al. 1997; Malacrida et al. 1998; Villablanca et al. 1998; Davies et al. 1999; He & Haymer 1999). Questions about the putative origin of the pest and possible colonization pathways remain unsolved.

In the present work, a fragment of 1.75 Kb corresponding to Cu, Zn Sod gene has been sequenced in individuals from 11 different populations. The nucleotide diversity detected occurs in the intron sequences, where evolutionary constraints are fewer than in coding or regulatory sequences (Villablanca et al. 1998). Six allelic variants have been identified based on nucleotide sequence changes. Other studies of this species, using markers such as mtDNA or allozymes have revealed less genetic diversity (Malacrida et al. 1992; McPheron et al. 1994; Baruffi et al. 1995; Gasparich et al. 1997).

The dendrogram of alleles (Fig. 1) reveals that, with the present information, there is no phylogeographic structure of the populations, but some interesting results are shown. Allele A1 is the most common; it is shared among all the samples surveyed (including the California sample that would correspond to a recent infestation), except the Réunion sample and the outgroup. A1 is probably an ancestral allele. Alleles A2, A3 and A4 are rare, each present only once, in California (CAL), Málaga (MAL) and Menorca (MEN), respectively, and only in heterozygous individuals. Allele A5 is exclusive to the homozygote individual of Réunion (REU).

In summary, the analysis of the Cu, Zn Sod gene constitutes an efficient tool for detecting genetic diversity within and among populations of *C. capitata*. In the near future, a greater resolution about the putative origin, relationships among Medfly populations and possible colonization pathways of this pest could be provided by additional sequence data of samples from these same regions as well as from other regions of the world.

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**REFERENCES**


