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Mitochondrial and nuclear diversity in the green oak leaf roller *Tortrix viridana* L. (*Lepidoptera, Tortricidae*) from Sardinia

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The Green oak leaf roller *Tortrix viridana* is one of the most harmful pest of the Palearctic oaks. The young larvae of *T. viridana* develop inside the flushing buds and their egg hatch is synchronized with the bud burst of the oaks. The aim of the study was to investigate the genetic structure and variation of mitochondrial and nuclear loci in Sardinian populations associated with deciduous (*Quercus pubescens*) and evergreen (*Q. suber* and *Q. ilex*) oaks. We performed multilocus analyses (COI, COII mitochondrial genes and eight microsatellites) in about two hundreds individuals collected on all the three oak species coming from the most important forest districts in Sardinia Island, Italy. The populations were also characterized by egg hatching, after comparison in semi-field tests for egg development. Eighty-five mitochondrial haplotypes were found, with three ones shared among all populations, except the most southern one. A high haplotype diversity (H =0.90) and a low nucleotide diversity (π =0.003) have been observed. Microsatellites were highly variable with alleles ranging from 4 to 56 for locus and polymorphic information content values varying from 0.510 to 0.945. AMOVA tests were performed by structuring samples in different groups (geographic districts/oak species/egg development). The highest values of Fixation indexes resulted grouping populations according to early or late egg hatching. Furthermore, we tested for sex-biased dispersal, estimating pairwise F\(_{ST}\), F\(_{IS}\), and relatedness \(r\) at nuclear loci separately for the sexes. We produced evidence for non random patterns of gene flow probably mediated by male biased dispersal, with values significantly higher in females than in males and females the phylopatric sex. In support of a male biased dispersal, nuclear and mitochondrial markers provided different results in the matter of genetic structure, with mitochondrial loci, unlike microsatellites, highlighting significant differentiation levels among populations.
Mitochondrial and nuclear diversity in the green oak leaf roller Tortrix viridana L. (Lepidoptera, Tortricidae) from Sardinia

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The green oak leaf roller Tortrix viridana is one of the most harmful pest of the Palaearctic oaks. The young larvae of T. viridana develop inside the flushing buds and their egg hatch is synchronised with the bud burst of the oaks. The aim of the study was to investigate the genetic structure and variation of mitochondrial and nuclear loci in Sardinian populations associated with Quercus pubescens and Quercus ilex and (Q. suber and Q. Anx) oaks. The wide variability in budding within the different oak species determines the evolution of T. viridana populations which are adapted to a particular phenological type of oak and are characterized by different duration of summer diapause and hatching dates (Du Merle, 1999; Ivasov et al., 2002; Serra & Lentini, 2012).

We performed multilocus analyses in 362 individuals for COI, COII mitochondrial genes (total 2180 bp) and in 201 individuals for 8 microsatellites. Moths were collected in 24 localities with puceptene (QCP), cork (QS) or holm oak (QI) of seven Sardinian forestry districts. Individuals were characterized for sex, geographic origin, host species, and early or late on the basis of the duration of the summer diapause and the hatching time of the eggs after comparison in semi-field tests for egg development (Serra & Lentini, 2012).

### Mitochondrial genetic diversity and differentiation

<table>
<thead>
<tr>
<th>STR</th>
<th>Gallura</th>
<th>Gossano</th>
<th>Abbasanta</th>
<th>Iglesiente</th>
<th>Nuorese</th>
<th>Marmilla</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>362</td>
<td>42</td>
<td>70</td>
<td>37</td>
<td>52</td>
<td>66</td>
</tr>
<tr>
<td>ST</td>
<td>149</td>
<td>23</td>
<td>24</td>
<td>23</td>
<td>22</td>
<td>25</td>
</tr>
<tr>
<td>RST</td>
<td>0.04142</td>
<td>0.27823</td>
<td>0.3272</td>
<td>0.28022</td>
<td>0.33232</td>
<td>0.32675</td>
</tr>
<tr>
<td>Φ̂</td>
<td>0.00968</td>
<td>0.0391</td>
<td>0.04331</td>
<td>0.03819</td>
<td>0.0125</td>
<td>0.1576</td>
</tr>
<tr>
<td>F̂</td>
<td>0.090</td>
<td>0.0028</td>
<td>0.0055</td>
<td>0.0052</td>
<td>0.0038</td>
<td>0.06041</td>
</tr>
</tbody>
</table>

We found 149 haplotypes out of 362 collected specimens. Among them, two haplotypes (1, 2) showed a high frequency (23%, 15%, in red and blue respectively) and are present in all six districts but with different frequency in each population. We found 140 haplotypes with frequency <1%.

The haplotype 1 (in red) prevailed on evergreen QP and QI oaks populations in respect to deciduous (QP) population in the latter geographic area. A high haplotype diversity (H = 0.92) and a low nucleotide diversity (θ = 0.004) have been observed, with little differences across the observed populations (Table 1).

### Nuclear analysis

A Bayesian clustering method was used to examine population differentiation Applying STRUCTURE and the Evanno method to the entire data set, the best number of clusters given the microsatellite data was two. All df procedure suggested that there were K = 2 clusters, with individual assignment probabilities to individual clusters were low and nearly uniform across clusters. This outcome indicates that the analysis procedure overestimated the true number of clusters and that a large admixture among populations does exist.

We used factorial Correspondence Analysis (FCA) to examine genetic differentiation of multilocus genotypes. The analysis did not reveal any differentiation among populations that appeared clustered in an unique group. Outliers concern individuals from different populations.

### AMOVA tests for mitochondrial data

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Percentage of Variation</th>
<th>Fixation Index</th>
<th>ST values below diagonal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Among groups</td>
<td>14.32</td>
<td>Φ̂ = 0.443</td>
<td>21.30</td>
</tr>
<tr>
<td>Within groups</td>
<td>85.68</td>
<td>F̂ = 0.231</td>
<td>17.75</td>
</tr>
</tbody>
</table>

### AMOVA tests for microsatellites data

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Percentage of Variation</th>
<th>Fixation Index</th>
<th>ST values below diagonal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Among populations</td>
<td>0.16</td>
<td>F̂ = 0.002</td>
<td>0.09</td>
</tr>
<tr>
<td>Within populations</td>
<td>0.84</td>
<td>F̂ = 0.008</td>
<td>0.85</td>
</tr>
</tbody>
</table>

The numbers in violet are the % of individuals not migrant.

### Results and Discussion

Mitochondrial and nuclear loci were used to infer sex-biased dispersal and estimated pairwise FST, FST and relatedness to nuc locus separately for each oak locality.

Sex dispersal rate

We tested for sex-biased dispersal, estimating pairwise FST, FST and relatedness to nuc locus separately for each oak locality. We produced evidence for non random patterns of gene flow probably mediated by male biased dispersal, with values significantly higher in females than in males (p<0.05), showing females more philopatric than males.

In support of a male biased dispersal, nuclear and mitochondrial markers provided different results: a genetic structure could be found in mitochondrial but not in microsatellites profiles.

Dispersal

Many populations have not proportion of not migrants (<1.6% except Iglesiente). The population with a very high self recruitment is Iglesiente (0.066). The high red values corresponding to Iglesiente % of immigrant individuals. Iglesiente receives immigrants from populations of the high violet value means that it doesn’t contribute to the other populations. The dispersal rate is low and invers is lower than 0.10 (with the exception of Iglesiente). It is not well understandable the reason of this predominant unidirectional migration to the southern forested area dominated by evergreen oaks and late egg hatching moth populations. In addition, climatic conditions, like the predominant mistral wind that drives towards the Island, and an ecological barrier consisting of a strip of about 20km without oaks between Iglesiente and the others districts, may limit dispersal along the S-N direction.

AMOVA results showed that genetic differentiation among Sardinian populations of Tortrix viridana is associated with egg development and, consequently, with bud burst phenology of different oak populations. In addition, geographic origin, latitude and altitude contribute to differentiate late-early egg-hatching populations on the same oak species, with a genetic structure more identifiable at mitochondrial loci than on nuclear traits.

Accordingly with ecological studies that described females as badly flyers and stationary and males as good flyers (Du Merle & Piegat, 1982), differences in structure of nuclear traits associated to sex indicated that green oak leaf rollers are characterized with a sex mal-biased dispersal.

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**References**