

GENETIC VARIABILITY OF MTDNA FROM *DIABROTICA SPECIOSA* (GERMAR, 1824) (COLEOPTERA: CHYSOMELIDAE) BRAZILIAN POPULATIONS

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Genetic variability of populations of *Diabrotica speciosa* (Germar, 1824) (Coleoptera: Chrysomelidae) collected from different localities and plant hosts was evaluated by using mitochondrial DNA (mtDNA) sequencing. Samples were collected in Campo Mourão, PR (wheat), Cachoeira Dourada, RS (soybean and corn), Rolândia, PR (soybean and corn) and Pelotas, RS (corn). DNA was extracted using a CTAB protocol. We studied two fragments from the cytochrome oxidase subunit I (COI) and cytochrome B (CytB). Amplifcons (total ca. 1097 bp) were purified by using ExoSap-IT enzyme and sequenced in an ABI 3100 automatic sequencer. Sequences were aligned with Bioedit v. 7.1.3 and edited in Mega 5. Analysis of molecular variance (AMOVA) was performed in Arlequin v.3.5 to access the genetic structure among and within populations. The relatedness among haplotype as well their distribution was estimated in TCS v. 1.21 to infer genealogies, considering localities and plant hosts. We sequenced 87 specimens of *D. speciosa* and 24 haplotypes were identified. Samples from Cachoeira Dourada (RS) share at least two haplotypes with the samples from Rolândia (PR) and Campo Mourão (PR), but do not share haplotypes with another locality (Pelotas, RS) within the same state. We found that haplotype differentiation was not related to the plant host. AMOVA analysis detected that 78.03% of the total variation was found within populations and 21.97% was due to among population variation ($P \leq 0,001$). F_{ST} (0.22) index shows that *D. speciosa* populations are structured.



**Genetic variability of mtDNA from *Diabrotica speciosa*
(Germar, 1824) (Coleoptera: Chrysomelidae) Brazilian
populations**

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Introduction

Corn rootworm is an important corn and soybean pest. Attack root in corn cause a characteristic symptom called “Gus neck”.

Knowledge on population structure is useful for IRM (Insect Resistance Management).



Objective

Understand the population structure of *D. speciosa* populations

Questions

Are haplotypes associated with geographic origin?

Are *D. speciosa* populations structured?

Which populations share more haplotypes?

Genetic variability of populations of *Diabrotica speciosa* collected from different localities and plant hosts was assessed by using mitochondrial DNA (mtDNA) sequencing.

Materials and Methods

Populations and hosts of samples of *D. speciosa* :

- 1) Campo Mourão, PR (wheat);**
- 2) Cachoeira Dourada, MG (soybean and corn);**
- 3) Rolândia, PR (soybean and corn);**
- 4) Pelotas, RS (corn).**

DNA extraction was performed with CTAB salt base protocol;



Material and Methods

Genes

- **Subunit I of the cytochrome oxidase I (COI);**
- **Cytochrome oxidase II (COII);**
- **Cytochrome B (CytB);**

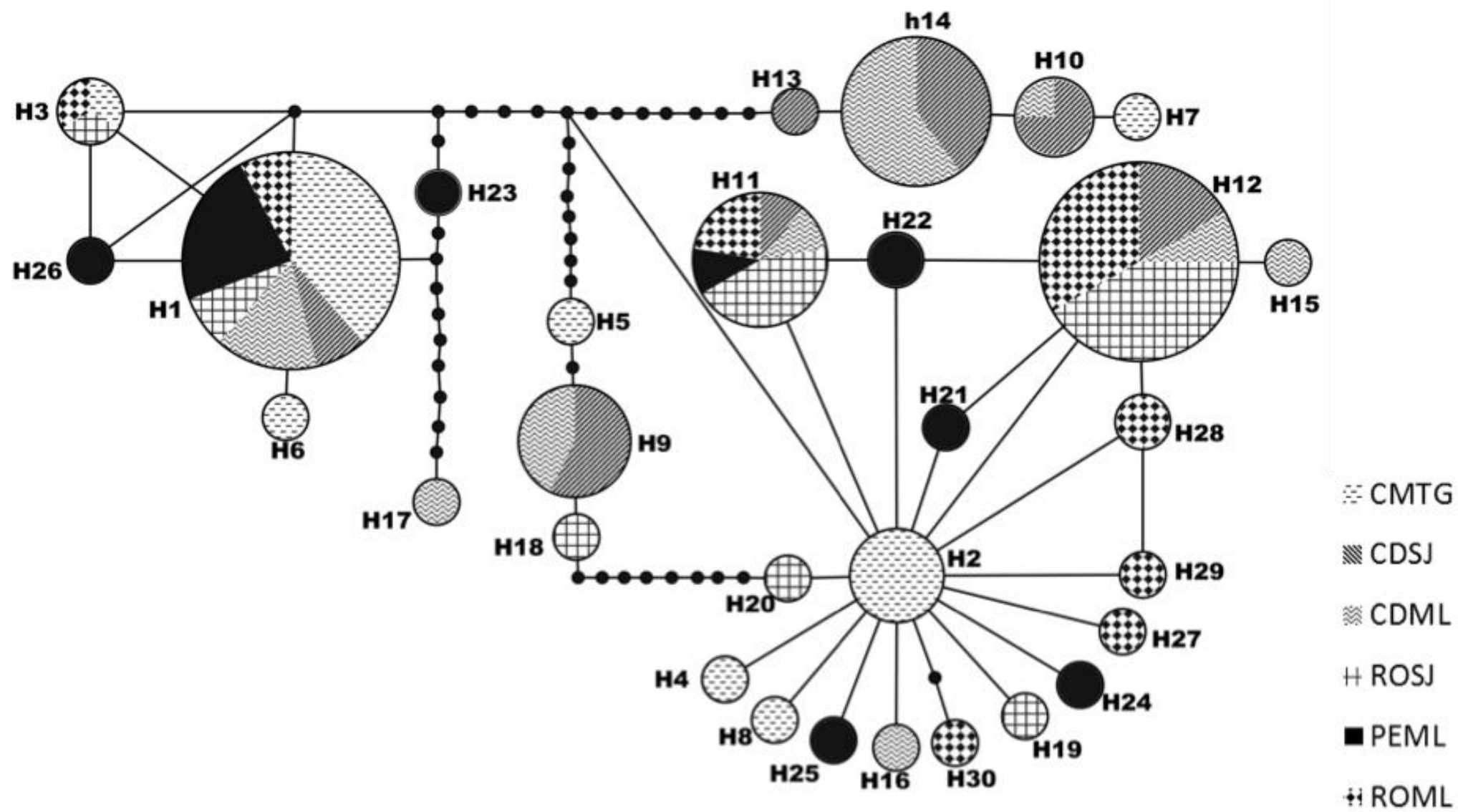
Material and Methods

- Sequences were aligned with Bioedit v. 7 1.3 and edited in Mega 5;
- Analysis of molecular variance (AMOVA) was performed in Arlequin v. 3.5 to access the genetic structure among and within populations;
- The relatedness among haplotype and their distribution were analyzed in TCS v. 1.21.



Results

- We sequenced 87specimens of *D. speciosa*;**
- 24 haplotypes were obtained after the network analysis;**
- Samples from Cachoeira Dourada (MG) share at least three haplotypes with the samples from Rolândia (PR) and Campo Mourão (PR);**
- AMOVA showed that 78.03% of the genetic variability was found within populations and 21.87% occurred among populations (Table).**



Haplotype network of *Diabrotica speciosa* using COI + COII + CytB partial genes.

Partition of the molecular variability among and within geographic populations of *Diabrotica speciosa*

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among populations	5	100.787	1.11184 V _a	21.87
Within populations	82	323.883	3.9480 V _b	78.03
Total	87	424.670	5.06164	
F _{ST}	0.2196			

Conclusions

***D. speciosa* populations are structured;**

Some haplotypes are shared by all the studied populations;

other haplotypes are characteristic of each geographic populations.