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High-level phylogeographic structuring of *Neoleucinodes elegantalis* Guenée (Lepidoptera, Crambridae) in Brazil: an important tomato pest

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ABSTRACT

Neoleucinodes elegantalis is an important tomato pest in Brazil, occurring throughout the country and resulting in economic losses in agriculture. In several species, biogeographic studies in Brazil indicate the structuring of populations, following the refuge model, with a split between the populations of the northeast and the southeast regions of Brazil. The objective of this work was to analyze the phylogeography of *N. elegantalis* in Brazil, understanding its population structure and the demographic patterns. Larvae were collected from eight locations throughout Brazil, and the mitochondrial cytochrome c oxidase subunit 1 gene was analyzed. A total of 628 bp in 51 individuals were obtained, showing 12 haplotypes with a haplotype diversity of 0.836. Spatial analysis of molecular variance (SAMOVA) and cluster analysis showed two populations, indicating population structuring between individuals from the northeast (population 1) and southeast (population 2) regions of Brazil. Phylogenetic analysis indicated that the clades corresponding to the groups defined by SAMOVA have a divergence time of 0.2–0.5 million years, suggesting isolation during climatic events and a separation of the two populations coinciding with the predicted refuges to the Atlantic forest.

Introduction

The small tomato borer (*Neoleucinodes elegantalis* Guenée, 1984, Lepidoptera: Crambridae) is a pest that has had great economic impact in Brazil, Venezuela, and Colombia (Badji et al., 2003; Picanço et al., 2007). High infestation levels of this pest make the fruits unsuitable for consumption and industrial processing (Badji et al., 2003; Benvenga et al., 2010; Picanço et al., 2007). Losses resulting from the damage can be as high as 90% of the total production (Miranda et al., 2005).

In Brazil, *N. elegantalis* is distributed throughout the country, associated with human migrations in tomato-producing regions. It extends from the cold regions of the southeast to the dry regions of the northeast, particularly in areas of dry seasonal forests (Caatinga). These regions show differences in climate, topography, floristic composition, and demographic events that can model the population structure of *N. elegantalis*. *N. elegantalis* has been controlled with the use of chemical insecticides and behavioral controls using sex pheromones (Badji et al., 2003). In controls using sex pheromones, population structuring is very important in determining the specificity of the pheromone types. For example, a variation in cuticular hydrocarbons associated with geographical distance (Bonelli et al., 2015) was detected in *Polistes biglumis* (Hymenoptera: Vespidae). In *N. elegantalis*, unpublished data suggest that pheromones have different effects on the populations of the southeast and the northeast of Brazil, indicating a geographical association with pheromone efficiency. These differences may be associated with population structure due to the influence of climate fluctuations as the biogeographic events.

Biogeographical events such as climate variations and changes in habitat are significant factors in explaining the geographical distribution of many species, particularly in climactic events in the Pleistocene. Using paleomodeling, Carnaval and Moritz (2008) showed the phylogeographic center of endemism in Brazil, suggesting distinct centers of endemism for different species, including butterflies. Thus, estimates of the spatial distribution of the populations of *N. elegantalis*, including demographic patterns and historical population parameters, enable an understanding of

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the dispersion of the species and contribute to pest management. Phylogeographic studies of insect species have been conducted using the cytochrome c oxidase subunit 1 (CO1) region, and it has become a standardized region for dispersion studies. The aim of this study was to analyze the phylogeographic structure of *N. elegantalis* in Brazil using the region of the CO1 gene. The results allow analyze the hypothesis about population structuring resulting from climate changes that may have occurred in the species, as well as contributing to a clearer understanding of distribution patterns of species resulting from successive climatic cycles during the Pleistocene.

Material and methods

Sampling and DNA extraction

Larvae were collected in eight locations (51 individuals) distributed from the southeast to the northeast of Brazil (Fig. 1). The samples were stored in 70% ethanol and conditioned at 4 °C. Two of the locations were in the southeast, in the states of São Paulo and Minas Gerais, and the other six locations were in the northeast of Brazil, in dry forest areas in the state of Pernambuco (Caatinga; map shown in Fig. 1A). The DNA was extracted using the CTAB protocol (Doyle and Doyle, 1987), quantified using spectrophotometry, and analyzed for quality using 1% agarose gel.

Amplification and sequencing

The region of the mitochondrial CO1 gene was amplified using LepF1/LepR1 primers (Hebert et al., 2004). The reactions were performed in a total of 50 μL, containing 5 μL reaction buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTP, 1.25 U Taq DNA polymerase, 0.5 μM of each primer, and 100–150 ng of DNA. The amplification was performed with an initial denaturation at 94 °C for 4 min, followed by 30 cycles at 94 °C for 40 s, 55 °C for 35 s, and 72 °C for 1 min, and a final extension at 74 °C for 4 min. The PCR products were amplified using a BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems®) in electrophoresis in a 3500 Genetic Analyzer (Applied Biosystems Inc., Foster City).

Analysis of the haplotypes

The forward sequences were edited using the software program Mega 5.2 and aligned with ClustalW and Muscle implemented in Mega 5.2. The alignment was performed using standard adjustments and manual optimization when necessary. The haplotype diversity (h) and nucleotide diversity (π), along with Fu and Li's D and Tajima's D tests, were calculated using DnaSP 5.10.01 software (Librado and Rozas, 2009). Estimates with significant negative values are expected from the Fu and Li's D and Tajima's D tests in populations that have undergone recent demographic expansion.

The best adjusted nucleotide substitution model was obtained using the jModelTest 2.1.4 program (Darriba et al., 2012) to help select the molecular evolution model, and the HKY model was used for posterior phylogenetic inference using Bayesian Analysis and Maximum Likelihood (ML). The Bayesian analysis was performed using Beast v1.8.0 (Drummond and Rambaut, 2007) and the posterior distribution was approximated using Markov Chain Monte Carlo (MCMC) for 50 million steps. The convergence of the parameters was checked using Tracer 1.5 software (Rambaut, 2009). The time to most recent common ancestor (TMRCA) was calculated assuming a relaxed molecular clock (uncorrelated lognormal), following the parameters described by Papadopoulou et al. (2010): (ucld.stdev = 0.2571; a coefficient of variation = 0.2609; and a substitution rate of 0.0168 per million years ago, Ma) (outgroup Lepidoptera sp. – GenBank: JF843940). Genealogical relationships among the haplotypes were estimated using the median-joining method, implemented in Network 4.613 software (Bandelt et al., 1999).

Population structuring

Measurements of population differentiation (G_{ST} and N_{ST}) were calculated using DnaSP 5.10.01 (Librado and Rozas, 2009). When the N_{ST} estimates were greater than those of the G_{ST}, phylogeographic structuring was assumed, with closely related haplotypes being detected more frequently in the same area than remotely correlated ones. This approach has been used in other studies to detect phylogeographical structure (Guicking et al., 2011; Liu et al., 2012; Chiu et al., 2013). A spatial analysis of molecular variance was conducted using the spatial analysis of molecular variation (SAMOVA) software program (Dupanloup et al., 2002). This software uses simulation to identify groups of populations (k) that are geographically homogeneous and those that maximize the differences between groups, allowing variation between the groups (F_{CT}), between the locations within each group (F_{SC}), and between the locations in relation to the total sample (F_{ST}), to be obtained. SAMOVA analyses were conducted with 1000 interactions for $k = \{2, \ldots, 8\}$ groups.

A cluster analysis was constructed using the "Bayesian approach to phylogeographic clustering," a Bayesian phylogeographic and ecological clustering (BPEC) package (Manolopoulou et al., 2011) implemented on R software (Team, 2012), using the parameters ds = 0, maximum number of migrations = 5, and 50 million steps in MCMC.

Ecological niche modeling

Ecological niche modeling was performed using MAXENT (Version 3.3.3k; Phillips et al., 2006). The climatic niches used were the 19 BIOCLIM variable available in the WorldSIM data base (http://www.worldclim.org). The environmental data contain three different periods: bioclim layers for the period from 1950 to 2000 at a resolution of 30 arc s, the last glacial maximum (LGM; <21,000 years BP) in the climatic conditions at a resolution of 2.5 arc min, and the last interglacial (LIG: <120,000–140,000 years BP) at a resolution of 30 arc s. To construct the ecological niches, runs were conducted with the parameters convergence threshold (0.00001), maximum iterations (500), and default prevalence (0.5). The figures were produced using the R software raster package (Team, 2012).

Results

Haplotype distribution and analysis

The region of the CO1 gene was sequenced in 51 individuals of *N. elegantalis*, and the analyzed fragments with 628 bp showed 17 polymorphic sites with a total of 12 different haplotypes (Fig. 1A), $h = 0.836 \pm 0.032$ and $\pi = 0.06608 \pm 0.00122$. The haplotype distribution showed a clear distinction between the southeast and northeast region locations in that haplotypes H6 and H10 that occurred only in the populations from the southeast of Brazil, whereas the other haplotypes occurred only in the northeast region (Fig. 1A). In the northeast region locations, haplotypes H1 and H5 occurred with higher frequency, whereas haplotypes H2, H3, H9, and H12 were less common (Fig. 1A). According to the NETWORK results, haplotype H10 had the highest number of substitutions in relation to the most frequently occurring haplotypes (H1 and H5) (Fig. 1A).

The phylogenetic analyses showed four clades, two of them containing haplotypes from the southeast and two of them with only haplotypes from the northeast of Brazil (Fig. 1B). The analysis of the