

GENETIC DIVERSITY OF *STURMIOPSIS PARASITICA* CURRAN (DIPTERA: TACHINIDAE)

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Abstract

The African sugarcane stalk borer, *Eldana saccharina*, is reported to show high levels of genetic differentiation in its indigenous range. This evoked the hypothesis that one of its biological control agents, *Sturmiopsis parasitica*, might have undergone genetic differentiation in response to the differentiation in its host. To confirm this hypothesis, mitochondrial DNA sequences in cytochrome oxidase I were sequenced. Phylogenetic analysis of the sequences using maximum parsimony clustered the specimens into two groups. The genetic divergence observed suggests the presence of intraspecific polymorphism in *S. parasitica*.

Keywords: *Sturmiopsis parasitica*, mitochondrial DNA, biotype, biological control, molecular systematics, phylogenetics, *Eldana saccharina*, sugarcane

Introduction

The African sugarcane stalk borer, *Eldana saccharina* Walker (Lepidoptera: Pyralidae) has for over 100 years been a pest of graminaceous crops in Africa, where it feeds on a variety of crops (Conlong, 2001). Currently it is the most economically important insect pest in South African sugarcane, causing substantial losses of the crop (Conlong, 1994, 2001). Surveys for natural enemies of this borer conducted in east, west and southern African countries indicated that there is a disparity in the type of natural enemies attacking *E. saccharina* in different geographic regions (Conlong, 2001). In the same study, *E. saccharina* was found to show a clear discrepancy in behaviour from region to region, suggesting the presence of biotypes. The existence of biotypes was further supported by recent molecular studies conducted on the borer (King *et al.*, 2002).

King *et al.* (2002), using mitochondrial DNA (mtDNA), found that *E. saccharina* had at least two different biotypes. This genetic divergence may have an implication on the natural enemies that attack different biotypes. Hence, it was considered important to look into the genetic variation that may exist in *Sturmiopsis parasitica* Curran (Diptera: Tachinidae), which is a widely spread parasitoid of various stem borers including *Eldana saccharina*, *Busseola fusca* and *Sesamia calamistis* in western, eastern and southern Africa (Conlong, 2000; Chinwada *et al.*, 2004). This experiment thus proposed to investigate whether there is genetic diversity between populations of *S. parasitica* in its geographic areas of distribution.

Materials and methods

Taxon sampling

The taxon sample included seven *S. parasitica* specimens from Benin, one *S. parasitica* specimen from Senegal, two *S. parasitica* specimens from Kenya, four *S. parasitica* specimens from Zimbabwe and one anonymous *S. parasitica*.

Laboratory techniques

DNA was extracted from individual specimens using the Qiagen DNeasyTM Tissue Kit. Polymerase chain reaction (PCR) was accomplished using the end primers C1-J-154 and C1-N-2173, and C1-J-2183 and TL2-N-301. PCR amplifications were performed on a Perkin Elmer GeneAmp PCR System 2400 under the following conditions: 95°C for 10 minutes, 35 cycles of (94°C for 30 seconds, 50°C for 30 seconds, 72°C for 2 minutes) 72°C for 7 minutes, 4°C hold. Each 50 µl PCR reaction mix was prepared using 10xPCR Buffer, 25 mM MgCl₂, 10 µM of each dNTP, 15 pmol of each PCR primer, 1 unit of Supertherm Gold DNA Polymerase, and approximately 250 ng of genomic DNA/RNA mix. The PCR product was purified using a QIAquickTM PCR Purification Kit. Cycle sequencing was performed using an ABI BigDyeTM Terminator v3.0 Cycle Sequencing Kit under the following conditions: 32 cycles of (96°C for 10 seconds, 50°C for 30 seconds, 60°C for 4 minutes) 4°C hold. Cycle sequencing products were cleaned using the Ethanol/Sodium Acetate Precipitation protocol.

Analysis

DNA sequence chromatograms were edited and checked for base-calling errors using Staden package (Staden, 1996). Sequences were automatically aligned using ClustalX (Thompson *et al.*, 1997) and then manually corrected using BioEdit (Hall, 1999). The remaining analyses were performed using PAUP*4.0b10 (Swofford, 1998).

Results

DNA sequence variation

Approximately 500 bp were obtained from 15 specimens of *S. parasitica*. Sequence analysis revealed the presence of genetic divergence between *S. parasitica* specimens ranging from 0% (within the Benin specimens) to 5.3% (between the northern and the southern groups).

Maximum parsimony analysis

A heuristic search performed under the parsimony criterion for the 500bp COI sequences showed the presence of two groups (northern and southern), with a bootstrap value of 90% in the northern clade and 100% in the southern clade (Figure 1).

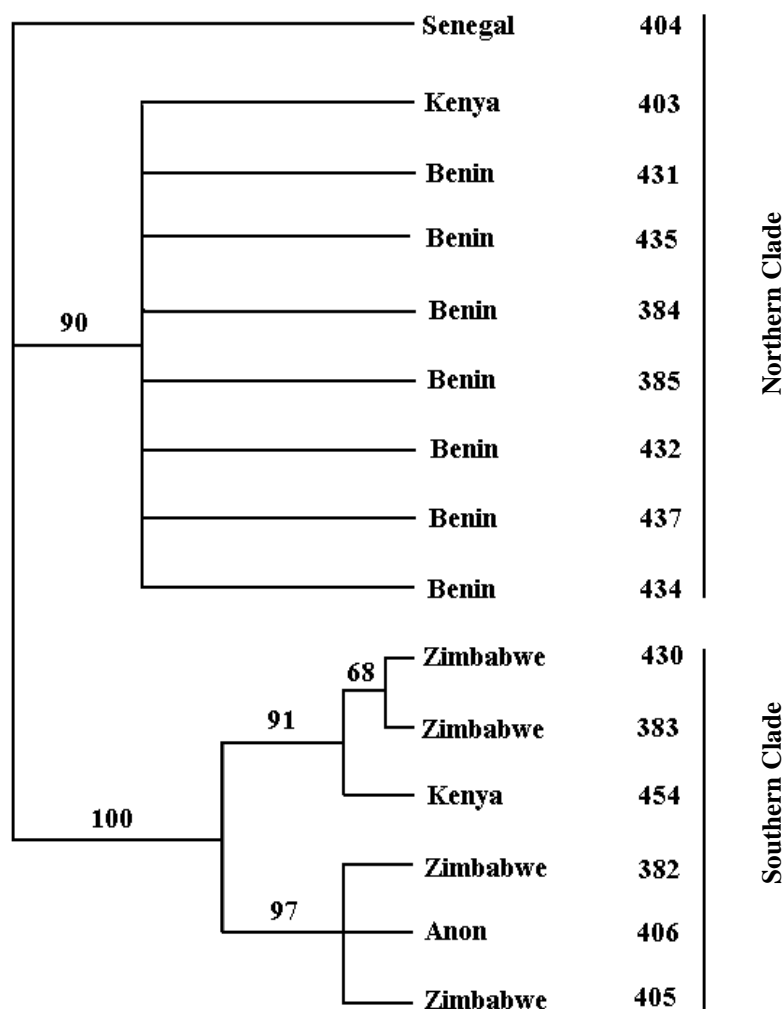


Figure 1. Strict consensus tree of *S. parasitica* 500 bp COI data derived by phylogenetic analysis using parsimony (PAUP version 4.0). Numbers above branches indicate percentage bootstrap support for a particular branch (1000 replications). Bootstrap values of less than 50% are not given on the tree and specimens are named according to their country of origin, with the numbers referring to the DNA extraction number. ‘Anon’ indicates that the country of origin is unknown. The tree is unrooted.

Discussion

Uncorrected pairwise divergence between COI sequences of the 15 specimens analysed showed that there is up to 5.3% sequence divergence. This is strong evidence for the presence of different biotypes in the populations of *S. parasitica* studied. Hebert *et al.* (2003) showed that intraspecific divergences for invertebrates are rarely greater than 2% and most are less than 1%. In a similar study on COI-COII sequences of the spruce budworm species complex (*Choristoneura* spp.), Sperling and Hickey (1994) reported 2.7-2.9% sequence divergence to be sufficient to clearly distinguish between species and a sequence divergence of less than 1% to be only between haplotypes. These studies are further supported by results of other molecular studies (Brower, 1994; Sperling and Hickey, 1994; Kruse and Sperling, 2001; Simmons and Scheffer, 2004). Hence the percentage sequence divergence obtained in this experiment (5.3%) is far more than that expected within haplotypes, and suggests the existence of different biotypes of *S. parasitica*. The strong bootstrap support for the northern (90%) and the southern clade (100%) (Figure 1) further strengthens the idea of two distinct

populations (biotypes) within *S. parasitica*. This implies that the biological information from northern populations may not necessarily be true for the southern populations. Therefore care should be taken in designing and implementing pest control strategies and in using the parasitoid for biocontrol of *E. saccharina*.

As results of one experiment from one gene could be misleading, it is important to confirm the consistency of this result using unrelated and slowly evolving nuclear genes on larger samples from diverse geographical areas.

Conclusions

Further research including nuclear genes on a larger geographic scale will be necessary to confirm the results of this study.

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