

MITOCHONDRIAL DNA SEQUENCE VARIATION AMONG POPULATIONS OF SUGARCANE BORER *ELDANA SACCHARINA* WALKER (LEPIDOPTERA: PYRALIDAE)

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Abstract

Studies on *Eldana saccharina* have shown that populations from western Africa have distinct behavioural differences when compared with populations from eastern and southern Africa. In addition, the parasitoid guilds attacking these populations in the different regions are markedly different. The parallel geographical variation in these patterns between several widespread populations of *E. saccharina* evoked the hypothesis of diversification. To evaluate this hypothesis, a molecular analysis of the Cytochrome Oxidase c subunit I (COI) region of the mitochondrial DNA was conducted on populations of *E. saccharina* from various parts of Africa. Results of the current study reveal the presence of genetic variation in *E. saccharina* populations, which is related to geographical distribution.

Keywords: *Eldana saccharina*, mitochondrial DNA, sugarcane, Rift Valley, biotype, phylogenetics

Introduction

The African sugarcane borer, *Eldana saccharina* Walker (Lepidoptera: Pyralidae) is indigenous to Africa where it feeds on cultivated crops, several wild grasses and sedges (Conlong, 1994). The insect is a key pest of sugarcane in western, eastern and Southern Africa (Conlong, 2001; Atkinson, 1980). Studies conducted have reported the insect to exhibit considerable behavioral variation, display differential responses to control agents (Carnegie *et al.*, 1985) and feed on different host plants in various parts of Africa (Conlong, 2001).

Insect species that are often morphologically very similar, but have contradicting behavioral attributes and even different natural enemies in different regions, can be separated by mitochondrial DNA sequencing (Evans *et al.*, 2000; Scheffer, 2000; King *et al.*, 2002). King *et al.* (2002) provided an overview of the genetic structure of natural populations of *E. saccharina* and the genetic variation between these populations from different parts of Africa. The present study examines whether this feature operates on a much larger geographic scale, and investigates the role of geographical barriers and shift in host plants on the genetic diversity of the species.

Materials and methods

Sample collection and DNA extraction

The deoxyribonucleic acid (DNA) sequence analysis of regions of the mitochondrial Cytochrome Oxidase c subunit I (COI) gene was performed on *E. saccharina* samples collected from Ethiopia, Kenya, Uganda, Benin and South Africa. Genomic DNA was extracted from the thorax using the Qiagen DNeasy™ Tissue Kit.

DNA amplification and sequencing

Polymerase Chain Reaction (PCR) amplification was performed in a Perkin Elmer GeneAmp PCR System 2400. Each reaction contained 36.8 µl of distilled water, 5 µl of 10 X PCR buffer, 1 µl of dNTPs (10 µM of each dNTP), 3 µl of forward PCR primer (15 pmol), 3 µl of reverse PCR primer (15 pmol), 0.2 µl of Super-Therm Gold Taq DNA polymerase (1U unit/reaction) and 1 µl of genomic DNA in a total volume of 50 µl. The thermal cycle conditions detailed in King *et al.* (2002) were used for most of the samples. In some samples these conditions failed to produce a product. For these cases, the following conditions were used: denaturation at 95°C for 11 min followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 50°C for 30 sec and extension at 72°C for 90 sec and hold at 4°C. Successful amplification was confirmed by examining a 5 µl aliquot of the amplification product using agarose gel electrophoresis. Amplified DNA was purified using the Qiagen QIAquick™ PCR purification kit, following the manufacturer's protocol. Samples were then sequenced using ABI PRISM® BigDye™ Terminator v3.0 Ready Reaction Cycle Sequencing Kit, and sequences were visualized on an ABI 3100 Genetic Analyzer.

Sequence analysis

Editing and assembling DNA sequence chromatograms was completed using a Staden package (Staden, 1996). Sequences were aligned using ClustalX (Thompson *et al.*, 1997) and manually corrected using BioEdit sequence alignment editor (Hall, 1999). Uncorrected pairwise sequence divergence was calculated using PAUP* v4.0b8 (Swofford, 1998). Phylogenetic analysis of the samples was performed by Neighbourhood Joining (NJ) method with Tamura-Nei model. Tree reliability was assessed by the bootstrap method with 1000 replications using the MEGA2 (Molecular Evolutionary Genetics Analysis, Version 2) software package (Kumar *et al.*, 2001).

Results

Phylogenetic analysis

Uncorrected pairwise sequence divergence among the nucleotide sequences ranged from 0 to 5.37%. The NJ showed two clusters of sampling localities and one unique locality as the third group. The first group has a mixed distribution of sequences from Benin, Uganda, Ethiopia and the western part of Kenya. This is the largest clade and has a strong bootstrap support (78%). The second group is a South African clade with one sequence from the eastern part of Kenya. This clade has a very low bootstrap support (46%). One sample from the Rift Valley in Kenya was found to be different from all the other specimens in the study and formed the third clade.

Discussion

Evidence from molecular (King *et al.*, 2002) and ecological studies (Conlong, 2001) suggested that the Great Rift Valley might be a geographic barrier to gene flow between *E. saccharina* populations. There is now strong evidence for this: the largest portion of genetic diversity revealed in this study is distributed among Kenyan populations. Kenyan specimens from the Great Rift Valley, east of Rift Valley and west of Rift Valley fall into three separate clades.

Uncorrected pairwise divergences within *E. saccharina* suggest the presence of distinct lineages within this species. Variation in COI observed between the Kenyan specimen from within the Rift Valley and the other two clades (up to 5.37%) is greater than values previously reported for other pest species (Sperling *et al.*, 1999; Evans *et al.*, 2000; Scheffer and Lewis, 2001). This differentiation between the clades suggests that *E. saccharina* may even contain a cryptic species. However, it is too early to say that the observed genetic difference reflects interspecific diversity. As reported by Landry *et al.* (1999), per cent mitochondrial sequence divergence between closely related sister species of Lepidoptera is highly variable and is not necessarily a good predictor of whether two unknown populations constitute reproductively isolated species. Therefore, any revision to the current taxonomy would be premature because observations are based on few specimens and only one gene. Further studies using nuclear markers need to be conducted. In addition, sampling from a range of habitats and cross-mating adults from different regions will be necessary to fully determine whether the observed genetic differences of this study reflect interspecific genetic divergence or not.

Conclusions

The genetic divergence determined in this study provides strong support for the existence of different biotypes and even potentially separate species within *E. saccharina*. However, a single gene tree is not indisputable evidence for the presence of a cryptic species. Therefore, a larger geographic sample is necessary to confirm the consistency of this genetic difference and the role of the Great Rift Valley as a geographic barrier. Analysis using nuclear markers and cross-mating adults could also provide supporting evidence.

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REFERENCES

- Atkinson PR (1980). On the biology, distribution and natural host-plants of *Eldana saccharina* Walker (Lepidoptera: Pyralidae). *J ent Soc Afr* 43: 171-194.
- Carnegie AJM, Conlong DE and Graham DY (1985). Recent introduction of parasitoids against *Eldana saccharina* Walker (Lepidoptera: Pyralidae). *Proc S Afr Sug Technol Ass* 59: 160-163.
- Conlong DE (1994). A review and perspectives for the biological control of the African sugarcane stalk borer *Eldana saccharina* Walker (Lepidoptera: Pyralidae). *Agric Ecosyst Environ* 48: 9-17.

- Conlong DE (2001). Biological control of indigenous African stemborers: what do we know? *Insect Sci Applic* 21: 1-8.
- Evans JD, Pettis JS and Shimanuki H (2000). Mitochondrial DNA relationships in an emergent pest of honey bees *Aethina tumida* (Coleoptera: Nitidulidae) from the United States and Africa. *Ann Entomol Soc Am* 93: 415-420.
- Hall TA (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. <http://www.mbio.ncsu.edu/BioEdit/bioedit.html>.
- King H, Conlong DE and Mitchell A (2002). Genetic differentiation in *Eldana saccharina* (Lepidoptera: Pyralidae): evidence from the mitochondrial cytochrome oxidase I and II genes. *Proc S Afr Sug Technol Ass* 76: 321-328.
- Kumar S, Tamura K, Jakobsen IB and Nei M (2001). MEGA2: Molecular Evolutionary Genetics Analysis software. *Bioinformatics* 17(12 2001): 1244-1245.
- Landry B, Powell JA and Sperling FAH (1999). Systematics of the *Argyrotaenia franciscana* (Lepidoptera: Tortricidae) species group: Evidence from mitochondrial DNA. *Ann Entomol Soc Am* 92(1): 40-46.
- Scheffer SJ (2000). Molecular evidence of cryptic species within *Liriomyza huidobrensis* (Diptera: Agromyzidae). *J Econ Entomol* 93: 1146-1151.
- Scheffer SJ and Lewis ML (2001). Two nuclear genes confirm mitochondrial evidence of cryptic species within *Liriomyza huidobrensis* (Diptera: Agromyzidae). *Ann Entomol Soc Am* 94: 648-653.
- Sperling FAH, Raske AG and Otvos IS (1999). Mitochondrial DNA sequence variation among populations and host races of *Lambdina fiscellaria* (Gn.) (Lepidoptera: Geometridae). *Insect Mol Biol* 8: 97-106.
- Staden R (1996). The Staden Sequence Analysis Package. *Mol Biotech* 5: 233-241.
- Swofford DL (1998). PAUP phylogenetic analysis using parsimony (version 4.0). *Sinauer Sunderland, MA*.
- Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F and Higgins DG (1997). The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nuc Acids Res* 24: 4876-4882.