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Research Article

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Determining genetic variations in *Busseola fusca* Fuller (Lepidoptera: Noctuidae) and *Chilo partellus* Swinhoe(Lepidoptera: Crambidae) from Swaziland and South Africa through sequences of the mtDNA Cytochrome Oxidase Sub Unit I (COI) gene

***Assefa Y. and Dlamini T.**

Department of Crop Production, University of Swaziland, Luyengo Campus, P. O.Luyengo M205, Swaziland

*Corresponding author: *yaarmide@uniswa.sz*

Abstract

Understanding the impact of climate variability and landscape fragmentation on host and herbivore interaction is essential for designing and implementation of pest management strategies. To examine the effects of variation in climate and agricultural landscape on insect herbivores, this study assessed the genetic diversity in *B. fusca* and *C. partellus* from maize and sugarcane in Swaziland and South Africa. Molecular analysis of these stem borers' specimens from the two countries using fragment of the mitochondrial cytochrome oxidase sub-unit I revealed no detectable genetic differentiation in *B. fusca* and *C. partellus* populations from maize and sugarcane cultivated in different climatic areas and agricultural landscapes. The genetic distances between haplotypes of both stem borer species (0.22%) were within the range of genetic divergence expected from individuals of the same population in cereal stem borers. This analysis showed that *B. fusca* and *C. partellus* populations residing in maize and sugarcane fields in different parts of Swaziland and South Africa are in contact with each other and there is a continuous gene flow between these populations.

Keywords: Genetic diversity,maize, mtDNACOI region, stem borers, Swaziland.

Introduction

Animal mitochondrial DNA is believed to strictly follow maternal inheritance and is highly variable within a species (Li *et al.*, 2013). DNA sequences of the mitochondrial DNA have long been recognized as important tools for phylogenetic inference and for analyses of genetic diversity. Different regions of the mitochondrial DNA have been used for different taxonomic groups and in different laboratories. The cytochrome *c* oxidase subunit 1 (COI) mitochondrial DNA (mtDNA) gene has served as a barcode for identification the unknown in animal kingdom (Hebert *et al.*,2003; Shokralla *et al.*, 2014). The gene is a relatively fast evolving and practical locus from which to derive recent evolutionary genetic histories. The COI gene has also been successfully applied previously to assess population structure for *B. fusca* (Sezonlin *et al.*, 2006, Assefa *et al.*, 2015) and for other indigenous African stem borers (Assefa *et al.*, 2006, Ong'amo *et al.*, 2008).

This study analyses the genetic diversity in populations of *B. fusca* and *C. partellus* from maize and sugarcane in Swaziland and South Africa using sequences of the cytochrome-c oxidase I gene of

mitochondrial DNA. Most of the areas in South Africa and all of the sample collection sites in Swaziland from where the specimens of these stem borers were collected fall under the Maputaland-Pondoland-Albany biodiversity hotspot region (Perera *et al*.. 2011) but the climate and landform of Swaziland are different from the sites in South Africa from where specimens of the borers were collected. Such variations in landscape and in climatic conditions known to increases the spatial isolation of plant populations and increased inter population genetic divergence in herbivore insects due to increased random genetic drift (Young *et al*., 1996; Keller and Largiader, 2003). We examined the two stem borer species from Swaziland maize which have not been studied yet and evaluated their genetic relatedness with the South African populations from sugarcane and maize. The study examines the effects of land fragmentation and variations in climatic conditions on gene flow between populations of stem borers residing in maize and sugarcane. Results of this study are useful to evaluate the effects of climate and landscape on species genetic diversity and assist in designing effective biocontrol programme in the management of these borers in the two countries.

Materials and Methods

Study sites and insect specimen collection

Materials examined (Table 1) were collected from maize and sugarcane crops at localities in Swaziland and South Africa. Selection of sampling sites was based on the climatic variation and agricultural landscapes. Localities in Eastern Cape Province of South Africa are areas situated on the coast of the Indian Ocean and are part of the Maputo-Pondoland-Alabany biodiversity hotspot (Perera *et al*., 2011). These areas are characterized by small-scale farming with subsistence maize plots and few stands of sugarcane plants that rarely exceed 0.01 ha in size (Assefa, 2015). In contrast to the Eastern Cape Province, sites in North-West Province of South Africa are large scale farms located inland (Kruger *et al*., 2012) and relatively cooler areas outside the Maputo-Pondoland-Alabany biodiversity hotspot (Perera *et al*., 2011). The Highveld and Middleveld sites in Swaziland are small scale maize fields in relatively warmer inland areas located inside the Maputo-Pondoland-Alabany biodiversity hotspot (Perera *et al.*, 2011).

Sample specimens from South African sugarcane and maize were collected between May and June 2014 and were sequenced in October 2014. The Swaziland specimens from maize were collected in February 2016 and their sequences were generated in March 2016. The collected specimens from both countries were kept frozen in 95% alcohol in sealed 30ml glass vials until used for DNA sequencing.

DNA extraction, Amplification and Sequencing

Extraction of DNA, amplification and sequencing of specimens collected from South Africa was done following the method described in Assefa *et al.* (2015). Insect specimens collected from maize in Swaziland were sent to Inqaba Biotech (a genomics company), Pretoria, South Africa where the target region of the mtDNA cytochrome Oxidae sub Unit I gene was sequenced.

Table 1 Localities in Swaziland and South Africa from where specimens of *Busseola fusca* and *Chilo partellus* were collected.

Sample name	Stem borer Species	Country	Province/AEZ	No of localités	Host Plant	No of spécimens
SA-EC-MAIZE	*B. fusca*	South Africa	Eastern Cape	13	Maize	39
SA-EC-CANE	*B. fusca*	South Africa	Eastern Cape	5	Sugarcane	15
SA-EC-CANE	*C. partellus*	South Africa	Eastern Cape	5	Sugarcane	11
SA-NW-MAIZE	*B. fusca*	South Africa	North-West	2	Maize	2
SD-HV-MAIZE	*B. fusca*	Swaziland	Highveld	2	Maize	2
SD-HV-MAIZE	*C. partellus*	South Africa	Highveld	1	Maize	1
SD-MV-MAIZE	*B. fusca*	South Africa	Middleveld	1	Maize	1
SD-MV-MAIZE	*C. partellus*	South Africa	Middleveld	1	Maize	1

Sequence Analysis

Editing and assembling DNA sequence chromatograms was completed using the Staden package (Staden, 1996). Sequences were then aligned using ClustalX (Thompson *et al.*, 1997) and manually corrected using BioEdit sequence alignment editor (Hall 1999). Haplotype polymorphism was analysed using DnaSP (Librad and Rozas, 2009) and only one representative from localities, host plant and country was used for reconstruction of a phylogenetic tree. The neighbour joining (NJ) phylogenetic tree (Saitou and Nei, 1987)was constructed using the MEGA 6.0 program (Tamura *et al.*, 2013) with a Kimura 2-parameter model (Kimura, 1980) and node support was assessed by the bootstrap method with 10,000 replications (Felsenstein, 1985). *Eldana saccharina* Walker (Lepidoptera: Pyralidae) sequences from our collection were used as an out-group in the analysis.

Results

Phylogenetic analysis

After trimming, the final for which all the 72 aligned sequences were having data was 452bp in length. The neighbor-joining tree generated based on the sequences obtained in this study together with the sequences of *Eldana saccharina* from our collection using MEGA 6.0 program is presented in Figure 1.Two distinct cladeseach representing a species of stem borer were observed. The first clade consisted *B. fusca* specimens collected from maize and sugarcane in all sites of Swaziland and South Africa. This clade was subdivided into to small groups (B1 and B2) with Swaziland specimens from maize are grouped in the biggest group (B1) of the clade. The second clade was comprised of *C. partellus* from small scale sugarcane fields of Eastern Cape in South Africa and specimens from maize grown in Highveld and Middleveld areas Swaziland. This clade was also sub grouped into C1 and C2 with specimens from High and Middleveld maize of Swaziland were clustered in sub clade C2 with some specimens from Eastern Cape sugarcane.

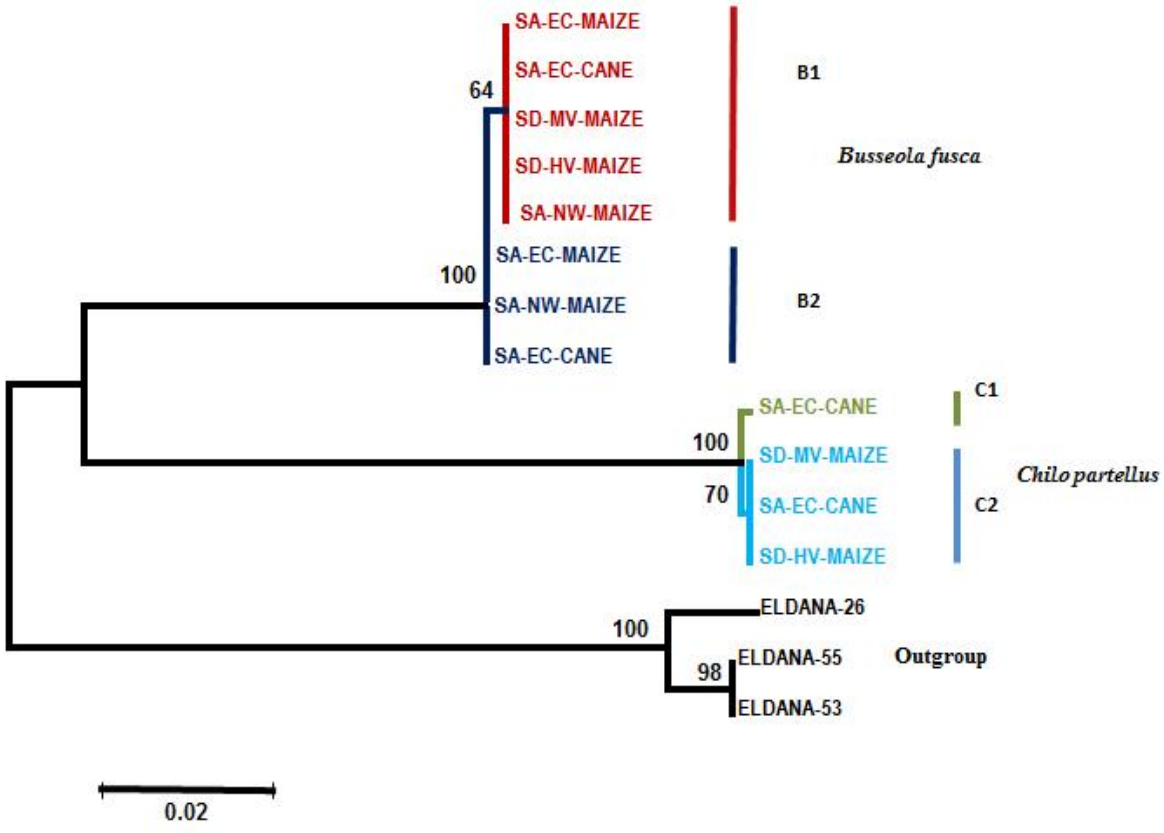

Figure 1.A NJ phylogenetic tree showing the relationship between the *B. fusca* and *C. partellus* sequences and *E. saccharina* as an out-group. SA stands for South Africa, SD for Swaziland, EC for Eastern Cape Province, NW for North-West Province, HV and MV for Highveld and Middleveld Agro-Ecological Zones of Swaziland. The haplotypes for the two species are indicates as B1, B2, C1 and C2.

Haplotypes

Four different haplotypes (two from *B. fusca* (B1 and B2) and two from *C. partellus* (C1 and C2)) were identified. The analysis involved mitochondrial sequences from all four haplotypes. The haplotypes diversity (Hd) for the whole data set was 0.4566. The most haplotype (B1) was represented by52 sequences of *B. fusca* collected from Swaziland and South Africa. This haplotype was found in samples collected from maize and sugarcane in both provinces of South Africa and also in samples from maize in Highveld andMiddleveldareas of Swaziland. The second *B. fusca* haplotype was represented in seven sequences from South African maize and sugarcane. With the exception of one sequence from maize in the North-West Province all sequences in this haplotype were from small scale maize and sugarcane fields of the Eastern Cape Province. This haplotype has sequence divergence of only 0.22%from haplotype B1. The third (C1)and fourth (C2) haplotypes were represented by sequences from South Africansugarcane and samples from Swaziland maize in Highveld and Middleveld. The common *C. partellus* haplotype (C1) was represented in nine of the sequenced individuals from South Africa and has 0.22% sequence divergence from the other haplotype which contain the two sequences frommaize in Highveld and Middleveld areas of Swaziland.

Discussion

The Cytochrome Oxidase Sub-Unit I (COI) gene of the mitochondrial DNA has been very widely used by geneticists to analyse the phylogenetic relationships at inter-species level in stem borers (Le Ru *et al.*, 2014; 2015) and their natural enemies (Assefa *et al.*, 2008; Muirhead *et al*., 2012). It has also been used to investigate the intra species genetic variation in stem borers (Sezonlin *et al.*, 2012; Assefa *et al.*, 2015). This gene has been proven to be effective in identification of previously unknown species of indigenous African cereal stem borers (Assefa *et al.*, 2007) and evaluation of host plant and/or habitat associated genetic differentiation in stem borers (Ong'amo *et al.*, 2012; Assefa *et al.*, 2015).

Analysis of the Cytochrome Oxidase Sub-Unit I (COI) gene in this study indicate lack of evidence for host plant, climate and/or agricultural landscape associated genetic differentiation in *B. fusca* and *C. partellus* populations from maize and sugarcane in Swaziland and South Africa. All phylogenetic analyses conducted separated *B. fusca* and *C. partellus* sequences into two closely groups/haplotypes. The genetic distance between the haplotypes of *B. fusca* and *C. partellus* were equal (both 0.22% sequence divergence) and they are within the range of genetic variation reported from studies on closely related stem borer species. In recent study conducted on *B. fusca*, Assefa *et al*. (2015) reported a within population variation of upto 0.95% and a between population genetic divergence of 2.86%. Similarly, Ong'amo *et al*. (2012) reported upto 0.95% haplotype genetic diversity in *B. segata* population. This indicates that *B. fusca* and *C. partellus* in maize and sugarcane in the two countries are part of the same population.

Busseola fusca and C. *partellus* have diversified host ranges that include maize and sorghum and sugarcane which are increasingly availability due to extensive cultivation of these crops in the region (Kassie *et al*., 2012; Singles *et al*., 2013). It is likely that the habitat modification and fragmentation of natural ecosystems to plant maize, sorghum and sugarcane in the region is providing a continuous supply of food and bring the populations of these pests in the region together than limiting exchange of individuals and genetic material. The movement of *B. fusca* and *C. partellus* moths between host plants in different habitats may be attributed to their host use plasticity (Nylinand Gotthard, 1998).The extensive cultivation of these crop hosts may give *B. fusca*a great advantage as it is specialized to use limited number of hosts (Assefa *et al.,* 2015).The continued demand for agricultural land and associated clearing of uncultivated fragments which affects their role as a refugee for natural enemies is, therefore, likely to benefit these pests are and may result in an increase in the frequency of outbreaks of these pests in small scale farms. It is, therefore, advisable to implement habitat management options that encourage the population of natural enemies that could keep the populations of these pests in check.

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