

Phylogeography and Population Structure of the Mediterranean Corn Borer, *Sesamia nonagrioides* (Lepidoptera: Noctuidae), Across Its Geographic Range

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Abstract

Sesamia nonagrioides (Lefèbvre) (Lepidoptera: Noctuidae), is a widespread insect pest in Africa, the Middle East, and Europe. However, its pest status varies across its distribution range. It is a major pest of maize in Europe and of sugarcane in Iran. In Africa, it is a major pest of maize in West Africa but not considered as a pest in East Africa. Recent surveys conducted in 2015 recorded *S. nonagrioides* to be a major pest of sugarcane in Ethiopia and reported the species for the first time in Botswana, outside its known geographic range. The genetic relationship of these records with the previously recorded population of *S. nonagrioides* was investigated using the cytochrome oxidase subunit I region of the mitochondrial genome. In total, 113 individuals across the geographic range of the species were analyzed and 63 haplotypes were identified. Phylogenetic analysis separated the populations into two clades with no distinct geographic distribution pattern. The genetic differentiation was also not associated with host plants and geographic distances. Results of the molecular analysis revealed the long-time establishment of *S. nonagrioides* population in Botswana and identified the newly recorded sugarcane population from Ethiopia as part of the wild host population in the country. The phylogeographic patterns observed among population of *S. nonagrioides* have probably been shaped by Pleistocene's climatic oscillations and geographic range expansions from different refugia with secondary contact and admixture. Possible reasons for the host-plant expansion by the Ethiopian population are discussed.

Key words: genetic diversity, mitochondrial DNA, stem borer, sugarcane, wild host plant

The Mediterranean corn borer, *Sesamia nonagrioides* (Lefèbvre) (Lepidoptera: Noctuidae), is one of the widespread lepidopteran stem borer species found in sub-Saharan Africa from Côte d'Ivoire in West Africa to Kenya and Ethiopia in East Africa, and Zambia in Austral Africa (Moyal et al. 2011). In the Palearctic region, it spreads from North Africa to Western Europe and Iran (Moyal et al. 2011, Cherghali et al. 2015). The insect is indigenous to Africa and feeds on a wide range of host plants (Le Ru et al. 2006, Kergoat et al. 2015). *Sesamia nonagrioides* is considered as one of the major insect pests of maize in many countries around the Mediterranean basin (Margaritopoulos et al. 2007, De La Poza et al. 2008). It is also a major pest of sugarcane and maize in Iran (Cherghali et al.

2015). However, its pest status varies across its distribution range in Africa. For instance, it is a major pest of maize in West Africa (Buadu et al. 2002), but not in East Africa (Moyal et al. 2011) where it is mainly reported from wild host plants in wetlands (Moyal et al. 2011). However, in Ethiopia, *S. nonagrioides* was recorded for the first time in 2015 as the only sugarcane borer in a newly established sugarcane estate in Kesem and as the dominant borer in two old sugarcane estates in Wonji and Metehara. It has to be noted that previous surveys conducted in 2003 and 2004 at Finchawa, Metehara, and Wonji sugarcane estates of Ethiopia reported *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae), *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae), and *Sesamia calamistis* Hampson (Lepidoptera:

Noctuidae) as the only lepidopterous stem borer pests (Assefa 2006). In addition, *S. nonagrioides* was recovered for the first time from several wild host plants in Botswana, outside its known geographic range. The genetic relationship of these new records with the previously recorded population of *S. nonagrioides* was investigated using the mitochondrial DNA of cytochrome oxidase subunit I (COI) gene.

In species with wide geographic distributions and extensive host ranges, it is common to find the substantial genetic difference (Seyahooei et al. 2011, Barman et al. 2013). Thus, the newly recorded populations of *S. nonagrioides* could be genetically distinct from the previously known populations, due to the variation in eco-geographic factors and pest status of the insect across its geographic range. Insect pest populations may become genetically different due to historical processes, geographic barriers, and ecological isolations (Assefa et al. 2015, Bennett et al. 2016, Gofitshu et al. 2016, Joyce et al. 2016). Genetically distinct populations of insect pests may differ in their damage potential and/or susceptibility to pest management practices (Joyce et al. 2014). Therefore, gaining insight into *S. nonagrioides* phylogeography, including the spatial distribution and genetic relatedness among populations aids in designing sound insect pest management strategies.

Recent genetic diversity study undertaken on *S. nonagrioides* populations of sub-Saharan Africa and the Palearctic region revealed the presence of two isolated clades (the West African and East African clades; Moyal et al. 2011). According to this study, the Palearctic population invaded Mediterranean Europe from Africa, and its mitochondrial gene was identical to the East African clade (Moyal et al. 2011). However, the study by Moyal et al. (2011) was based on specimens collected from only 22 sampling sites in sub-Saharan Africa, 3 eastern and 1 West African countries. Moreover, out of these locations, samples were taken from 13 different locations in Kenya and West Africa was represented only by one location, making their results likely biased and uninformative at a wider scale. A similar study that covered the entire species ranges across sub-Saharan Africa, and representative samples from Palearctic region were conducted by Kergoat et al. (2015). The study provided valuable insights into the ecological genetics of *S. nonagrioides*, showing that populations of *S. nonagrioides* have both structured and differentiated throughout its geographic range. Unlike in Moyal et al. (2011), where the genetic diversity of *S. nonagrioides* populations were associated from the perspectives of geographic locations only, Kergoat et al. (2015) compared the population of *S. nonagrioides* based on host-plant information from which the insect pest was recovered. The study of Kergoat et al. (2015), however, emphasized delimitation of species related to *S. nonagrioides* and the sampling was biased to wild habitats. Host plants play a major role in the population structure and genetic differentiation of phytophagous insects (Barman et al. 2012). Phytophagous insects of the same species may specialize on different plants at different geographic areas, and hitherto the species may be considered as polyphagous when the whole geographic spread is considered (Fox and Morrow 1981). Thus, it is important to study host-related genetic variation all over the geographic distribution of a species (Barman et al. 2012). Examining such difference may have significant implications for management of major insect pest species and justifies a continued effort to describe patterns of genetic variation (Agunbiade et al. 2014, Lesieur et al. 2016).

To build on the previous findings, the present study is a reassessment and expansion of data reported in Moyal et al. (2011) and Kergoat et al. (2015), where the newly obtained populations of *S. nonagrioides* from wild hosts in Botswana and sugarcane from Ethiopia were compared with the previously collected populations of sub-Saharan Africa and Palearctic regions. The addition

of a new population may considerably change genetic relationships (Bucheli and Wenzel 2005). Thus, we aimed at 1) analyzing the origin of *S. nonagrioides* population infesting sugarcane in Ethiopia; 2) characterizing the genetic structure of *S. nonagrioides* populations and infer its evolutionary history in its current geographic range; 3) investigating the impact of host plants on the genetic diversity of *S. nonagrioides* population in Ethiopia and across its geographic scale; and 4) identifying the genetic relationships of the Ethiopian and Botswana population of *S. nonagrioides* with the previously recorded populations of sub-Saharan and Palearctic region.

Materials and Methods

Insect Specimens

Sesamia nonagrioides larvae from Ethiopia were collected from young ratoon sugarcane (2–3 mo old) during April 2015 in a survey made at sugarcane plantations of Metehara, Kesem, and Wonji estates in the Great Rift Valley. Specimens from Botswana were collected from *Echinochloa pyramidalis*, *Echinochloa stagnina*, *Oriza longistamina*, and *Vossia cuspidata* in June 2015 and March 2016.

The mitochondrial DNA COI sequences of individuals collected from localities in sub-Saharan Africa (Benin, Cameroon, Democratic Republic of Congo, Republic of Congo, Ghana, Kenya, Rwanda, Tanzania, Uganda, and Zambia) and from the Palearctic regions (France, Iran, Italy, and Turkey) by NSBB project (Noctuid Stem Borer Biodiversity IRD-icipe) since 2006 (Le Ru et al. 2006, Kergoat et al. 2015) were included in this study to reassess their genetic relationships with the newly collected populations from Ethiopia and Botswana. The collection localities (Fig. 1), host plants, and DNA extraction number of each sequence presented in this study are found in Supp Table S1 (online only).

DNA Extraction, Polymerase Chain Reaction, and Sequencing

For the newly collected specimens from Ethiopia and Botswana, DNA was extracted from hind legs using the Macherey Nagel Nucleospin Extraction Kit. Polymerase chain reaction (PCR) amplifications of the COI gene region of mitochondrial DNA and sequencing of specimens were done at Laboratoire Evolution, Genome and Speciation at Gif-sur-Yvette in France using the primers and methods indicated in Gofitshu et al. (2016).

Phylogenetic Reconstruction

The mitochondrial DNA COI sequence data were aligned with ClustalX (Thompson et al. 1997) and edited manually by BioEdit (Hall 1999). The resulting 655-bp fragment of the cytochrome oxidase I sequences of populations collected from each distinct geographic region was compared with each other.

The phylogenetic relationship among haplotypes originated from each country was assessed by maximum likelihood (ML) and neighbor joining (NJ) in MEGA6 (Tamura et al. 2013). ML analyses were performed using experiential approach found automatically by using Neighbor-Join and BioNJ procedures to a matrix of pairwise distances assessed with the maximum composite likelihood method. The bootstrap consensus tree was inferred from 10,000 replications (Felsenstein 1985). The NJ tree was constructed using the *p*-distance method. The percentage of replicate trees in which the associated taxa clustered together were statistically tested by a bootstrap test containing 10,000 replications.

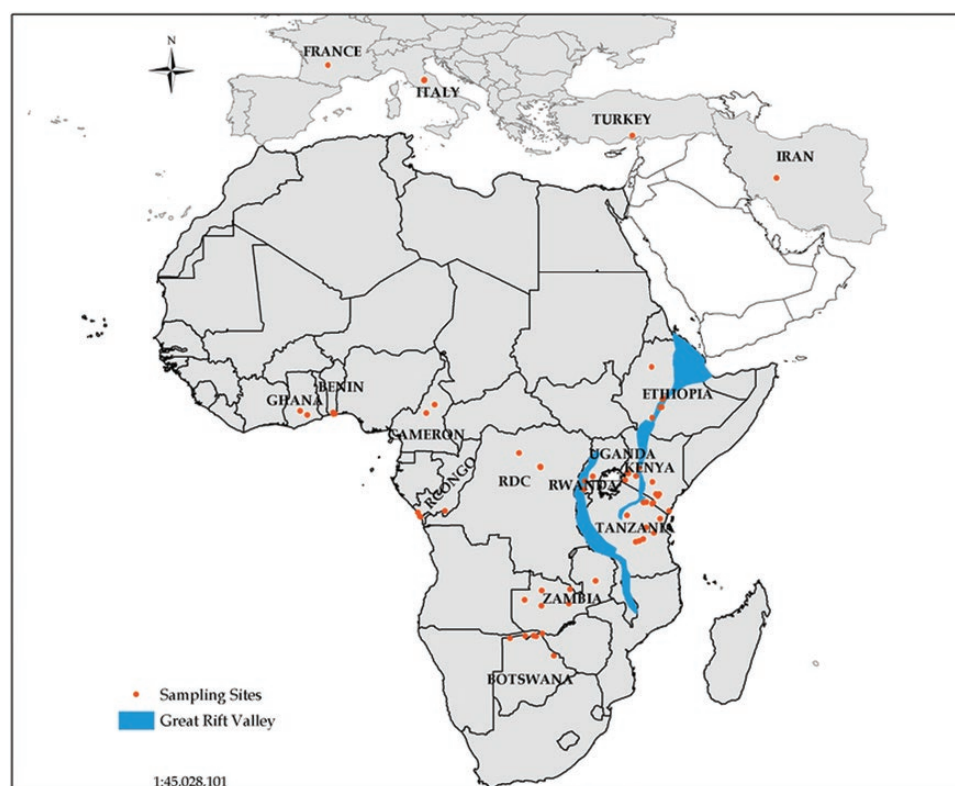


Fig. 1. Geographic distribution of localities where *Sesamia nonagrioides* was sampled. Figure is best viewed online in color.

Genetic Diversity, Network Analyses, and Demographic History

The magnitude of genetic diversity among populations was estimated with DnaSP v.5.1.0 (Librado and Rozas 2009) based on the number of haplotypes (h), haplotype diversity (H_d), nucleotide diversity (π), number of polymorphic sites (S), and average number of differences between sequences (K). To evaluate the genetic relationships of geographic and host-plant populations, a median-joining network was generated using NETWORK 4.6 (Bandelt et al. 1999).

To investigate the demographic history of each clade, mismatch distributions, Tajima's D (Tajima 1989) and Fu's F_s (Fu 1997), were evaluated using DnaSP v.5.1.0 (Librado and Rozas 2009).

Genetic Structure and Isolation-by-Distance

To evaluate the magnitude of genetic variation among/between populations of various groups, three different statistical tests, namely, F_{ST} , K_{ST} , and S_{nn} , were calculated using DnaSP v.5.1.0 (Librado and Rozas 2009). F_{ST} is the coefficient of genetic differentiation and measures the level of interpopulation diversity. K_{ST}^* is a statistic that considers variation in nucleotide sequences between different haplotypes (Hudson et al. 1992). The nearest-neighbor statistic (S_{nn}) is a measure of the frequency of the nearest neighbors of sequences found in the same populations (Hudson 2000). These three statistical tests more or less vary to specific data set features. Accordingly, we used them all together for robust detection of genetic variation (Dobigny et al. 2013). For each test, statistical significances were performed using 10,000 permutation test.

A Mantel test was performed with the program, isolation-by-distance (IBD, version 3.23; <http://ibdws.sdsu.edu/~ibdws/>). IBD predicts that genetic similarity among populations increases as the geographic distance between them decreases (Jensen et al. 2005).

According to Rousset (1997), IBD among populations over the distribution area could be calculated as the linear regression of genetic distance [$F_{ST}/(1 - F_{ST})$] against the geographic distance. The geographic distance among populations was calculated using the straight and shortest distance between populations based on their longitudes and latitudes (<http://www.gpsvisualizer.com/calculators>). The Palearctic population was excluded from the IBD analyses, owing to small sample sizes of haplotypes.

Results

Phylogenetic Reconstruction

The ML and NJ trees showed a similar topology (Fig. 2). Sequences of 655 base pairs from 113 individuals of *S. nonagrioides* were used in the analysis. Of the 655 nucleotide sites in the data set, 74 were variable, of which 55 were informative in parsimony analysis. The phylogenetic tree analysis separated the *S. nonagrioides* populations into two major clades: clade I and clade II. Clade I comprise haplotypes from Central Africa (Cameroon), East Africa (Ethiopia, Kenya, Rwanda, Tanzania, and Uganda), Palearctic region (France, Iran, Italy, and Turkey), and very few haplotypes from Democratic Republic of Congo, Republic of Congo, Southern Africa, and Botswana. This is the largest clade encompassing 57.2% of the total haplotypes. Clade II includes haplotypes dominantly collected from Central Africa (Democratic Republic of Congo, Republic of Congo), Southern Africa (Botswana and Zambia), and West Africa (Benin and Ghana), and very few haplotypes from East Africa (Kenya and Tanzania; Fig. 2). The Ethiopian population of *S. nonagrioides* all grouped under clade I. Out of the total haplotypes found in Botswana, only one haplotype (H_{14}) was grouped in clade I, while the remaining six haplotypes were categorized under clade II. Uncorrected pairwise differences among all haplotypes within clade

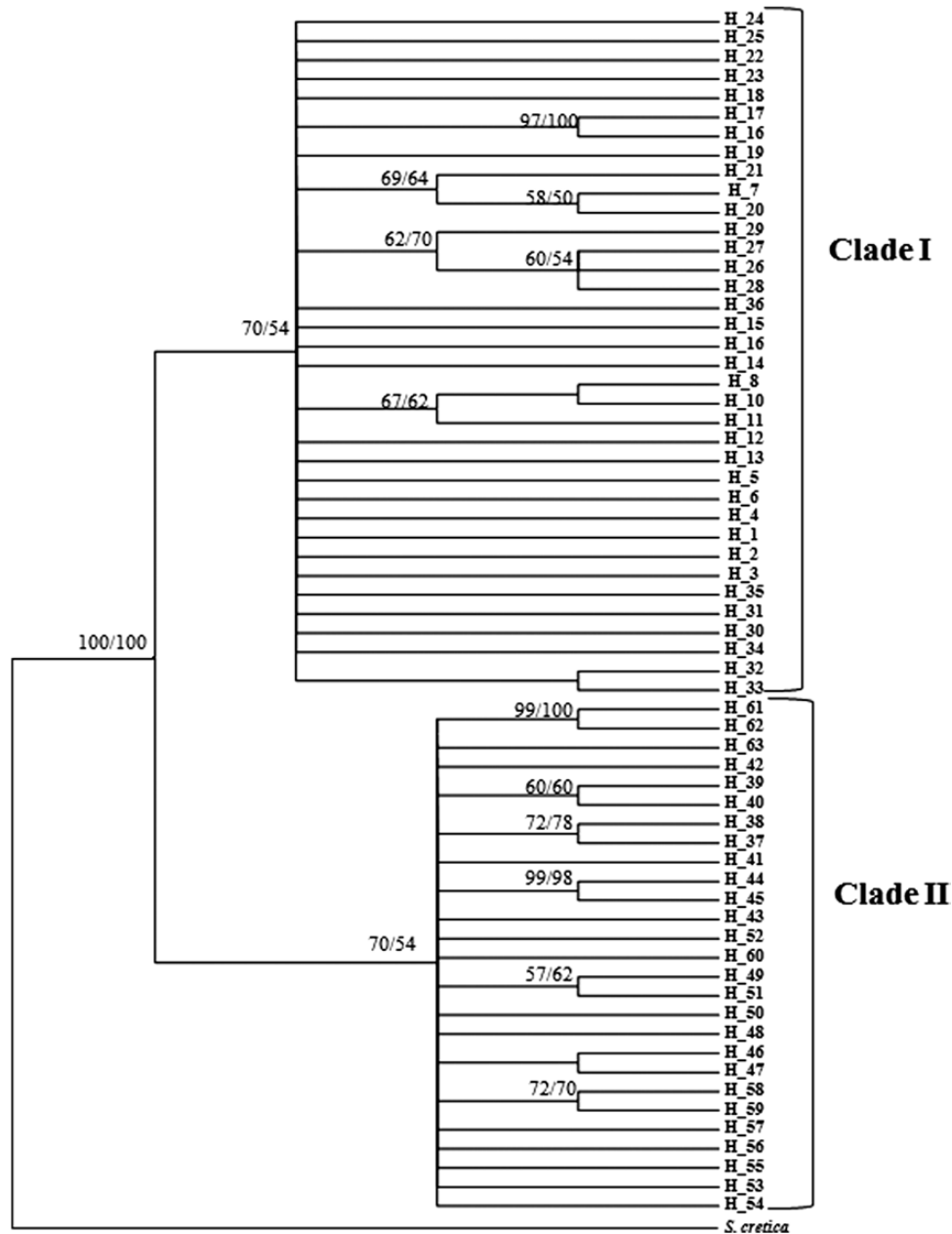


Fig. 2. Neighbor-joining (NJ) and maximum likelihood (ML) tree showing the relationships among haplotypes of *Sesamia nonagrioides*. Bootstrap support > 50% in both NJ (first number) and ML (second number) are given for the relevant nodes. Country of origin and host plants where the haplotypes were recovered are indicated in Fig. 3a–c and SuppTable S1 (online only). The tree is rooted using *Sesamia cretica* as the outgroup taxon.

Table 1. Uncorrected pairwise distances among haplotypes of *Sesamia nonagrioides* populations

	Clade I	Clade II	Ethiopia	Botswana
Clade I	0.15–1.83			
Clade II	0.76–2.75	0.15–2.60		
Ethiopia	0.15–1.83	1.22–2.75	0.15–1.22	
Botswana	0.15–2.60	0.15–2.60	0.61–2.60	0.15–1.98

The bold value show the pairwise distances found within each clade (Clade I and Clade II) or within each country (Ethiopia and Botswana).

I ranged from 0.15 to 2.75% and from 0.15 to 2.6% within clade II (Table 1). The genetic divergence between clade I and the Ethiopian

population ranged from 0.15 to 1.83%. Intrapopulation sequence divergence of 0.15–1.22% was recorded within the Ethiopian populations. Similarly, the pairwise genetic distance among all the Botswana haplotypes ranged between 0.15 and 1.98% (Table 1).

Haplotype Network

Molecular analyses of the whole population samples revealed 63 different mitochondrial haplotypes. Clade I was represented by 36 haplotypes, and clade II consisted of 27 haplotypes (Table 2). In addition, 14 potential median vectors were built within the median-joining network (Fig. 3a). Out of the total haplotypes identified 36 were unique (represented by a single individual). In clade I, a specimen from Ethiopia, two specimens from Tanzania, and six specimens from Kenya were represented in the most common haplotype (H_32;

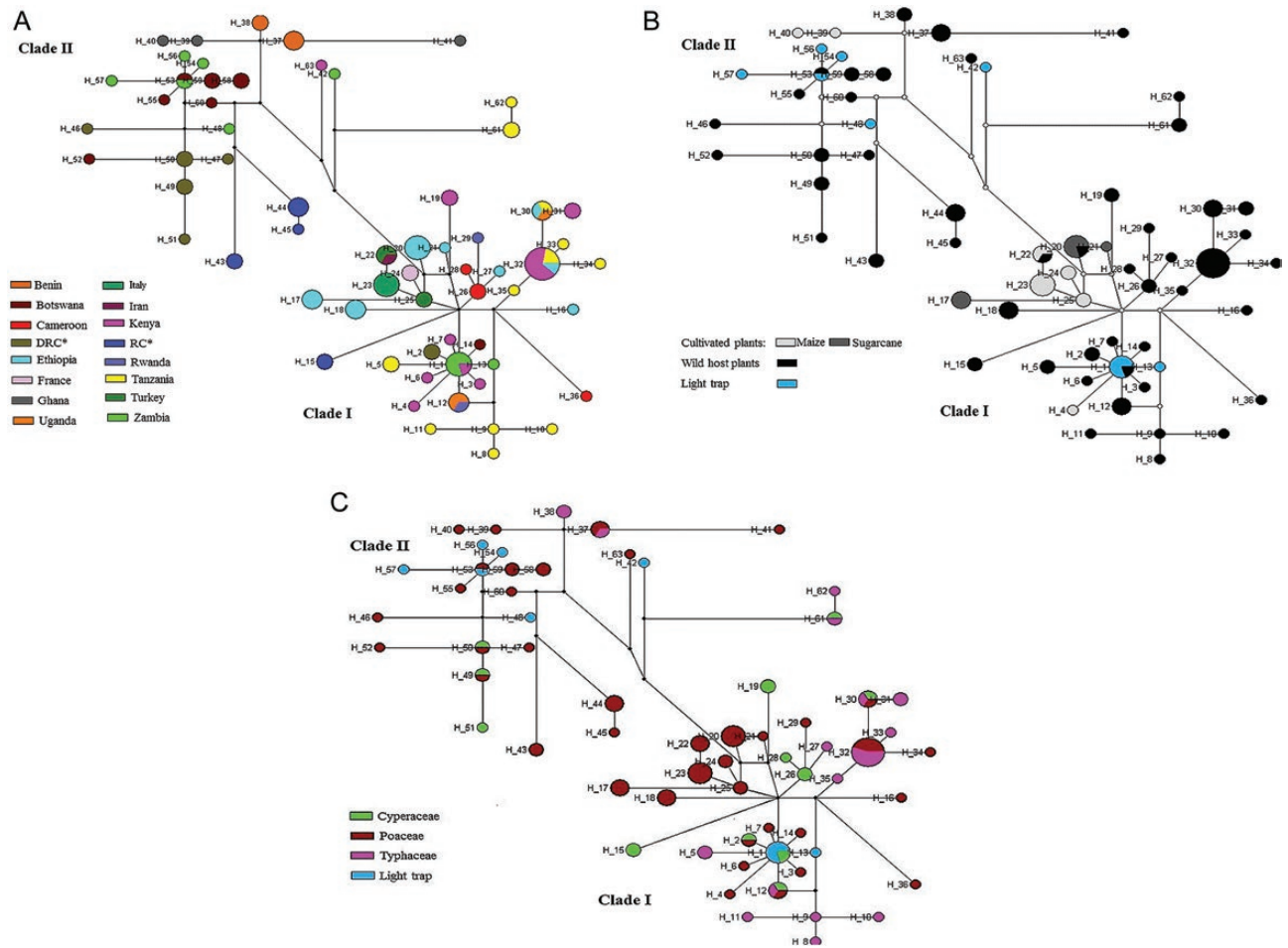


Fig. 3. (a) mtDNA haplotype network of populations of *Sesamia nonagrioides* across the collected localities. Circles represent haplotypes. Colors represent the geographic origin of specimens. The black dot represents missing steps between observed haplotypes. Circle diameter is relative to the number of specimens carrying a particular haplotype. (b) mtDNA haplotype network of populations of *S. nonagrioides* across the collected localities. Circles represent haplotypes. Colors represent where the specimens were (type of host plants and light trap) collected. Circle diameter is relative to the number of specimens carrying a particular haplotype. White circles represent median vectors. (c) mtDNA haplotype network of populations of *S. nonagrioides* across the collected localities. Circles represent haplotypes. Colors represent where the specimens were (host-plant families and light trap) collected. Circle diameter is relative to the number of specimens carrying a particular haplotype. Black circles represent median vectors. Figure is best viewed online in color.

Table 2. Genetic diversity (mean ± SD) of the cytochrome oxidase I gene in *Sesamia nonagrioides* populations

Parameters tested	All populations	Clade I	Clade II	Botswana	Ethiopia
Number of sequences (<i>n</i>)	113	74	39	9	16
Number of polymorphic sites (<i>S</i>)	74	45	44	17	16
Number of haplotypes (<i>h</i>)	63	36	27	7	8
Haplotype diversity (<i>H_d</i>)	0.984 ± 0.004	0.967 ± 0.009	0.981 ± 0.010	0.944 ± 0.070	0.867 ± 0.060
Nucleotide diversity (<i>π</i>)	0.0140 ± 0.0057	0.0081 ± 0.0004	0.0131 ± 0.0009	0.0078 ± 0.0025	0.0075 ± 0.0006
Average number of nucleotide difference (<i>K</i>)	9.14 ± 1.761	5.27 ± 1.360	8.46 ± 1.723	5.10 ± 1.451	4.88 ± 1.358

Fig. 3a). In this clade, four other haplotypes viz H₁ (Kenya and Zambia), H₁₂ (Rwanda and Uganda), H₂₂ (Iran and Turkey), and H₃₀ (Ethiopia, Uganda, and Tanzania) shared between neighboring countries. However, in clade II, only one haplotype (H₅₃) shared between Botswana and Zambia (Fig. 3a). Similarly, in clade I, all the three host-plant families were shared by two haplotypes (H₁₂ and H₃₂; Fig. 3c). Among the 36 mitochondrial haplotypes of clade I, eight haplotypes have been recorded in Ethiopia, out of which six matches to haplotypes reported in the previous study (Kergoat et al. 2015) and two new haplotypes (H₁₇ and H₂₁) were found from sugarcane plant in the present study.

Out of the eight haplotypes recorded in Ethiopia, six of them are specific to this country (Fig. 3a). Within the Ethiopian population of *S. nonagrioides*, only one haplotype (H₂₀) was common between sugarcane and wild hosts (Fig. 3b). The genetic difference within the Ethiopian *S. nonagrioides* haplotypes collected from Metehara (H₁₇) and Wonji (H₂₀) sugarcane estates, which are separated by a distance of 100 km, is eight mutational steps. However, the genetic difference between the closest haplotype of the Palearctic region recovered from maize (H₂₅) and haplotypes collected from Ethiopian sugarcane (H₂₀ and H₂₁) ranged between three to four mutational steps, respectively. The genetic difference between the

Botswana haplotype found in clade I (H₁₄) and its closest sister haplotype in clade II (H₆₀) was 14 mutational steps.

Genetic Diversity and Demographic History

The genetic diversity and structure within and among populations were analyzed based on phylogenetic tree grouping and host plants (cultivated vs wild hosts and also host-plant families from which the specimens were collected viz. Cyperaceae, Poaceae, and Typhaceae) for the whole data set and for the Botswana and Ethiopian populations. Accordingly, grouping based on phylogenetic tree revealed highest molecular diversity in clade II (27 of 63 mitochondrial haplotypes, 39 individuals, $H_d = 0.981$, $\pi = 0.0131$) than clade I (36 of 63 mitochondrial haplotypes, 74 individuals, $H_d = 0.967$, $\pi = 0.0081$; Table 2). However, a comparable level of molecular diversity (Poaceae [$H_d = 0.977$, $\pi = 0.0142$], Cyperaceae [$H_d = 0.967$, $\pi = 0.0147$], and Typhaceae [$H_d = 0.940$, $\pi = 0.0114$]) was observed among individuals collected from different host-plant families (Supp Table S2 [online only]). Comparatively higher genetic diversity ($H_d = 0.978$, $\pi = 0.0149$) was recorded within population of wild host plants than cultivated (maize and sugarcane) plants ($H_d = 0.913$, $\pi = 0.0074$; Supp Table S2 [online only]), which may correspond to the diversity of plants from which the specimens were collected. Likewise, the Ethiopian population recovered from wild host plants revealed higher genetic diversity than population collected from sugarcane plant (Supp Table S2 [online only]). High genetic diversity ($H_d = 0.944$, $\pi = 0.0078$) was recorded in Botswana populations (Table 2).

Results of Tajima's D and Fu's F_s statistics revealed negative values for both clades.

Genetic Structure and Isolation-by-Distance

Comparisons made between clades and groups of populations show significant genetic differentiation for all statistics (F_{ST} , K_{ST}^* , and S_{nn} ; Table 3; Supp Table S3 [online only]). Consequently, the molecular analysis showed significantly higher ($F_{ST} = 0.469$; $P < 0.0001$) genetic differentiation between the two clades (Table 3). This was further supported by the highest S_{nn} value (1.00; $P < 0.0001$). Likewise, significant structuring ($F_{ST} = 0.272$; $P < 0.05$) was observed within the Ethiopian populations collected from sugarcane and wild hosts (Supp Table S3 [online only]). Contrary to this, the genetic differentiation was negligible ($F_{ST} = 0.048$; $P < 0.05$) among populations recovered from different plant families (Cyperaceae, Poaceae, and Typhaceae).

The Mantel test revealed insignificant association between genetic and geographic distances at the continental/entire geographic distribution level ($r = 0.0448$; $P = 0.0950$) and for clade II ($r = 0.0749$; $P = 0.1420$). However, the pattern of IBD for clade I revealed a weak but significant correlation ($r = 0.288$, $P = 0.0240$).

Table 3. Genetic differentiation analyses between populations of *Sesamia nonagrioides*

Groups	F_{ST}	K_{ST}^*	S_{nn}
Clade I vs clade II	0.469***	0.144***	1.000***
Clade I vs Botswana	0.577***	0.091***	0.988***
Clade II vs Botswana	0.081*	0.026**	0.813**
Clade I vs Ethiopia	0.154**	0.044***	0.913***
Clade II vs Ethiopia	0.504***	0.141***	1.00***
Botswana vs Ethiopia	0.608***	0.233***	0.960***

Significant with * $P < 0.01$, ** $P < 0.001$, *** $P < 0.0001$.

Discussion

Genetic Structure and Phylogeographic Pattern of *S. nonagrioides*

All phylogenetic analyses separated *S. nonagrioides* populations into two major clades. However, a strict geographic separation of haplotypes was not observed between clades. Few haplotypes collected from very close geographic locations were found to belong to distinct clades. Applying a molecular clock of 2.3% substitution per million years for insects (Brower 1994), the two clades would have diverged approximately 1.37 Mya. This is long before the introduction of maize (Miracle 1965) and sugarcane (Osborne 1964) in Africa, suggesting that the observed genetic diversification has little or no relation with agricultural intensification. Furthermore, an IBD model is not supported for *S. nonagrioides* at the continental level; therefore, the explanation for the detected phylogeographic pattern must be sought in past incidences of geographic and/or ecological factors.

The geological history of Africa has been characterized by the presence of diverse geographic barriers, largely related to past volcanic and tectonic activities (deMenocal 2004), which may have played a role as a putative barrier to dispersal and gene flow among *S. nonagrioides* populations. The climate oscillations that occurred during the Pleistocene, which significantly affected the African biomes (deMenocal 2004), may also have contributed to the divergence of *S. nonagrioides* population. Moreover, insect herbivores associated with different host-plant species can experience different selection pressures, which may create ecological barriers to gene flow (Martel et al. 2003), resulting in host-associated lineages (Barman et al. 2012).

Underlying Factors Maintaining the Current Population Structure

Host Plant Associated Genetic Differentiation of *S. nonagrioides*

Studying the processes underlying variation is a fundamental question in evolutionary biology. Access to new host plants provides chances for adaptive speciation and is thought to be the main driving mechanism for host-associated differentiation (HAD) in herbivorous insects (Joyce et al. 2016).

The Mediterranean corn borer, *S. nonagrioides*, is a polyphagous species with 42 known host plants belonging to the families Poaceae, Cyperaceae, and Typhaceae (Kergoat et al. 2015). Our study revealed the absence of HAD among *S. nonagrioides* populations. Numerous haplotypes collected from a specific host-plant species (Supp Table S1 [online only]) but yet belonged to different genetic clades poorly support the HAD hypothesis. Moreover, the presence of shared haplotypes collected from different host-plant families (Fig. 2c) from wide geographic areas also argues against host race formation. It is thus improbable that host-plant range caused the observed genetic difference in this study. Our result is in agreement with Kergoat et al. (2015), who reported that host plants did not significantly influence the genetic structure of *S. nonagrioides*.

In spite of broader geographic coverage and sampling of more than 20 host plants, we are not able to find any genetic variations related to host plants. The conclusion that *S. nonagrioides* has not differentiated into separate lineages on different host-plant species in the same locality suggests that gene flow has been high enough to prevent the trade-offs in fitness between *S. nonagrioides* populations attacking different host plants from creating isolation. A similar pattern of host use diversification without undergoing genetic differentiation has been reported in *Busseola segeta* Bowden (Lepidoptera: Noctuidae) (Ong'amo et al. 2012).

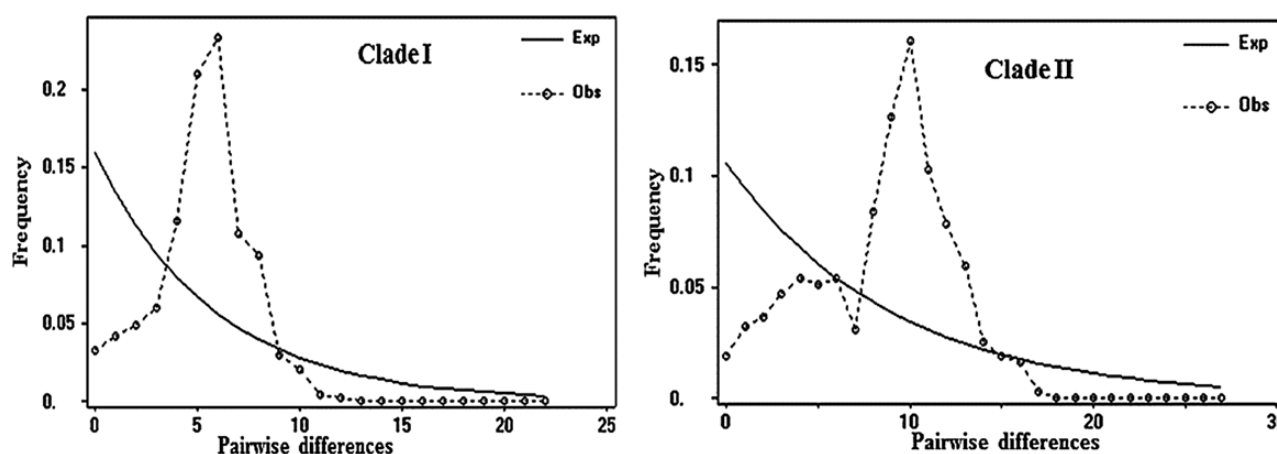


Fig. 4. Mismatch distribution for the two *Sesamia nonagrioides* population. Solid line indicates expected frequency; dashed line with circles indicates observed frequency.

Impact of Geography and Climate on Genetic Differentiation

Geographic Structuration of *S. nonagrioides*

The molecular analysis revealed significant structuring between the two clades. However, the levels of genetic differentiation among *S. nonagrioides* population at the continental scale were unrelated to the geographic distances separating these population. Multiple lines of evidence argue against the existence of IBD in *S. nonagrioides* population across sub-Saharan Africa. First, and most convincing, the haplotypes that were collected in very close localities (e.g., in Democratic Republic of Congo and Zambia) belong to two distinct lineages (as indicated in the median-joining network and phylogenetic trees; Figs. 3a and 2, respectively). This occurred in the absence of geographic boundaries and physical barriers that prevent gene flow among the population of *S. nonagrioides*. Second, the distances among the survey locations in Cameroon (Central Africa) and West African countries (e.g., Benin, Ghana and Republic of Congo that belong to clade II) are less than the survey locations of Cameroon and other East African populations that predominate clade I population. However, all haplotypes originated from Cameroon belonged to clade I.

Impact of the Rift Valley on *S. nonagrioides* Populations

Geographic barriers to dispersal were identified as a major physical barrier to sustain the population structuring of vertebrate and invertebrate animal species in Africa (Pitra et al. 2002, Alpers et al. 2004, Sezonlin et al. 2006). Among the natural barriers, the Rift Valley was frequently reported as a physical barrier reinforcing population structure of cereal stem borers in Africa (Assefa et al. 2006, 2015; Sezonlin et al. 2006; Gofishu et al. 2016). Even though the Eastern Rift Valley has been anticipated as a barrier for gene flow in cereal stem borers, in this study, haplotypes originated from very close localities occurred on either side of the Rift Valley. According to our result, the Rift Valley did not completely prevent the occurrence of the admixture of geographically isolated haplotypes as depicted in the haplotype network and phylogenetic tree.

Impact of Climate

Contrary to HAD and physical barriers, the refuge theory best explains the phylogeographic pattern observed in *S. nonagrioides*. The refuge theory describes that climate-driven oscillations play a

significant role for the fragmentation of species (Anthony et al. 2007, Dobigny et al. 2013).

Estimated divergence time between the two lineages of *S. nonagrioides* probably related to Pleistocene climatic oscillations, characterized by an intense climatic change in sub-Saharan Africa (deMenocal 2004). The period of climatic instability started some 3 Mya, fluctuating between warmer, wetter and cooler, drier periods (deMenocal 2004, Trauth et al. 2009, Dupont 2011) with major climatic changes occurring in sub-Saharan Africa near 2.8, 1.7, and 1.0 Mya (deMenocal 1995). The cyclic aridification instances that occurred during the Pleistocene have led to the recurrent reductions of the wettest environments (i.e., lake shores, rivers, and wetland areas; Trauth et al. 2009), appropriate environments for *S. nonagrioides* host plants, such as *Cyperus* spp., *Echinochloa* spp., *Panicum* spp., *Pennisetum* spp., and *Typha* spp., which were reduced to refugial areas. The divergence of *S. nonagrioides* populations thus seems to be the effect of vicariance related to expansion and contraction of suitable environments. The vegetation shifts related with the Pleistocene climatic cycles played a major role in contraction and expansion of population in African savanna species (Muwanika et al. 2003). Our observations are in line with Sezonlin et al. (2006) and Assefa et al. (2015), who related the differentiation of maize stem borer, *B. fusca*, into geographic populations with the influence of climatic cycles that happened in Africa during the Pleistocene.

The separation into two clades, clade I, haplotypes dominantly collected from East African countries, and clade II, predominantly recovered from Central and West African regions, is a pattern observed in insect (Bennett et al. 2016) and many African mammals (Flagstad et al. 2001, Muwanika et al. 2003). Convergent phylogeographic patterns could show the presence of shared refugia for many African savanna species during climatic fluctuations (deMenocal 1995), suggested to be situated in East, Southern, and West Africa (Lorenzen et al. 2012, Dobigny et al. 2013, Smitz et al. 2013). In this study, the mtDNA populations are geographically separated in most part of the range of *S. nonagrioides* but came into contact in some geographic regions as observed in haplotype network (Fig. 3a). The most probable hypothesis for the observed distribution pattern of *S. nonagrioides* population might be because of segregation and genetic variation within refugia throughout the unfavorable times and subsequent range expansion during favorable ones, with secondary contact among diverged population when overlapping occurred. We therefore suggest *S. nonagrioides* survived unfavorable times throughout the Pleistocene in at least one refuge situated

in Eastern and one refuge situated in Western Africa, with overlapping in the present-day Tanzania. Seven percent of the total land surface of Tanzania is covered with water bodies (NEMC/WWF/IUCN 1990). The country possesses the top three largest lakes (Victoria, Tanganyika, and Malawi) of Africa (Hassan and Jin 2014). The surrounding areas of these water bodies harbor diverse species of grasses and sedges (Ruffo et al. 2002). These various host plants found in a large area of the Tanzanian region probably harbor *S. nonagrioides* populations from Central, Southern, and Western Africa through the interconnected network of lake and river systems in the Western Rift Valley. Therefore, the increased genetic diversity recorded in the Tanzanian population of *S. nonagrioides* might be the outcome of the migration of the Central, Southern, and Western population into Tanzania. Thus, this country could be considered as a hybrid or overlapping zone (Zamudio and Savage 2003, Bilgin 2011) where the two lineages of *S. nonagrioides* came in contact. The East African region was described as secondary contact zones for the African sugarcane stem borer, *Eldana saccharina*, where main biogeographic populations from diverse African countries meet and overlap (Assefa et al. 2006).

IBD indicates limited dispersal among populations of clade I, supporting a more recent origin of the clade (Lorenzen et al. 2010). In contrast, the combination of higher nucleotide diversity and absence of IBD among populations of clade II reflect the possible ancestral locations of this clade. Althoff and Pellmyr (2002) proposed the ancestral location of bogus yucca moth, *Prodoxus decipiens* (Lepidoptera: Prodoxidae), based on the highest genetic diversity measures (haplotype and nucleotide diversity) and the absence of IBD. Accordingly, we hypothesize that Central Africa (the Congolian basin) as the possible ancestral location of *S. nonagrioides* population because of the highest level of nucleotide diversity ($\pi = 1.5\%$) recorded in the area. Our hypothesis corresponds well with Smitz et al. (2013) who suggested the location of the ancestral lineage in African Buffalo (*Syncerus caffer*) based on higher nucleotide diversity. Kergoat et al. (2015) also suggested the Congolian basin as the origin of *S. nonagrioides* based on the diversification and final split of *S. nonagrioides* from its sister species, *Sesamia congoensis*. The overall nucleotide diversity in this study reaches 1.4%, which is high in comparison to the maize stem borer, *B. fusca*, so far studied from a similar region (Sezonlin et al. 2006).

Mismatch Distribution

The mismatch distribution, Tajima's *D* and Fu's *F_s*, indicated that both clades of *S. nonagrioides* populations were in demographic expansion phase as found for the maize stem borer, *B. fusca* (Sezonlin et al. 2006, Assefa et al. 2015), and greater sugarcane borer, *Sesamia cretica* (Goftishu et al. 2016), in sub-Saharan Africa.

Records of *S. nonagrioides* in Botswana and Ethiopian Sugarcane

Sesamia nonagrioides is recorded for the first time from sugarcane estates of Ethiopia and attained the status of a pest in Ethiopia in particular and East Africa at large. Moreover, the insect recorded for the first time as a pest of sugarcane in Africa. Kergoat et al. (2015) during their study collected sample specimen of *S. nonagrioides* from maize in Kenya, albeit, the insect did not reach pest status. This indicates the chance of this insect invading maize and/or sugarcane exists in East African countries, provided environmental factors are conducive. Whether or not it will invade cultivated crops such as maize and sorghum in the region remains to be seen, but its adaptation to

sugarcane in Ethiopia is an indication that the possibility certainly exists.

The record of *S. nonagrioides* on sugarcane in Ethiopia might be described by the presence of a new biotype that is genetically different from the wild host-plant population or host range expansion of the wild population to sugarcane. Molecular analysis, however, indicated a significant ($F_{ST} = 0.272$; $P < 0.05$) genetic variation between the Ethiopian population of *S. nonagrioides* recovered from sugarcane and wild host plants. Contrary to this, the genetic distance (Table 1), phylogenetic tree (Fig. 2), and haplotype networking (Fig. 3b) showed the absence of noticeable genetic variation between the two populations. The observed genetic structuration between the two populations is most likely an artifact related to the sampling size. Since F_{ST} is the ratio of two variances, it is hardly possible to quantify it precisely without a large data set (Whitlock and McCauley 1999). Results based on uncorrected genetic distance, phylogenetic tree, and haplotype networking argues contrary to the hypothesis of a new population of *S. nonagrioides* in Ethiopia. Rather the pest expanded its host range to include sugarcane. This is further supported by the shared haplotype between the sugarcane and wild host population and the low genetic diversity observed in the sugarcane population when compared with the population of the wild host. Ancestral populations are likely to have higher haplotype diversity than recently derived populations (Althoff and Pellmyr 2002).

The presence of *S. nonagrioides* on a wide range of host plants and its rapid adaptation to sugarcane without genetic difference explains the presence of host use plasticity (Ong'amo et al. 2012, Assefa et al. 2017). Phytophagous insects with a wider diet breadth usually overcome plant defense without undergoing genetic adaptation (Nylin and Gotthard 1998). The majority of sugarcane stem borers in Africa are indigenous to the continent (Leslie 2004). The evolution of preference for and enhanced performance on sugarcane by native African stem borers was reported several years after the introduction of maize and sugarcane in Africa (Dick 1945, Assefa et al. 2010, Assefa et al. 2017). The host-plant expansion by *S. nonagrioides* to include sugarcane as a novel host is likely to be a function of several factors. First, the spatiotemporal availability of sugarcane that provides continuous supply of food (Goebel and Sallam 2011). Second, the disruption of effective natural enemies in sugarcane plantations. Insect herbivores appear to adapt to novel hosts and cropping systems more easily than do their natural enemies (Pélissier et al. 2010). Third, the destruction of natural habitats for large-scale sugarcane production may have promoted sugarcane colonization. In recent years, the old sugarcane estates of Ethiopia expanded their sugarcane production areas by displacing farmer's crop lands, pasture lands, and the natural vegetation. We observed very high infestation of *S. nonagrioides* on these newly expanded plantation areas. Destruction of natural habitats for large-scale sugarcane production could be a possible reason for the incursion of sugarcane by *S. nonagrioides*. A similar trend of invasion has been hypothesized in the greater sugarcane borer, *S. cretica*, that switched from wild hosts to sugarcane in Tendhau sugar estate, Ethiopia (Goftishu et al. 2016). Mass clearing of natural habitat to establish new sugarcane plantations coupled with intensive agronomic practices and lack of natural enemies plays a major role to the host expansion and outbreak of insect pests in sugarcane (Conlong 1997, Goebel and Sallam 2011).

This study also recorded *S. nonagrioides* for the first time outside of the known species geographic range in Southern Africa—Botswana. The pairwise genetic distance (1.53–1.98%) and the high level of haplotype diversity observed in the Botswana population suggests that *S. nonagrioides* might have a long-time establishment with an ancient history in the country. Until now *S. nonagrioides* has never been reported in South

Africa (Kergoat et al. 2015) despite hosting three endemic sister species with very similar ecological needs. It seems Botswana might be the southern limit of the species in Africa. According to Kulongoski et al. (2004), central regions of South Africa became mainly inhospitable due to lower temperature that existed during the Pleistocene. Moreover, the Kalahari Desert that formed during the last 3 Mya (Moore et al. 2012) dominated the regional plateau of the interior regions of Southern Africa (Meadows 2001). These environmental conditions might limit the expansion of the insect further to South Africa, as the species is particularly well adapted to wet and hot climate. Our hypothesis could be further supported by the absence of the sister species of *S. nonagrioides*, namely *Sesamia capensis*, *Sesamia natalensis*, and *Sesamia typhae* in the interior regions of Austral Africa (Kergoat et al. 2015). A similar pattern of distribution has been observed in the South African puff adder (*Bitis arietans*; Barlow et al. 2013). According to Barlow et al. (2013), interior regions of South Africa became largely 'unsuitable' because of lower temperatures.

Despite the geographic spread and occurrence of *S. nonagrioides* in Southern Africa and its host range expansion to include sugarcane in eastern Africa, all the newly collected samples fall into the two clades previously reported by Moyal et al. (2011). The genetic distance reported in the present study (2.75%), however, is greater than the previous record (2.30%; Moyal et al. 2011). This increase should not be a surprise as a large number of samples collected from diverse host plants across the geographic range of the species were used in this study.

Conclusion

This study represents the comprehensive investigation of the global-scale phylogeography of the Mediterranean corn borer. Pleistocene's climatic oscillations appear to have influenced the evolutionary history of *S. nonagrioides*, particularly the sub-Saharan populations. The study provides the first evidence that *S. nonagrioides* is invading sugarcane in Ethiopia. In addition, the study revealed the presence and long-time establishment of *S. nonagrioides* in Botswana for the first time. *Sesamia nonagrioides* did not exhibit host-associated genetic variation. The presence of *S. nonagrioides* in a wide range of host plants without genetic variation strongly suggests the existence of host use plasticity. Results of this study assist in understanding the evolutionary forces that may be responsible for the spatial distribution, genetic divergence and host range expansion of *S. nonagrioides* population. Genetically diverse populations of pests differ in their response to several pest management methods (Lesieur et al. 2016). Thus, the genetic differences in *S. nonagrioides* population should be carefully considered while designing pest management strategies. Furthermore, sugarcane farmers in sub-Saharan Africa should be aware that *S. nonagrioides* has expanded its diet breadth and became a major insect pest of sugarcane in Ethiopia. Thus, they should be advised to monitor sugarcane farms and take appropriate measures to prevent invasion by *S. nonagrioides*.

Supplementary Data

Supplementary data are available at *Journal of Economic Entomology* online.

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