

PHYLOGEOGRAPHY OF *SESAMIA NONAGRIOIDES*
(LEPIDOPTERA: NOCTUIDAE) IN IRAN
INFERRED FROM MITOCHONDRIAL DNA

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Although the stem borer *Sesamia nonagrioides* (Lefèbvre, 1827) is one of the most destructive agricultural pests in South and Southwest Iran, the structure of these populations is not yet well understood. In this study, we sequenced two mitochondrial genes (CO1 and Cyt-*b*) of populations collected in cultivated sugarcane and rice in South and Southwest Iran. These genetic data of Iranian populations were compared with those of already published data from West Palaearctic (Africa and Europe). To testing the intra- and inter-population variations, different population analyses (e.g. haplotype network, haplotype and nucleotide diversity) were executed. Our results indicate a considerable genetic variation in Iranian populations of *S. nonagrioides*, which supports the long-term establishment of *S. nonagrioides* populations in Iran versus a recent invasion. Close genetic relationship of Iranian population of *S. nonagrioides* with a population of Central Ethiopia supports the scenario of the origin of this species from Africa to western Asia through the Trans-Arabian bridge. Our results help to explain the variation in pest status of *S. nonagrioides* observed across its distribution range in Iran.

Key words: CO1, Cyt-*b*, gene flow, haplotype, population genetics.

INTRODUCTION

The stem borer *Sesamia nonagrioides* (Lefèbvre, 1827) (Lepidoptera: Noctuidae) is one of the most damaging sugarcane pests in sugarcane agro-industries of Khuzestan province (Southwest Iran) since 1950's. It is also a pest of maize and rice in provinces of Khuzestan and Fars (South Iran) where it completes four to five generations annually (KHANJANI 2004, CHERGHALI *et al.* 2015). The pest status of *S. nonagrioides* on different crops varies across its distribution range in Iran (CHERGHALI *et al.* 2015). Pest management strategies of this economic pest in sugarcane fields of south-western Iran include releasing the egg parasitoid wasp *Telenomus busseolae* Gahan (Hym., Scelionidae) (JAMSHIDNIA *et al.* 2010). However, this wasp cannot perform a successful biologi-

cal control of the pest especially during hot summers in Khuzestan province or in sensitive sugarcane varieties (JAMSHIDNIA, pers. comm.). Pesticides are used by farmers in the case of heavy damage to maize and rice. Management practices of *S. nonagrioides* in Iran ignore the possible existence of genetic diversity among populations of this pest and its range of distribution.

The species *S. nonagrioides*, is considered one of the most important pests of maize in many countries around the Mediterranean region where it is known as the Mediterranean corn borer or corn stalk borer (DE LA POZA *et al.* 2008). This pest has been traditionally regarded as a rather sedentary pest (ALBAJES *et al.* 2004, EIZAGUIRRE *et al.* 2004, MARGARITOPoulos *et al.* 2007), likely to develop genetic isolation between geographical regions. Genetic differentiation between *S. nonagrioides* populations in European countries has been reported using different molecular markers (BUES *et al.* 1996, KOURTI 2006, LENIAUD *et al.* 2006, MARGARITOPoulos *et al.* 2007, DE LA POZA *et al.* 2008). MOYAL *et al.* (2011) reconstructed the evolutionary history of Palaearctic and Sub-Saharan populations of *S. nonagrioides* using one nuclear and two mitochondrial genes and concluded that the isolated Iranian population appears to have recently originated from a population of eastern Europe origin.

Sesamia nonagrioides seems not to be a recently introduced pest, but changed from wild host plants to cultivated sugarcane during the first establishment of sugarcane agro-industries in south-western Iran in 1950's (ESFANDIARI *et al.* 2011a, b). CHERGHALI *et al.* (2015) found a considerably high genetic variation with Random Amplified Polymorphic DNA (RAPD) among Iranian *S. nonagrioides* populations which supported the "host-plant switching" hypothesis versus the recent invasion of *S. nonagrioides* by infested sugarcane cuttings to Iran. Concerning the disadvantages of RAPD (SCHLÖTTERER 2004), a deeper understanding of the population genetic structure of this pest could greatly benefit management decisions related to this species in Iran (e.g. improve monitoring and biological control strategies). In the present article, we used mitochondrial DNA (mtDNA) sequences of cytochrome *b* (Cyt-*b*) and cytochrome *c* oxidase, subunit 1 (CO1) to evaluate the population structure and genetic variability of the stem borer *S. nonagrioides* in Iran. These details were further analysed with sequences available in NCBI GenBank from those already published from West Palaearctic (Africa and Europe) (MOYAL *et al.* 2011).

MATERIAL AND METHODS

Sampling – Specimens of *S. nonagrioides* were collected across its area of distribution in Iran from infested host plants of sugarcane and rice in three main locations (Table 1): Haft-tappeh and Imam Khomeini agro-industries in North Khuzestan, Amir Kabir agro-industry in South Khuzestan and Firuzabad suburbs area in Fars. After collection, specimens were preserved in 96% ethanol and stored at -20°C for DNA extraction. Specimens were identified by using identification keys (e.g. TAMS & BOWDEN 1953, MEIJERMAN & ULENBERG 1996).

Table 1. Locations of *Sesamia nonagrioides* collections from agricultural fields in south and south-west Iran and quantity of analysed specimens (N).

Location	Province	Geocoordinate	Host plant	Sampling date	N (CO1, Cyt-b)
Hafttappeh	Khuzestan	32°05'N, 48°21'E	sugarcane	Aug. 2011	6, 6
Imam Khomeini	Khuzestan	31°47'N, 48°43'E	sugarcane	Oct. 2013	5, 4
Amir Kabir	Khuzestan	31°03'N, 48°18'E	sugarcane	Jun. 2007	8, 9
Firuzabad	Fars	29°00'N, 52°33'E	rice	Sept. 2011	4, 4

DNA extraction, PCR amplification and sequencing – DNA was isolated mostly from last instar larvae and in some cases from adults. Total genomic DNA was extracted from the head and thorax of each specimen using the DNeasy Blood & Tissue Kit (QIAGEN GmbH) following the manufacturer's protocol for insects. Two mitochondrial genes Cytochrome c oxidase subunit 1 (CO1) and Cytochrome b (Cyt-b) were amplified. For amplifying Cyt-b with nested PCR procedure, two primer pairs obtained from MOYAL *et al.* (2011) were used. External primers were TRs (5'-TCTATCTTATGTTTTCAAAG-3') and CP1 (5'-GATGATGAAATTTGGATC-3') while internal primers were CB3H (5'AGCAAATAAAAAATAT-CATC-3') and CB1 (5'TATGTACTACCATGAGGACAAATATC-3'). The products of internal primers were used for sequencing. Fragment of CO1 was amplified by primers Hobbes (5' AAATGTTGNGGRAAAAATGTTA-3') and Ron (5'-GGATCACCTGATATAG-CATTCC-3') (MOYAL *et al.* 2011). The PCR reaction was performed in a total volume of 25 µl containing 2.5 µl PCR buffer 10X, 1.5 µl MgCl₂ 50 mM, 0.5 µl dntp 10 mM, 1 µl of each primer 10 µM, 2 unit of Taq DNA polymerase (CinnaGen technologies-Iran) and 2 µl of extracted DNA (100–200 ng). The PCR program for the two genes was as follows: 92°C for 5 min, 35 cycles of 1 min at 92°C, 1.3 min at temperature gradient from 40 to 50°C and 1.3 at 72°C, followed by 5 min at 72°C. The PCR products were visualized by means of electrophoresis in 1% agarose gel. Sequencing was performed mainly with an ABI3730XL capillary sequencer (Macrogen, Seoul, Korea).

Data analyses – Nucleotide sequences of Cyt-b and CO1 of additional *S. nonagrioides* specimens from the study by MOYAL *et al.* (2011) were obtained from GenBank (NCBI) (Table 2). Sequences were edited in BioEdit 7.1.3 (HALL 1999) and successfully aligned with MAFFT 6 (KATOHO *et al.* 2002) by using default settings. All sequences were deposited in GenBank (accession numbers KT583674-KT583719, see supplementary table). Statistical parsimony networks of CO1 and Cyt-b for populations of *S. nonagrioides* were reconstructed with TCS v.1.3 (CLEMENT *et al.* 2000). This analysis separated the sequences into a network of closely related haplotype groups (with connected branches with less than 95% probability). Population diversity indices including haplotype diversity (*h*), nucleotide diversity (π) (NEI & MILLER 1990) and F_{st} for each population of *S. nonagrioides* were calculated using Arlequin 3.5.1.2 (EXCOFFIER & LISCHER 2010). The results of analyses of molecular variation (AMOVA) were visualized using a script of R statistic program integrated in the Arlequin.

RESULTS

Sequence variation – In total, 653 bp of CO1 mtDNA of 59 individuals and 369 bp of Cyt-b mtDNA of 62 individuals of *S. nonagrioides* were successfully aligned and analysed from Iran (Table 1), Europe and Africa (Table 2).

Table 2. Analysed sequences of *Sesamia nonagrioides* with sampling site details obtained from NCBI GenBank accession numbers (JF274085–JF274205) and quantity of analysed specimens (N).

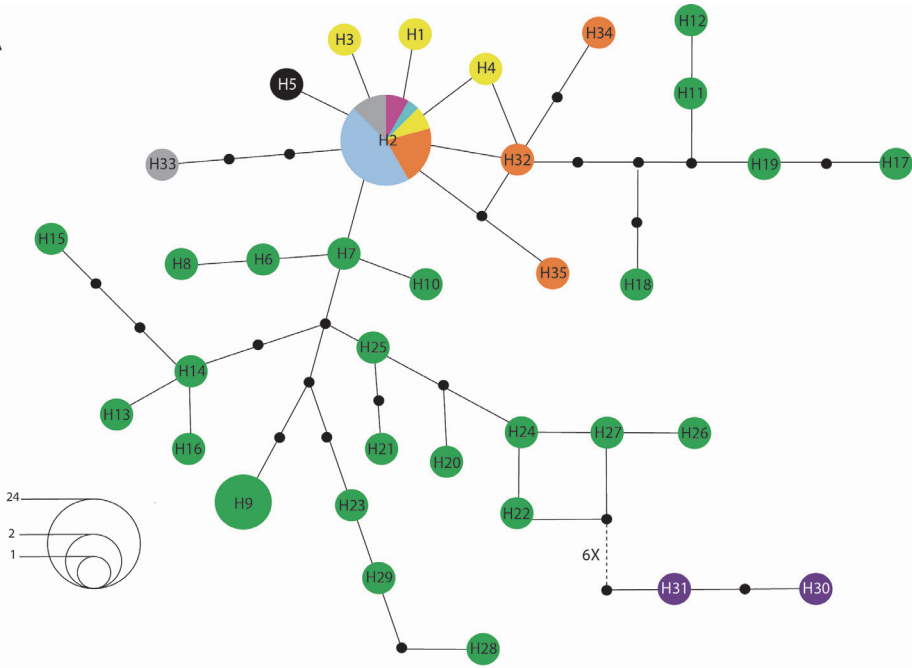
Country	N (CO1, Cyt- <i>b</i>)	Locality	Geocoordinates
Ivory Coast	(2, 8)	Abobo	05°19'N, 04°01'W
Rwanda	(1, 3)	Kitikinyoni	01°35'S, 29°36'E
Kenya	(3, 4)	Kisumu	00°06'S, 34°19'E
	(6, 7)	Lwanda	00°29'S, 34°18'E
	(3, 3)	Bogoria	00°13'N, 36°02'E
	(0, 1)	Kiboko	02°12'S, 37°42'E
	(2, 1)	Bahar Dar	11°22'N, 37°14'E
Ethiopia	(2, 5)	Andasa	11°18'N, 37°17'E
	(1, 0)	Black water	07°03'N, 38°17'E
	(2, 4)	Omolante	06°06'N, 37°24'E
	(4, 3)	Awasa	07°02'N, 38°17'E
	(1, 0)	Chamoleto	05°33'N, 37°19'E
Turkey	(1, 0)	Adana	37°01'N, 35°19'E
Greece	(2, 0)	Serres	41°05'N, 23°33'E
Spain	(1, 0)	Lleida	41°37'N, 00°35'E
France	(1, 0)	Pierrelatte	44°23'N, 04°42'E
	(2, 0)	Carcassonne	43°12'N, 02°20'E
	(2, 0)	Saint-Martin de Hinx	43°34'N, 04°42'W

The CO1 fragment contained 41 variable sites and revealed 35 haplotypes and Cyt-*b* with 30 variable sites and 33 haplotypes (Fig. 1).

Population genetic analyses – The general pattern of haplotype and nucleotide diversities of both CO1 and Cyt-*b* in the populations was highly similar. Unlike other Iranian populations, those from North Khuzestan showed no haplotype and nucleotide diversity in both genes (see Table 3). Haplotype and nucleotide diversities ranged from 0.0000 to 0.9960 and 0.000000 to 0.007687 for CO1, respectively (Table 3). These statistics ranged from 0.0000 to 0.9849 and 0.000000 to 0.010327 for Cyt-*b*, respectively (Table 3).

Fig. 1. Haplotype network based on partial mtDNA COI (A) and Cyt-*b* (B) of *Sesamia nonagrioides* analysed populations at the 95% level. Each haplotype is represented by a circle, and is identified by a number. Colors indicate representative populations and the size of the colored slices and circles is proportional to the number of individuals carrying the haplotype. Due to the low number of individuals per populations, all European countries (showed with Eu. in parenthesis) were pooled together in AMOVA analyses

A



B

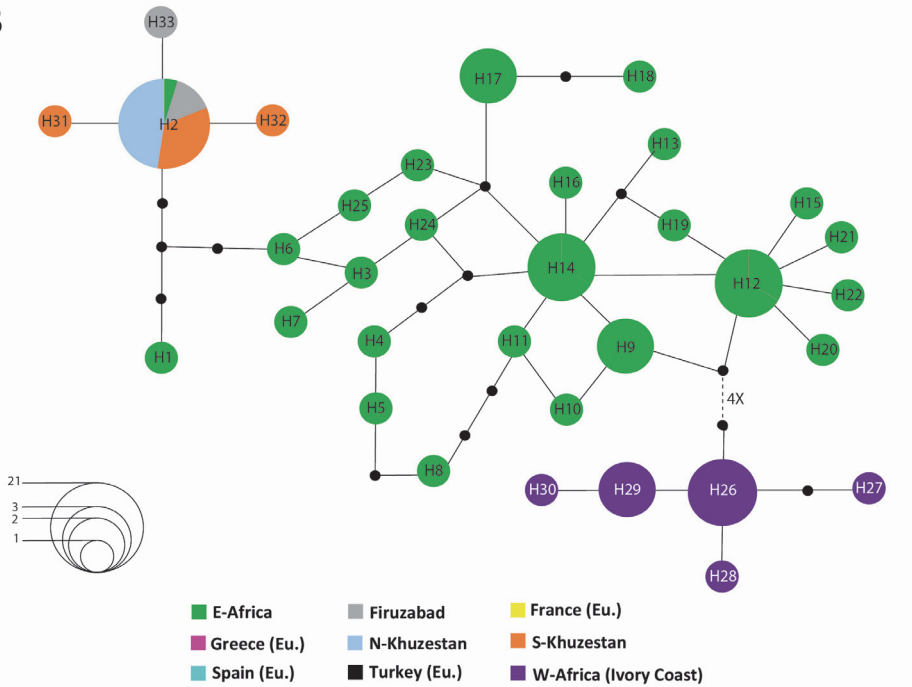


Table 3. Sampling sites, quantity of analysed specimens (n), and identified CO1 and Cyt- b haplotypes of the studied populations of *Sesamia nonagrioides* (H). Statistical parameters indicate haplotypic diversity h (mean \pm SD) and nucleotide diversity π (mean \pm SD) observed for the CO1 and Cyt- b fragments of the studied populations.

Marker		n	H	Haplotype diversity (h)	Nucleotide diversity (π)
CO1*	N Khuzestan	11	1	0.0000 \pm 0.0000	0.000000 \pm 0.000000
	S Khuzestan	8	4	0.6429 \pm 0.1841	0.002461 \pm 0.001849
	Firuzabad	4	2	0.5000 \pm 0.2652	0.002297 \pm 0.002050
	Europe	9	5	0.7222 \pm 0.1592	0.001361 \pm 0.001183
	East Africa	25	24	0.9960 \pm 0.0142	0.007687 \pm 0.004323
Cyt- b *	N Khuzestan	10	1	0.0000 \pm 0.0000	0.000000 \pm 0.000000
	S Khuzestan	9	3	0.4167 \pm 0.1907	0.001204 \pm 0.001333
	Firuzabad	4	2	0.5000 \pm 0.2652	0.001355 \pm 0.001680
	East Africa	31	24	0.9849 \pm 0.0124	0.010327 \pm 0.005942
	West Africa	8	5	0.8571 \pm 0.1083	0.004162 \pm 0.003160

*Not estimated for West Africa (in CO1) because only two sequences of CO1 were obtained in GenBank. Also not estimated for Europe (in Cyt- b) because no sequences were found in GenBank

The highest nucleotide diversity was recorded in East Africa population (CO1: 0.007687 \pm 0.004323; Cyt- b : 0.010327 \pm 0.005942) while the lowest was observed in North Khuzestan population (0.000000 \pm 0.000000 for both genes). The lowest haplotype diversity was also recorded in North Khuzestan population (0.000000 \pm 0.000000) for both CO1 and Cyt- b , while the highest value was recorded in East Africa population (CO1: 0.9960 \pm 0.0142; Cyt- b : 0.9849 \pm 0.0124).

Fixation index (F_{ST}) showed no significant genetic differentiation between Iranian populations according to both studied genes. The East African population showed significant difference with the Iranian populations in CO1. According to Cyt- b , both East and West African populations were also significantly different from the Iranian populations. The distances between

Table 4. Pairwise genetic differentiation (F_{ST}) between studied populations of *Sesamia nonagrioides* for COI ($p \leq 0.05$).

	N Khuzestan	S Khuzestan	Firuzabad	Europe	East Africa
N Khuzestan	0.00000				
S Khuzestan	0.13177	0.00000			
Firuzabad	0.27072	0.02041	0.00000		
Europe	0.02343*	0.04884	0.04202	0.00000	
East Africa	0.30873*	0.24301*	0.19016*	0.27430*	0.00000

*indicates a significant value at the 5% level

Table 5. Pairwise genetic differentiation (F_{ST}) between studied populations of *Sesamia nonagrioides* for Cyt-*b* ($p \leq 0.05$).

	N Khuzestan	S Khuzestan	Firuzabad	East Africa	West Africa
N Khuzestan	0.00000				
S Khuzestan	0.01235	0.00000			
Firuzabad	0.24528	0.00907	0.00000		
East Africa	0.47984*	0.46538*	0.41259*	0.00000	
West Africa	0.92141*	0.89186*	0.86405*	0.67750*	0.00000

*indicates a significant value at the 5% level

West African population and Iranian populations were about two times more than between East African and Iranian populations. The two African populations were significantly different from each other (Tables 4 & 5, Fig. 2).

Haplotype analyses – The relationships among mitochondrial haplotypes were reconstructed by haplotype networks for both CO1 and Cyt-*b* (Fig. 1). Only one haplotype (H2, the most common haplotype in both networks) was traced from North Khuzestan. Apart from H2, the remaining haplotypes were each unique to one population. The phylogenetic analysis of CO1 gene revealed 35 haplotypes separated into two main clades. The first clade consists of individuals from Ivory Coast in West Africa and the second one included all other populations in which the Iranian populations were placed in the group close to the European populations. This group is close to some haplotypes of East Africa origin (from central Ethiopia). Haplotype 2 was well distributed and contained specimens from Firuzabad, North and South Khuzestan as well as from Europe.

In haplotype network of Cyt-*b*, 33 haplotypes showing similar pattern as CO1 and were separated in two main clades. The West African population from Ivory Coast separated (with 8 mutation steps in CO1 and 6 in Cyt-*b*) from other populations (Fig. 1). Haplotype H2 was well distributed and shared with Firuzabad, North and South Khuzestan. This haplotype was also found in East Africa.

DISCUSSION

The present study was an attempt to investigate the variation in mtDNA of *S. nonagrioides*, one of the most destructive agricultural pests in South and Southwest Iran. The results of both CO1 and Cyt-*b* haplotype networks revealed that populations of *S. nonagrioides* in Iran are close or identical to those from Central Ethiopia, East Africa. Iranian *S. nonagrioides* populations also showed very close relationship with European population, but seemed to have higher diversification than the European population.

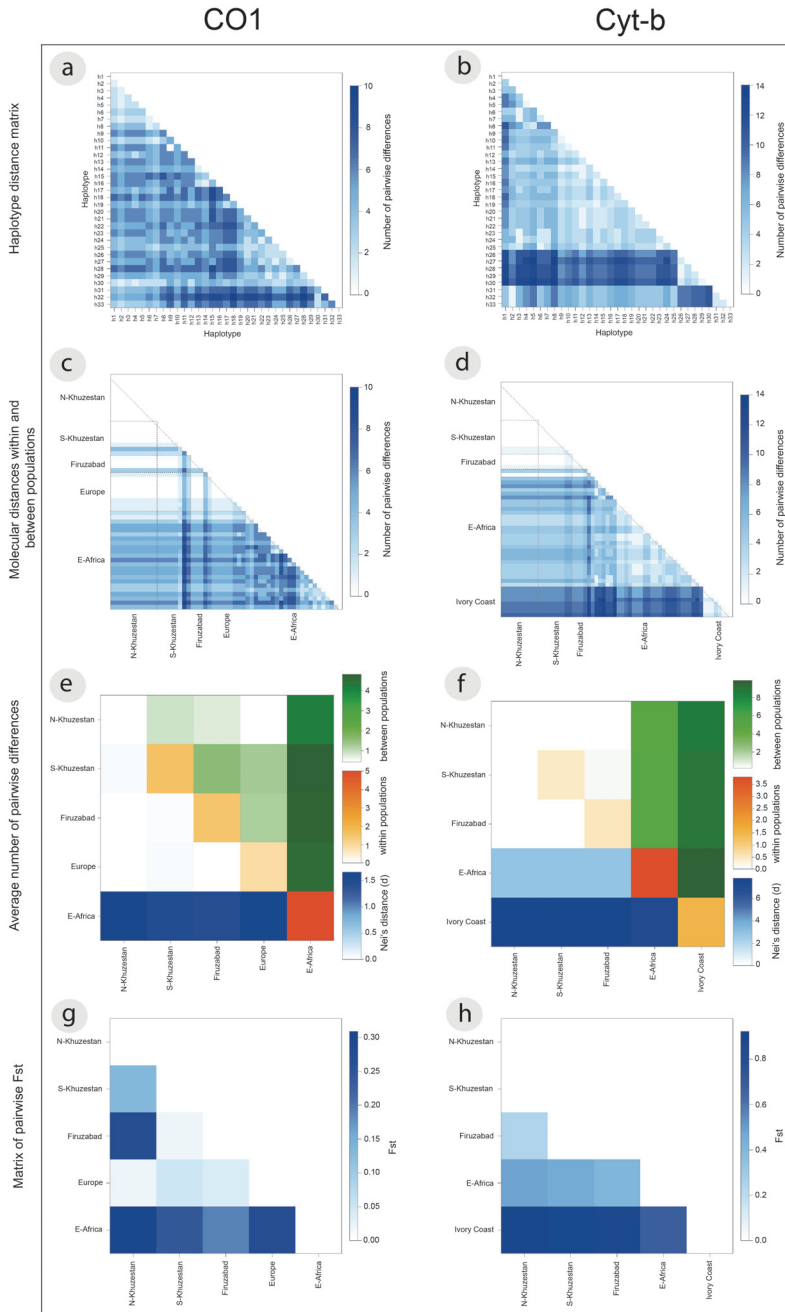


Fig. 2. Results of AMOVA for both studied genes of *Sesamia nonagrioides*. a and b: Haplotype distances matrix; c and d: Nei's within and between population distances; e and f: number of pairwise differences between localities; g and h: comparison of F_{st} values of all populations

MOYAL *et al.* (2011) reconstructed the evolutionary history of Palaearctic and Sub-Saharan populations of *S. nonagrioides* using one nuclear and two mitochondrial genes. They found only two haplotypes among 54 sequences of *Cyt-b* across southern Europe. In the present study we found 4 haplotypes among 23 sequences of *Cyt-b* in South and Southwest Iran which may be indicative of higher diversification in Iranian (vs European) populations. However, CO1 was more diversified than *Cyt-b* in the southern European populations and revealed 9 haplotypes among 65 sequences (MOYAL *et al.* 2011). We found 5 haplotypes among 23 sequences of CO1 in Iran. Moreover, haplotype diversity index (*h*) was also considerably higher in Iranian populations (Table 3) compared to European ones (MOYAL *et al.* 2011: table 2). This may support not only higher diversification, but also longer history of *S. nonagrioides* in Iran. It is worth saying that the sampling areas in southern Europe covered more geographical gradient and range of over 3000 km², whereas in our study, sampling localities in South and Southwest Iran were limited to a distance of 500 km².

Previous studies revealed high levels of differentiation among geographically close populations of *S. nonagrioides*, which could be indicative of the sedentary nature and moderate dispersal behavior of this pest, even when no remarkable geographical barriers exist (DE LA POZA *et al.* 2008, MOYAL *et al.* 2011, CHERGHALI *et al.* 2015). Ecological observations regarding flight behavior and dispersal capacity of *S. nonagrioides* validate the low dispersal ability of this pest too (ALBAJES *et al.* 2004, EIZAGUIRRE *et al.* 2004). In our study, only one haplotype of each CO1 and *Cyt-b* in Iranian populations were shared among localities (Fig. 1). Similar trend exists in African populations (MOYAL *et al.* 2011: figs 1 & 2). This may also support the fact that *S. nonagrioides* is a fairly sedentary species. Personal observations of the first author have shown that during the cold season, plant stubbles and residues contain a large number of *S. nonagrioides* larvae. Most probably, the pest population in the farms comes from plant stubbles and residues at the same site rather than migrating from other areas.

It has been suggested that *S. nonagrioides* might be native to south-western Iran rather than an exotic recently imported pest, having adopted sugarcane as a host after its cultivation in Iran about 70 years ago (ESFANDIARI *et al.* 2011a, CHERGHALI *et al.* 2015). Sugarcane cultivation in Khuzestan was common since 2000 years ago until it became obsolescent about 600 years ago due to unknown reasons (ADAMS 1962). High haplotype diversity in our result also support the hypothesis that the genetic structure of *S. nonagrioides* populations in Iran does not arise from a recent invasion of a common gene pool but may be due to a long time establishment with an ancient history. DANIALI (1985) reported some natural enemies such as the egg parasitoid *Telenomus busseolae* on *S. nonagrioides* in Khuzestan. Such natural enemies may have re-

sulted in limited rapid population build-up of this species in the past, but the pest adopted sugarcane and other crops to outbreak in recent decades. Despite reports on infestation of wild host plants by this pest in Iran, especially *Echinochloa colona* in North Khuzestan (DANIALI 1985), the stem borer seems to be uncommon in wild habitats. This could be attributed to poor nutritive value of wild plants.

Khuzestan sugarcane plantations and their environs are surrounded by two long rivers Karun and Karkheh and their tributaries. The main plants which inhabit such wetlands and tidal flats are reed species of *Phragmites australis* (CAV.) TRIN. Ex Steud and *Typha angustifolia* L. and sedges such as *Juncus* species (HOBERLANDT 1983, LINNAVUORI 2009). Such plants may be the original wild host-plants of this insect before vast cultivations of sugarcane and cereals such as maize and rice in southern Iran. The preference of *S. nonagrioides* for sugarcane and severity of its damage in this crop suggest that it may be originally associated with wild species close to sugarcane in Iran.

MOYAL *et al.* (2011) concluded that the isolated Iranian population appear to have recently originated from a population from Eastern Europe and does not belong to the Sub-Saharan sub-species *S. nonagrioides botanephaga* Tams et Bowden. MOYAL *et al.* (2011) only included 2–4 Iranian individuals per gene from North Khuzestan (Hafttappeh and neighbouring areas). However, more individuals included in our study show no haplotype and nucleotide diversities in both CO1 and *Cyt-b* haplotype networks (see Fig. 1). According to the results of haplotype network analyses in the present study, mitochondrial genes of Iranian population were identical to population of East Africa (individuals from Central Ethiopia), providing evidence for another plausible scenario for the spread of *S. nonagrioides* in the northeast part of its range. According to this scenario, this species may have spread into Southwest Iran through the Trans-Arabian bridge (as it also distributed in Yemen) and spread towards the north (northwest) along the Persian Gulf and also towards Europe. These populations had a stronger (more natural) genetic polymorphism, which was gradually lost during the spreading process (or not survived the subsequent crossing with the newly introduced, much less polymorphic populations). Moreover, the supposed natural reduction of polymorphism may explain the reduced haplotype diversity of the southern European population. In this case, the European population may have originated either from such pioneer populations expanding to the northern Mediterranean from East Africa (Arabian Peninsula) or from West Africa following a forceps-like colonisation. However, this scenario of unique colonization of the Palaearctic region from East Africa as suggested recently by KERGOAT *et al.* (2015) requires more studies to be confirmed.

Larvae of *S. nonagrioides*, one of the most widespread noctuid stem borers, feed on many plants from different families such as Poaceae, Cyperaceae

and Typhaceae (DANIALI 1985, JEMSI & KAMALI 1992, HOLLOWAY 1998). Such plasticity in the feeding habit of this species has helped its expansion and more expansive species of the genus *Sesamia* occurring in subtropical and tropical Asia most probably had a similar spreading scenario with different routes towards the Himalayan region and the Pacific archipelago (LÁSZLÓ RONKAY, pers. comm.). The details of this expansion and the possible secondary and generally anthropogenic effects should be the aim of further studies and analyses.

Such genetic diversity found in this study may help explain the variation in pest status of the cereal stem borer *S. nonagrioides* observed across Iran. On the other hand, these results should be tested in other regions for the genome such as nuclear genes to increase our understanding of the genetic structure of this pest species in Iran.

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Appendix. List of analysed sequences of *Sesamia nonagrioides*, with site codes, host-plants, sampling sites collecting data and accession numbers.

Code	Seq. gene	Host-plant	Sampling site	Genbank Accession Nr.
NKHH1	COI	Sugarcane	Iran, Khuzestan, Hafttappeh, 70 m, 32° 05'N 48° 21'E, Aug. 2011	KT583674
SKhA38	COI	Sugarcane	Iran, Khuzestan, S Ahvaz, Amir Kabir agro-industry, 9 m, 31° 03'N 48° 18'E, Jun. 2007	KT583675
SKhA41	COI	Sugarcane	Iran, Khuzestan, S Ahvaz, Amir Kabir agro-industry, 9 m, 31° 03'N 48° 18'E, Jun. 2007	KT583676
WFAf3	COI	Rice	Iran, Fars, Firuzabad, Mahkuyeh, 1480 m, 29° 00'N 52° 33'E, Sept. 2011	KT583677
NKhH7	COI	Sugarcane	Iran, Khuzestan, Hafttappeh, 70 m, 32° 05'N 48° 21'E, Aug. 2011	KT583678
NKhH6	COI	Sugarcane	Iran, Khuzestan, Hafttappeh, 70 m, 32° 05'N 48° 21'E, Aug. 2011	KT583679
NKhH3	COI	Sugarcane	Iran, Khuzestan, Hafttappeh, 70 m, 32° 05'N 48° 21'E, Aug. 2011	KT583680
SKhA29	COI	Sugarcane	Iran, Khuzestan, S Ahvaz, Amir Kabir agro-industry, 9 m, 31° 03'N 48° 18'E, Jun. 2007	KT583681
NKhCH2	COI	Sugarcane	Iran, Khuzestan, Shoeibieh (Imam Khomeini agro-industry), 25 m, 31° 47'N 48° 43'E, Oct. 2013	KT583682
NKhCH3	COI	Sugarcane	Iran, Khuzestan, Shoeibieh (Imam Khomeini agro-industry), 25 m, 31° 47'N 48° 43'E, Oct. 2013	KT583683
NKhCH6	COI	Sugarcane	Iran, Khuzestan, Shoeibieh (Imam Khomeini agro-industry), 25 m, 31° 47'N 48° 43'E, Oct. 2013	KT583684
NKhH5	COI	Sugarcane	Iran, Khuzestan, Hafttappeh, 70 m, 32° 05'N 48° 21'E, Aug. 2011	KT583685
WFAf6	COI	Rice	Iran, Fars, Firuzabad, Mahkuyeh, 1480 m, 29° 00'N 52° 33'E, Sept. 2011	KT583686
NKhCH5	COI	Sugarcane	Iran, Khuzestan, Shoeibieh (Imam Khomeini agro-industry), 25 m, 31° 47'N 48° 43'E, Oct. 2013	KT583687
WFAf7	COI	Rice	Iran, Fars, Firuzabad, Mahkuyeh, 1480 m, 29° 00'N 52° 33'E, Sept. 2011	KT583688
SKhA28	COI	Sugarcane	Iran, Khuzestan, S Ahvaz, Amir Kabir agro-industry, 9 m, 31° 03'N 48° 18'E, Jun. 2007	KT583689
NKhH4	COI	Sugarcane	Iran, Khuzestan, Hafttappeh, 70 m, 32° 05'N 48° 21'E, Aug. 2011	KT583690
WFAf2	COI	Rice	Iran, Fars, Firuzabad, Mahkuyeh, 1480 m, 29° 00'N 52° 33'E, Sept. 2011	KT583691
SKhA27	COI	Sugarcane	Iran, Khuzestan, S Ahvaz, Amir Kabir agro-industry, 9 m, 31° 03'N 48° 18'E, Jun. 2007	KT583692
SKhA36	COI	Sugarcane	Iran, Khuzestan, S Ahvaz, Amir Kabir agro-industry, 9 m, 31° 03'N 48° 18'E, Jun. 2007	KT583693
SKhA39	COI	Sugarcane	Iran, Khuzestan, S Ahvaz, Amir Kabir agro-industry, 9 m, 31° 03'N 48° 18'E, Jun. 2007	KT583694
SKhA1	COI	Sugarcane	Iran, Khuzestan, S Ahvaz, Amir Kabir agro-industry, 9 m, 31° 03'N 48° 18'E, Jun. 2007	KT583695

Code	Seq. gene	Host-plant	Sampling site	Genbank Accession Nr.
NKhCH4	CO1	Sugarcane	Iran, Khuzestan, Shoebieh (Imam Khomeini agro-industry), 25 m, 31° 47'N 48° 43'E, Oct. 2013	KT583696
Hyn2	CO1	Unknown	France, Moyal et al. (2011)	JF274168
Hyn3	CO1	Unknown	France, Moyal et al. (2011)	JF274169
NF02Car	CO1	Unknown	France, Moyal et al. (2011)	JF274170
NF04Car	CO1	Unknown	France, Moyal et al. (2011)	JF274171
2063Pie	CO1	Unknown	France, Moyal et al. (2011)	JF274172
NE2Esp	CO1	Unknown	Spain, Moyal et al. (2011)	JF274173
Gr0321	CO1	Unknown	Greece, Moyal et al. (2011)	JF274174
Gr0342	CO1	Unknown	Greece, Moyal et al. (2011)	JF274175
T13Tur	CO1	Unknown	Turkey, Moyal et al. (2011)	JF274176
T81_3Awa	CO1	Unknown	Ethiopia, Moyal et al. (2011)	JF274177
1204_3Awa	CO1	Unknown	Ethiopia, Moyal et al. (2011)	JF274178
T81_2Awa	CO1	Unknown	Ethiopia, Moyal et al. (2011)	JF274179
0305_9Cham	CO1	Unknown	Ethiopia, Moyal et al. (2011)	JF274180
1204_32Awa	CO1	Unknown	Ethiopia, Moyal et al. (2011)	JF274181
1204_50Omo	CO1	Unknown	Ethiopia, Moyal et al. (2011)	JF274182
0305_10Omo	CO1	Unknown	Ethiopia, Moyal et al. (2011)	JF274183
1204_4Black	CO1	Unknown	Ethiopia, Moyal et al. (2011)	JF274184
1206_21Bah	CO1	Unknown	Ethiopia, Moyal et al. (2011)	JF274185
1206_22Bah	CO1	Unknown	Ethiopia, Moyal et al. (2011)	JF274186
1206_27And	CO1	Unknown	Ethiopia, Moyal et al. (2011)	JF274187
1206_28And	CO1	Unknown	Ethiopia, Moyal et al. (2011)	JF274188

Code	Seq. gene	Host-plant	Sampling site	Genbank Accession Nr.
2979Bogor	COI	Unknown	Kenya, Moyal et al. (2011)	JF274189
bog3	COI	Unknown	Kenya, Moyal et al. (2011)	JF274190
Bog2	COI	Unknown	Kenya, Moyal et al. (2011)	JF274191
N6_2Lwanda	COI	Unknown	Kenya, Moyal et al. (2011)	JF274193
N6_1Lwanda	COI	Unknown	Kenya, Moyal et al. (2011)	JF274194
N7_1Lwanda	COI	Unknown	Kenya, Moyal et al. (2011)	JF274195
N5_2Lwanda	COI	Unknown	Kenya, Moyal et al. (2011)	JF274196
N4_2Lwanda	COI	Unknown	Kenya, Moyal et al. (2011)	JF274197
N4_1Lwanda	COI	Unknown	Kenya, Moyal et al. (2011)	JF274198
1204_25Kisi2	COI	Unknown	Kenya, Moyal et al. (2011)	JF274199
1204_24Kisi2	COI	Unknown	Kenya, Moyal et al. (2011)	JF274200
1204_26Kisi2	COI	Unknown	Kenya, Moyal et al. (2011)	JF274201
0706_9Rwanda	COI	Unknown	Rwanda, Moyal et al. (2011)	JF274203
CI37	COI	Unknown	Ivory Coast, Moyal et al. (2011)	JF274204
CI39	COI	Unknown	Ivory Coast, Moyal et al. (2011)	JF274205
NKhCH2	Cyt-b	Sugarcane	Iran, Khuzestan, Shoeibieh (Imam Khomeini agro-industry), 25 m, 31° 47'N 48° 43'E, Oct. 2013	KT583697
NKhCH3	Cyt-b	Sugarcane	Iran, Khuzestan, Shoeibieh (Imam Khomeini agro-industry), 25 m, 31° 47'N 48° 43'E, Oct. 2013	KT583698
NKhCH5	Cyt-b	Sugarcane	Iran, Khuzestan, Shoeibieh (Imam Khomeini agro-industry), 25 m, 31° 47'N 48° 43'E, Oct. 2013	KT583699
NKhCH6	Cyt-b	Sugarcane	Iran, Khuzestan, Shoeibieh (Imam Khomeini agro-industry), 25 m, 31° 47'N 48° 43'E, Oct. 2013	KT583700
NKhH1	Cyt-b	Sugarcane	Iran, Khuzestan, Hafttappeh, 70 m, 32° 05'N 48° 21'E, Aug. 2011	KT583701
NKhH3	Cyt-b	Sugarcane	Iran, Khuzestan, Hafttappeh, 70 m, 32° 05'N 48° 21'E, Aug. 2011	KT583702
NKhH4	Cyt-b	Sugarcane	Iran, Khuzestan, Hafttappeh, 70 m, 32° 05'N 48° 21'E, Aug. 2011	KT583703
NKhH5	Cyt-b	Sugarcane	Iran, Khuzestan, Hafttappeh, 70 m, 32° 05'N 48° 21'E, Aug. 2011	KT583704

Code	Seq. gene	Host-plant	Sampling site	Genbank Accession Nr.
NKhH6	Cyt-b	Sugarcane	Iran, Khuzestan, Hafttappeh, 70 m, 32° 05'N 48° 21'E, Aug. 2011	KT583705
NKhH7	Cyt-b	Sugarcane	Iran, Khuzestan, Hafttappeh, 70 m, 32° 05'N 48° 21'E, Aug. 2011	KT583706
SKhA27	Cyt-b	Sugarcane	Iran, Khuzestan, S Ahvaz, Amir Kabir agro-industry, 9 m, 31° 03'N 48° 18'E, Jun. 2007	KT583707
SKhA28	Cyt-b	Sugarcane	Iran, Khuzestan, S Ahvaz, Amir Kabir agro-industry, 9 m, 31° 03'N 48° 18'E, Jun. 2007	KT583708
SKhA29	Cyt-b	Sugarcane	Iran, Khuzestan, S Ahvaz, Amir Kabir agro-industry, 9 m, 31° 03'N 48° 18'E, Jun. 2007	KT583709
SKhA36	Cyt-b	Sugarcane	Iran, Khuzestan, S Ahvaz, Amir Kabir agro-industry, 9 m, 31° 03'N 48° 18'E, Jun. 2007	KT583710
SKhA37	Cyt-b	Sugarcane	Iran, Khuzestan, S Ahvaz, Amir Kabir agro-industry, 9 m, 31° 03'N 48° 18'E, Jun. 2007	KT583711
SKhA38	Cyt-b	Sugarcane	Iran, Khuzestan, S Ahvaz, Amir Kabir agro-industry, 9 m, 31° 03'N 48° 18'E, Jun. 2007	KT583712
SKhA39	Cyt-b	Sugarcane	Iran, Khuzestan, S Ahvaz, Amir Kabir agro-industry, 9 m, 31° 03'N 48° 18'E, Jun. 2007	KT583713
SKhA40	Cyt-b	Sugarcane	Iran, Khuzestan, S Ahvaz, Amir Kabir agro-industry, 9 m, 31° 03'N 48° 18'E, Jun. 2007	KT583714
SKhA41	Cyt-b	Sugarcane	Iran, Khuzestan, S Ahvaz, Amir Kabir agro-industry, 9 m, 31° 03'N 48° 18'E, Jun. 2007	KT583715
WFAf2	Cyt-b	Rice	Iran, Fars, Firuzabad, Mahkuyeh, 1480 m, 29° 00'N 52° 33'E, Sept. 2011	KT583716
WFAf3	Cyt-b	Rice	Iran, Fars, Firuzabad, Mahkuyeh, 1480 m, 29° 00'N 52° 33'E, Sept. 2011	KT583717
WFAf6	Cyt-b	Rice	Iran, Fars, Firuzabad, Mahkuyeh, 1480 m, 29° 00'N 52° 33'E, Sept. 2011	KT583718
WFAf7	Cyt-b	Rice	Iran, Fars, Firuzabad, Mahkuyeh, 1480 m, 29° 00'N 52° 33'E, Sept. 2011	KT583719
G12_32_Awa	Cyt-b	Unknown	Ethiopia, Moyal et al. (2011)	JF274111
T81-3Awas	Cyt-b	Unknown	Ethiopia, Moyal et al. (2011)	JF274112
G12_31_Awa	Cyt-b	Unknown	Ethiopia, Moyal et al. (2011)	JF274113
G12_50_omo	Cyt-b	Unknown	Ethiopia, Moyal et al. (2011)	JF274114
G12_51_Omo	Cyt-b	Unknown	Ethiopia, Moyal et al. (2011)	JF274115
0305_10_Omol	Cyt-b	Unknown	Ethiopia, Moyal et al. (2011)	JF274116
T78-1D04Omo	Cyt-b	Unknown	Ethiopia, Moyal et al. (2011)	JF274117
0706_11_Rw	Cyt-b	Unknown	Rwanda, Moyal et al. (2011)	JF274118

Code	Seq. gene	Host-plant	Sampling site	Genbank Accession Nr.
G12_24_Kis	Cyt-b	Unknown	Kenya, Moyal et al. (2011)	JF274119
G12_25_Kis	Cyt-b	Unknown	Kenya, Moyal et al. (2011)	JF274120
G12_26_Kis	Cyt-b	Unknown	Kenya, Moyal et al. (2011)	JF274121
G16732	Cyt-b	Unknown	Kenya, Moyal et al. (2011)	JF274122
N4_1Lwanda	Cyt-b	Unknown	Kenya, Moyal et al. (2011)	JF274123
N4_2Lwanda	Cyt-b	Unknown	Kenya, Moyal et al. (2011)	JF274124
N5_1Lwanda	Cyt-b	Unknown	Kenya, Moyal et al. (2011)	JF274125
N5_2Lwanda	Cyt-b	Unknown	Kenya, Moyal et al. (2011)	JF274126
N6_1Lwanda	Cyt-b	Unknown	Kenya, Moyal et al. (2011)	JF274127
N7_1Lwanda	Cyt-b	Unknown	Kenya, Moyal et al. (2011)	JF274128
N6_2Lwanda	Cyt-b	Unknown	Kenya, Moyal et al. (2011)	JF274129
bog3	Cyt-b	Unknown	Kenya, Moyal et al. (2011)	JF274136
Bog1	Cyt-b	Unknown	Kenya, Moyal et al. (2011)	JF274137
Bog2	Cyt-b	Unknown	Kenya, Moyal et al. (2011)	JF274138
0706_9_Rw	Cyt-b	Unknown	Rwanda, Moyal et al. (2011)	JF274141
0706_10_Rw	Cyt-b	Unknown	Rwanda, Moyal et al. (2011)	JF274142
1206-1Anda	Cyt-b	Unknown	Ethiopia, Moyal et al. (2011)	JF274143
1206-2Andasa	Cyt-b	Unknown	Ethiopia, Moyal et al. (2011)	JF274144
1206-28Anda	Cyt-b	Unknown	Ethiopia, Moyal et al. (2011)	JF274145
1206-6Anda	Cyt-b	Unknown	Ethiopia, Moyal et al. (2011)	JF274146
1206-27Anda	Cyt-b	Unknown	Ethiopia, Moyal et al. (2011)	JF274147
1206-22Baha	Cyt-b	Unknown	Ethiopia, Moyal et al. (2011)	JF274148

Code	Seq. gene	Host-plant	Sampling site	Genbank Accession Nr.
0305_24_Kibo	Cyt-b	Unknown	Kenya, Moyal et al. (2011)	JF274159
CI13	Cyt-b	Unknown	Ivory Coast, Moyal et al. (2011)	JF274160
CI29	Cyt-b	Unknown	Ivory Coast, Moyal et al. (2011)	JF274161
CI45	Cyt-b	Unknown	Ivory Coast, Moyal et al. (2011)	JF274162
CI10	Cyt-b	Unknown	Ivory Coast, Moyal et al. (2011)	JF274163
CI11	Cyt-b	Unknown	Ivory Coast, Moyal et al. (2011)	JF274164
CI21	Cyt-b	Unknown	Ivory Coast, Moyal et al. (2011)	JF274165
CI33	Cyt-b	Unknown	Ivory Coast, Moyal et al. (2011)	JF274166
CI31	Cyt-b	Unknown	Ivory Coast, Moyal et al. (2011)	JF274167