Origin and taxonomic status of the Palearctic population of the stem borer *Sesamia nonagrioides* (Lefèbvre) (Lepidoptera: Noctuidae)

PASCAL MOYAL1*, PATRICE TOKRO2†, AHMET BAYRAM3, MATILDA SAVOPOULOU-SOUTLANI4, ERIC CONTI5, MATILDE EIZAGUIRRE6, BRUNO LE RÜ7, ARMAN AVAND-FAGHIH8, BRIGITTE FRÉROT9 and STEFANOS ANDREADIS4

1IRD – CNRS – LEGS – Avenue de la terrasse – BP 1 – 91198 Gif-sur-Yvette, Cedex, France
2Université de Cocody, Laboratoire de Zoologie et Biologie animale, Abidjan, Ivory Coast
3University of Dicle, Faculty of Agriculture, Plant Protection Department, 21280 Diyarbakır, Turkey
4Aristotle University of Thessaloniki, Faculty of Agriculture, Department of Plant Protection, Laboratory of Applied Zoology and Parasitology, 54124 Thessaloniki, Greece
5DSAA-Entomologia, Universita degli studi di Perugia, Borgo XX Giugno, 06121 Perugia, Italy
6Universitat de Lleida, Centre UdL-IRTA, 25198 Lleida, Spain
7ICIPE, PO Box 30772, Nairobi, Kenya
8Iranian Research Institute of Plant Protection, PO Box 1454, Tehran 19395, Iran
9INRA, UMR PISC, 1272 Route de Saint-Cyr, 78026 Versailles cedex, France

Received 8 December 2010; revised 3 February 2011; accepted for publication 5 February 2011

The major pest of maize in Mediterranean Europe, the stem borer *Sesamia nonagrioides* (Lefèbvre) (Lepidoptera: Noctuidae), has a fragmented distribution, north and south of the Sahara. The present study aimed: (1) to clarify the uncertain taxonomic status of the Palearctic and sub-Saharan populations which were first considered as different species and later on as subspecies (*Sesamia nonagrioides nonagrioides* and *Sesamia nonagrioides botanephaga*) and (2) to investigate the origin of the Palearctic population which extends from Spain to Iran, outside what is considered typical for this mainly tropical genus. We reconstructed the evolutionary history of both populations using one nuclear and two mitochondrial genes. The sub-Saharan taxon was fragmented in two isolated populations (West and East) whose mitochondrial genes were distant by 2.3%. The Palearctic population was included in the East African clade and its genes were close or identical to those of a population from Central Ethiopia, where the species was discovered for the first time. Similarly, in Africa, the alleles of the nuclear gene were distributed mainly in two West and East clades, whereas some Palearctic alleles belonged to the West clade. The Palearctic population originated therefore from East and West Africa and is the progeny of the cross between these two African populations. The main species concepts were in agreement, leading to the conclusion that the three populations are still conspecific. In the surveyed regions, the species therefore does not include two subspecies but three isolated populations. The Palearctic population suffered from severe bottlenecks that resulted in the fixation of one East African mitochondrial genome and the large reduction in its genetic diversity compared to the African populations. The data suggest that natural colonization of the Palearctic region was more plausible than human introduction. The allelic distribution of the Palearctic population was similar to that of species that survived the last glaciation. It is concluded that the African populations expanded during the last interglacial, crossed the Sahara and mixed in North Africa where fixation of the East mitochondrial genome occurred. The species then colonized Europe westward through only one eastern entrance. The coalescent-based estimate of the time to the ancestor of the Palearctic population was 108 000 years, which is consistent with this scenario. © 2011 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2011, 103, 904–922.

*Corresponding author. E-mail: pascal.moyal@ird.fr
†Patrice Tokro is deceased. This paper is dedicated to his memory.
INTRODUCTION

Sesamia nonagrioides (Lefèbvre) (Lepidoptera: Noctuidae), the major pest of maize in Mediterranean Europe, was discovered in 1824 in Sicily (Lefèbvre, 1827). Despite its economic importance, the taxonomic status of this species remains controversial. The genus Sesamia includes approximately fifty species that are distributed throughout the warm regions of the Old World, mainly in tropical Africa and Asia (Poole, 1989). Tams & Bowden (1953), who revised the African species and described several new ones, considered Sesamia nonagrioides to be limited to Europe and the Azores. Nye (1960), however, observed that S. nonagrioides was morphologically very close to one of the new sub-Saharan species they had described (i.e. Sesamia botanephaga) and indicated that they were to be regarded as two subspecies (i.e. Sesamia nonagrioides nonagrioides and Sesamia nonagrioides botanephaga) that were distributed north and south of the Sahara, respectively. The distribution area of the species was then fragmented, extending over Mediterranean Europe, Northwest Africa, and tropical and equatorial regions of West and East Africa (Commonwealth Institute of Entomology, 1979).

However, the respective distribution areas of both subspecies are not clearly defined at present. Indeed, the Palearctic population is not limited to Mediterranean Europe any more because the species has subsequently been recorded in Iran where it was nonetheless identified as S. n. botanephaga (Jemsi & Kamali, 1991; Lange et al., 2004). The Palearctic population therefore extends from Spain to Iran, and includes the two subspecies.

Furthermore, Holloway (1998), when reviewing the African pests of the genus, considered that this taxonomic status required further study, which suggests that both taxa might well be different species.

Not only the taxonomic status of this major agricultural pest is unclear, but also the origin of the Palearctic population has never been elucidated. Sesamia nonagrioides is the only species of the subtribe Sesamiina that is present in Europe, except for some rare temporary populations of the closely related species Sesamia cretica Lederer. During summer months, the latter species most likely immigrates from the dry East Mediterranean countries such as Egypt where it is a major pest (Moyal et al., 2002). Kunckel d’Herculais (1896, 1897), who discovered S. nonagrioides in North Africa, considered that it might have been introduced into Europe from Asia by Arab tradesmen during the 13th Century within stems of sugar cane. However, we know now that the species is not present in Asia, and, if introduced, it must have originated from Africa. Indeed, there are strong arguments supporting human introduction of S. nonagrioides. First, three molecular studies of the European population, using allozymes (Bues et al., 1996), restriction fragment length polymorphism (Margaritopoulos et al., 2007) and random amplified polymorphic DNA (RAPD) markers (De la Poza et al., 2008) concluded that the insect was sedentary and a poor flyer, with strong spatial genetic structure and limited genetic exchange between distant populations. Second, in sub-Saharan Africa, far from being a species of the northern dry savannas, the species is limited to southern wet localities. In East Africa, it is found mainly around lakes, ponds, and rivers (Nye, 1960), and it is not known in countries north of Kenya. In West Africa, it is a serious pest of maize only in the southern humid forest regions (Tams & Bowden, 1953; Pollet, Roon Van & Mauritz, 1978; Girling, 1980; Baudu et al., 2002). It therefore appears that it would have been difficult for a sedentary species, localized moreover mainly in humid regions in the southern parts of sub-Saharan Africa, to succeed in crossing the Sahara on its own.

Human introductions of various economically important stem borer species by way of plant material already occurred during previous centuries. Two of the major pests of cereals in North America were introduced in this way: the Hessian fly, Mayetiola destructor (Say) (Diptera: Cecidomyiidae) and the European corn borer, Ostrinia nubilalis (Hübner) (Lepidoptera: Crambidae) (Balachowsky & Mesnil, 1935).

However, there are also arguments that support natural expansion of the species. First, these famous introductions of stem borers took place between temperate countries, which may make the survival of the introduced insect easier; in addition, they resulted from major cereal transportation during wars that do not appear to have occurred between Africa and Europe, particularly before the 19th Century. Second, this insect, which is presently sedentary, has a very large geographic distribution throughout Mediterranean Europe up to Iran, North West Africa, and West and East sub-Saharan Africa. Such a vast distribution appears to be in contradiction to the sedentarity of the insect, which should be much more localized, as observed in several other species of the subtribe (Moyal et al., 2010, 2011a). This suggests that S. nonagrioides might be able to expand vastly on some
occasions (e.g. when environmental conditions become more humid). Desertification of the Sahara commenced at least 7 Mya (Schuster et al., 2006), raising a tremendous barrier to migration. However, there is evidence for periods of high humidity during which the desert turned into a well-vegetated savannah; for example, in the early Holocene (De Menocal et al., 2000) and during the last interglacial, the Eemian, between 0.130 Mya and 0.119 Mya (Hearty et al., 2007). Large range expansion of *S. nonagrioides* across the Sahara might have taken place during such very wet periods.

Both hypotheses (i.e. human introduction or natural colonization) therefore appear possible. In the present study, we attempt to solve the issues of the taxonomic status and spatio-temporal origin of the Palearctic population of *S. nonagrioides* by studying the evolution of one nuclear and two mitochondrial genes. These questions of delimiting species and unravelling the influence of dispersal on species genetic structure and speciation are central to systematics (Wiens, 2007) and evolutionary biology (Losos & Glor, 2003; Petit & Excoffier, 2009; Wilson et al., 2009). This species appears to be a particularly suitable model to address these questions. Clarifying its evolutionary history might also help to document the role of the Sahara in the speciation processes, which is poorly known (Guillaumet, Crochet & Pons, 2008).

**MATERIAL AND METHODS**

**INSECT SAMPLING**

Larvae of *S. nonagrioides* were collected from maize crops in Spain, France, Italy, Greece, Turkey, and Iran. In sub-Saharan West Africa, larvae were collected from sorghum crops in the Ivory Coast. In East Africa, the species is hardly ever found in crops, and so larvae were looked for in the stems of wild monocots in Kenya and Rwanda, where the insect had earlier been collected (Nye, 1960). During our surveys, we also searched for this species northwards of Kenya, in Ethiopia and Eritrea, where it was not previously recorded. Larvae were reared on an artificial diet (Onyango & Ochieng’Odero, 1994) until pupation and emergence of adults, the genitalia of which were dissected after a quick immersion in a boiling 10% potash bath to enable species identification.

The geographic coordinates of the localities in which the insects were collected are given in Table 1.

**DNA EXTRACTION, AMPLIFICATION, AND SEQUENCING**

Total DNA was extracted from the thorax or the first part of abdomen using a Qiagen DNeasy tissue kit. Fragments of three genes, two mitochondrial and one nuclear, were amplified: cytochrome *b* (*cyt* *b*) [949 nucleotides (nt)], cytochrome *c* oxidase, subunit 1 (CO1) (925 nt), and the gene coding for the pheromone binding protein 2 (PBP2) (685 nt). *Cyt* *b* was amplified using two primer pairs obtained from Simon et al. (1994) and Harry, Solignac & Lachaise (1998): CP1 (5’-GATGATGAAAAATTGTGGATC-3’) – CB3H (5’-AGCAAATAAAATATCCTAC-3’) and CB1 (5’-TAT GTA CCA CCA GGA CAA ATA TC-3’) – TRs (5’-TATCTTTATGTTTTCAAAAAG-3’). CO1 was amplified using the primers Ron (5’-GGATCACCTGATATAGCATTCCC-3’) and Hobbes (5’-AAATGTGNGRAAAAATGTATA-3’) (Monteiro & Pierce, 2001). The gene PBP2 was first identified in *S. nonagrioides* by de Santis et al. (2006). We designed two external (named PBP2E-SN) and two internal primers (PBP2I-SN) from the exon sequence: PB2E-SN-F (5’-CTGCACTGATCCGGCCATCATGTCG-3’), PB2E-SN-R (5’-CAAGACTTCTCCCACGATGAG-3’), PBP2I-SN-F (5’-TGTGCCCATACTGTCTCATCTAC-3’), PBP2I-SN-R (5’-CTTAAGGCTCTGGATCAGGGAGTC-3’). The external primers were used to amplify the whole gene and the internal ones for sequencing.

The polymerase chain reaction (PCR) profile for the mitochondrial genes was: 5 min at 92 °C; 35 cycles of 1 min at 92 °C, 1.3 min at 46 °C, and 1.3 min at 72 °C, followed by 5 min at 72 °C. For PBP2, the PCR profile was: 5 min at 94 °C; 40 cycles of 1 min at 94 °C, 1 min at 63 °C, and 2 min at 72 °C, followed by 5 min at 72 °C. The reaction mixture (50 μL) contained 3 mM MgCl2, 0.4 μM primers, 0.24 μM dNTPs, 2 U of Promega Taq polymerase, and 100 ng of DNA. The PCR product was purified by using a Qiagen QIAquick PCR purification kit. Sequencing reactions were carried out by using the Sanger dideoxy method (Sanger, Nicklen & Coulson, 1977), and sequences were run and detected on an ABI 377 automated sequencer: 186, 100, and 168 sequences were obtained for *cyt* *b*, CO1, and PBP2, respectively (Table 1). When heterozygotes could not be reliably identified as a combination of two known homozygotes, cloning of PBP2 was performed using an Invitrogen TA Cloning Kit Dual Promoter, with TOP 10 F’ chemically competent *Escherichia coli*.

Sequences have been deposited in GenBank under the accession numbers JF274085–JF274205.

**GENETIC DATA ANALYSIS**

**Testing for selection and recombination**

The neutral model of evolution is a prerequisite for correct interpretation of most of the analyses conducted in this study. Mitochondrial genes, which are mostly housekeeping genes, are generally considered in agreement with this model (Bachtrog et al., 2006),
although they were found to be under selection pressure in some species (Ballard & Rand, 2005). We therefore checked for evidence of selection by using the McDonald–Kreitman (MK) test (McDonald & Kreitman, 1991) implemented in DnaSP, version 4.5 (Rozas et al., 2003). This test is one of the most reliable for detecting selection and is independent of demographic events (Nielsen, 2001). Changes in synonymous and nonsynonymous substitutions were analyzed in comparison with the close species Sesamia calamistis Hampson. The PBP2 fragment sequenced was mostly made up of an intron, which, although not expected to undergo selection, might have been subjected to recombination events. We used two methods to trace them: the ZZ test (Rozas et al., 2001), which is little biased by homoplasy, implemented in DnaSP, and a method based on the comparison of phylogenetic trees suitable to detect mosaic sequences implemented in TOPALI (McGuire & Wright, 1998, 2000; Milne et al., 2004). Window sizes of 100 nt or 200 nt and a step size of 10 nt were used in the latter method.

### Phylogenetic trees and networks

Sequences were aligned using MULTALIN (Corpet, 1988). MODELTEST, version 3.7 (Posada & Crandall, 1998) was used in combination with PAUP 4.0b10 (Swofford, 1998) to select the best nucleotide substitution model using the Akaike information criterion (Posada & Buckley, 2004). Phylogenetic analysis was carried out using BEAST, version 1.4.8 (Drummond & Rambaut, 2007), which uses Markov chain Monte Carlo (MCMC) methods within a Bayesian framework to co-estimate genealogy and divergence times. A Yule process was used as tree prior. The diagnostic analysis of the MCMC output of BEAST was performed.

### Table 1. Localities in which the insects were collected and number of sequences per country

<table>
<thead>
<tr>
<th>Country</th>
<th>Locality</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Number of sequences</th>
<th>Cyt b</th>
<th>CO1</th>
<th>PBP2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spain</td>
<td>Lleida</td>
<td>41°37 N</td>
<td>00°35 E</td>
<td>6</td>
<td>7</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>France</td>
<td>Saint-Martin de Hinx</td>
<td>43°34 N</td>
<td>04°42 W</td>
<td>13</td>
<td>21</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Carcassonne</td>
<td>43°12 N</td>
<td>02°20 E</td>
<td>2</td>
<td>10</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pierrelatte</td>
<td>44°23 N</td>
<td>04°42 E</td>
<td>10</td>
<td>10</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Italy</td>
<td>Perugia</td>
<td>43°06 N</td>
<td>12°23 E</td>
<td>10</td>
<td>10</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Greece</td>
<td>Serres</td>
<td>41°05 N</td>
<td>23°33 E</td>
<td>12</td>
<td>12</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>Turkey</td>
<td>Adana</td>
<td>37°01 N</td>
<td>35°19 E</td>
<td>10</td>
<td>12</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Iran</td>
<td>Dezful</td>
<td>32°22 N</td>
<td>01°16 E</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Ethiopia</td>
<td>Black water</td>
<td>07°03 N</td>
<td>38°17 E</td>
<td>32</td>
<td>15</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Awasa</td>
<td>07°02 N</td>
<td>38°17 E</td>
<td>32</td>
<td>15</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Omolante</td>
<td>06°06 N</td>
<td>37°24 E</td>
<td>32</td>
<td>15</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Esbay Nuro</td>
<td>07°34 N</td>
<td>38°26 E</td>
<td>32</td>
<td>15</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chamoletto</td>
<td>05°33 N</td>
<td>37°19 E</td>
<td>32</td>
<td>15</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Andasa</td>
<td>11°18 N</td>
<td>37°17 E</td>
<td>32</td>
<td>15</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bahar Dar</td>
<td>11°22 N</td>
<td>37°14 E</td>
<td>32</td>
<td>15</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>Rwanda</td>
<td>Kitikinyoni</td>
<td>01°35 S</td>
<td>29°36 E</td>
<td>5</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Kenya</td>
<td>Aram</td>
<td>00°11 S</td>
<td>34°22 E</td>
<td>61</td>
<td>17</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bogoria</td>
<td>00°13 N</td>
<td>36°02 E</td>
<td>61</td>
<td>17</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Katunguma</td>
<td>02°27 S</td>
<td>37°59 E</td>
<td>61</td>
<td>17</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Kiboko</td>
<td>02°12 S</td>
<td>37°42 E</td>
<td>61</td>
<td>17</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Kimana</td>
<td>02°26 S</td>
<td>37°19 E</td>
<td>61</td>
<td>17</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Kilifi</td>
<td>03°05 S</td>
<td>40°05 E</td>
<td>61</td>
<td>17</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Muhaka</td>
<td>04°12 S</td>
<td>39°19 E</td>
<td>61</td>
<td>17</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mtito Andei</td>
<td>02°24 S</td>
<td>38°07 E</td>
<td>61</td>
<td>17</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Kwale</td>
<td>04°05 S</td>
<td>39°16 E</td>
<td>61</td>
<td>17</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Migori</td>
<td>00°33 S</td>
<td>34°19 E</td>
<td>61</td>
<td>17</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Nguruman</td>
<td>05°28 S</td>
<td>36°02 E</td>
<td>61</td>
<td>17</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Kisumu</td>
<td>00°06 S</td>
<td>34°19 E</td>
<td>61</td>
<td>17</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lwanda</td>
<td>00°29 S</td>
<td>34°18 E</td>
<td>61</td>
<td>17</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Ivory Coast</td>
<td>Abobo</td>
<td>05°19 N</td>
<td>04°01 W</td>
<td>35</td>
<td>2</td>
<td>22</td>
<td></td>
</tr>
</tbody>
</table>

Cyt b, cytochrome b; CO1, cytochrome c oxidase, subunit 1; PBP2, pheromone binding protein 2.
using TRACER, version 1.4 (Rambaut & Drummond, 2007). TREEANNOTATOR, version 1.4.8, was used to produce the summary information and the target tree with a 10% burn-in. The outgroup used was *S. calamistis*.

Haplotypic networks were constructed according to the statistical parsimony method of Templeton, Crandall & Sing (1992) with TCS, version 1.18 (Clement, Posada & Crandall, 2000). The probability of parsimony was set at 0.94 and 0.90 for PBP2 and the mitochondrial genes, respectively.

**Genetic diversity**

Haplotypic diversity (Nei, 1987), mean number of pairwise differences and nucleotide diversity (Tajima, 1983) were calculated using ARLEQUIN, version 3.1 (Excoffier, Laval & Schneider, 2005).

**Geographic distribution of alleles, gene flow, and expansion in the Palearctic**

The spatial analysis of molecular variance (SAMOVA) (Dupanloup, Schneider & Excoffier, 2002) implemented in SAMOVA software (available at http://cmpg.unibe.ch/software/samova) was used to identify the most likely geographic structure in the Palearctic region. Groups of populations were defined by using a simulated annealing approach. The most probable associations between populations were estimated through maximization of the $F_{CT}$ index, which is the proportion of total genetic variance as a result of differences between groups of populations (Excoffier, Smouse & Quattro, 1992).

The migrant number between populations per generation ($N_{m}$) was calculated sensu Slatkin (1991) using ARLEQUIN: one individual exchanged between two populations indicates a gene flow sufficient to prevent the fixation of neutral alleles (Slatkin, 1987).

Fuj’s $F_{ST}$ test (Fu, 1997) was used to check whether the European population was in demographic expansion, which leads to large negative values of $F_{ST}$.

**Dating the colonization of the Palearctic**

We estimated the time to the most recent common ancestor (tMRCA) of the Palearctic population using the coalescent-based method implemented in BEAST. The study was performed using the mitochondrial genes because they were the only ones to have diversified from their ancestors in Europe. Cyt $b$ and CO1 were concatenated. Previous studies suggested that the evolution rate of 1.15% per Myr for mitochondrial genes, common in insects (Brower, 1994), was likely in African noctuid stem borers because it explained most of their genetic fragmentations as a result of the impact of the major paleoclimatic events of the past million years (Moyal & Le Rü, 2006; Moyal et al., 2010). However, the annual number of generations of *S. nonagrioides* in Africa is six (P. Moyal, unpubl. data), whereas it is only three in Europe (Galichet, 1982) as a result of the winter diapause. Because we considered that the evolution rate was constant per generation (0.19% per million generations), we estimated it to be twice as slow per unit time in Europe compared to Africa. An expansion growth was used as tree prior because previous analyses indicated that the European population was (or had been in the recent past) growing. TRACER and TREEANNOTATOR were then used to analyze the results as described above.

**Morphological studies**

Tams & Bowden (1953) indicated two morphological differences between the Palearctic and sub-Saharan populations: a qualitative one (i.e. the presence of a flat cornutus on the vesica of the aedeagus only in the Palearctic taxon) and a quantitative one (i.e. the larger male genitalia in the sub-Saharan taxon). We examined 30 specimens from each region (Palearctic, East, and West Africa) to check these differences. The length of the genitalia (from saccus to uncus) and of their valva was measured and compared using the $F$-test.

**RESULTS**

In East Africa, *S. nonagrioides* was found only in the vicinity of wet areas and was collected from several wild host plants belonging to Poaceae, Cyperaceae and Typhaceae. Its main host plant was *Typha domingensis* (Typhaceae). This borer species was discovered for the first time in Ethiopia, up to the north of the country but was not found in Eritrea.

The gene for PBP2, sequenced in its entirety, included three exons and two introns. The fragment used included the end of exon 2 (30 nt) and most of intron 2 (655 nt). We found that intron 2 included a transposable element (MITE), a unique case among the *Sesamia* species that we have sequenced so far, which suggests that its insertion happened during the speciation process or shortly thereafter. This MITE, approximately 230 nt long, was found independently by Coates et al. (2009) in the course of sequencing the genome of the European corn borer, *O. nubilalis*. The phylogenetic analysis of PBP2 was carried out without the outgroup because discarding the MITE, whose evolution rate was a little quicker than that of the surrounding intron, resulted in the loss of many polymorphic sites.

**Selection and recombination**

No departure from the standard neutral model of molecular evolution was detected by the MK test either for cyt $b$ or CO1 or both concatenated.
genes (Fisher’s exact test $P$-values of 0.39, 0.14 and 0.42, respectively). No significant recombination was detected within PBP2 either by the ZZ test \[ ZZ = 0.0465, P(ZZ = 0.0465) = 0.906 \] or by the graphical method implemented in TOPALI.

**PHYLOGENETIC TREES AND NETWORKS**

The substitution models selected for the mitochondrial and nuclear genes were TrN with a proportion of invariable sites, $I = 0.82$, and a gamma distribution parameter, $\alpha = 2.78$, and Hasegawa–Kishino–Yano with $I = 0.61$ and $\alpha = 1.0$, respectively.

The phylogenetic analysis of the mitochondrial genes (Figs 1, 2, 3) revealed a first fragmentation that resulted in two clades genetically distant by 2.3%: the clade West, that included the individuals from Ivory Coast, and the clade East, which included the individuals from East Africa and the Palearctic. The clade East was subdivided into two sets: the first one including some specimens from Central Ethiopia and those from the Palearctic, and the other one the remainder of the East African individuals. All Palearctic individuals but one shared the same cyt $b$ sequence that was also found in some specimens from Central Ethiopia (Fig. 1). CO1 was more diversified than cyt $b$ in the Palearctic, with no haplotype in common with Central Ethiopia (Fig. 2).

The analysis of PBP2 revealed four Palearctic alleles and two main clades (Figs 4, 5). The first clade included most alleles from East Africa and one Palearctic allele (the latter one was connected to an allele from Central Ethiopia). This clade East, similar to the

---

**Figure 1.** Cytochrome $b$ network. The haplotype number is shown in bold; the number of individuals sharing the same haplotype when $> 2$ is shown in italics and parentheses. T, Turkey.
mitochondrial one, was well supported (posterior probability, \( PP = 0.99 \)). The second clade, which was not so well supported (\( PP = 0.81 \)), included four well-supported clades (\( PP = 1 \)). One of them, named clade West, included most alleles from West Africa and was therefore analogous to the mitochondrial clade West, except that it included also two alleles from the Palearctic population. The other three clades included (1) alleles from Central Ethiopia only (clade A), (2) alleles from East and West Africa (clade B), and (3) alleles from North Ethiopia and from the Palearctic (clade C) (Figs 4, 5).

**GENETIC DIVERSITY**

Genetic polymorphism was highly reduced in the Palearctic population in comparison with African populations (Table 2), except for nucleotide diversity and pairwise difference in PBP2, which were higher than in the Ivory Coast population. Both mitochondrial genes had similar diversity in Africa (not estimated for West Africa because only two sequences of CO1 were obtained) but CO1 was more diversified than \( \text{cyt } b \) in the Palearctic population.

**GEOGRAPHIC DISTRIBUTION OF ALLELES, GENE FLOW, AND EXPANSION IN THE PALEARCTIC**

The three major Palearctic haplotypes of CO1, h1–h3, were distributed over three regions (Fig. 6): West (Spain and France) (h1), Central (Italy, Greece, and Turkey) (h2), and East (Greece, Turkey, and Iran) (h3). However, one individual from Spain shared the central haplotype, h2, which was also closer to the
African haplotypes in the network (Fig. 2), suggesting that it might be ancestral. The SAMOVA (Table 3) showed a major fragmentation between populations east and west of the Alps. East of the Alps, where the distribution areas of the two main haplotypes h2 and h3 overlap, the SAMOVA separated only the Iran population, where the East allele h3 was the only one found. The Alps barrier was also indicated by the migrant number per generation (Table 4), which was high only within the West (Spain to France) and East (Italy to Iran) population groups.

Population expansion was detected only in populations west of the Alps (Fu's $F_{ST} = -3.69$, $P = 0.001$, for the set Spain to France).

The three major Palearctic PBP2 alleles were also distributed in three distinct regions: West, Central, and East (Fig. 7). The fourth Palearctic allele was found only in two heterozygotes, in Spain and in Italy. The central distribution area was different from that delimited by CO1, and stretched up to West France. In addition, the East allele extended up to East France, beyond the Alps. The West allele was limited to the

---

**Figure 3.** Cytochrome $b$ + cytochrome $c$ oxidase, subunit 1, Bayesian tree with posterior probabilities.
west of the Alps. The SAMOVA (Table 3) and the migrant number (Table 4) confirmed these observations. Both analyses indicated high gene flow between Italy and France, as well as between the regions east of the Alps. Eastern France, where the three distribution areas overlapped, was also the region with the highest heterozygote number (Fig. 7, Table 5).

**ESTIMATION OF THE TIME TO THE PALEARCTIC MRCA**

The tMRCA of the Palearctic population was estimated at 108 000 years (SE: ±1766) using coalescent-based analysis (Fig. 8). The inferred tree was divided into two main clades. The first one, well supported (PP = 0.94), included all the individuals west of the Alps except for three that were close to the African population in the network (haplotypes 2 and 5; Fig. 2). Its tMRCA was estimated at 37 000 years (SE: ±871). The other clade was little supported (PP = 0.38), and its tMRCA was estimated at 89 333 years (SE: ±1730). Nodes within the clades were little supported (PP < 0.01), except for the coalescence node of the individuals sharing the major East Palearctic haplotype, h3, which was well supported (PP = 0.99) with a tMRCA estimated at 18 545 years (SE: ±476).

**MORPHOLOGICAL DATA**

Our observations did not confirm the absence of a flat cornutus in the aedeagus of the sub-Saharan populations. We found it was present in all the specimens we observed. There was no significant difference between the genitalia sizes, their probability of being equal in the range 0.31–0.99.

**DISCUSSION**

**GEOGRAPHIC ORIGIN AND DIVERSITY OF THE PALEARCTIC POPULATION**

Our molecular study showed that *S. nonagrioides* was divided into two genetically isolated populations in sub-Saharan Africa, which is consistent with the known geographic distribution of the species. The
genetic fragmentation was evident from the mitochondrial data, as a result of the quick fixation of mitochondrial genes because of their low effective population size. The nuclear data also showed two similar geographic clades but did, however, reveal the presence of three other clades (clades A–C; Figs 4, 5). These three clades were fairly close in the network and also in the phylogeny for two of them but rather distant from the East and West clades. Moreover, one of them consisted of individuals from different African regions, suggesting that they might be the remnants of an ancestral polymorphism.

The geographically isolated Palearctic population was not genetically separated from the African populations. Its mitochondrial genes were very close or even identical to those of a population of Central

**Figure 5.** Pheromone binding protein 2 Bayesian tree with posterior probabilities. 1–25, allele number; European alleles are shown in bold.
Ethiopia, suggesting a recent origin from this region. However, the Palearctic alleles of PBP2 belonged not only to the clade East, but also to the clade West, providing evidence for the Palearctic population originating from West Africa as well.

Table 2. Diversity indices

<table>
<thead>
<tr>
<th>Gene</th>
<th>Region</th>
<th>(N)</th>
<th>(H)</th>
<th>(N_d \times 1000) (mean ± SE)</th>
<th>(H_d) (mean ± SE)</th>
<th>(PD) (mean ± SE)</th>
<th>(S)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyt b</td>
<td>Europe</td>
<td>54</td>
<td>2</td>
<td>0.04 ± 0.12</td>
<td>0.038 ± 0.036</td>
<td>0.04 ± 0.10</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>East Africa</td>
<td>100</td>
<td>50</td>
<td>9.06 ± 4.68</td>
<td>0.976 ± 0.006</td>
<td>8.55 ± 3.99</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>Ivory Coast</td>
<td>35</td>
<td>8</td>
<td>3.27 ± 1.93</td>
<td>0.750 ± 0.055</td>
<td>3.08 ± 1.64</td>
<td>13</td>
</tr>
<tr>
<td>CO1*</td>
<td>Europe</td>
<td>65</td>
<td>9</td>
<td>1.07 ± 0.81</td>
<td>0.711 ± 0.033</td>
<td>0.990 ± 0.68</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>East Africa</td>
<td>35</td>
<td>28</td>
<td>8.06 ± 4.29</td>
<td>0.987 ± 0.010</td>
<td>7.46 ± 3.57</td>
<td>49</td>
</tr>
<tr>
<td>PBP2</td>
<td>Europe</td>
<td>114</td>
<td>4</td>
<td>12.20 ± 6.30</td>
<td>0.652 ± 0.022</td>
<td>8.37 ± 3.90</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>East Africa</td>
<td>32</td>
<td>14</td>
<td>20.46 ± 10.48</td>
<td>0.934 ± 0.020</td>
<td>14.04 ± 6.47</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td>Ivory Coast</td>
<td>24</td>
<td>7</td>
<td>9.91 ± 5.40</td>
<td>0.823 ± 0.050</td>
<td>6.80 ± 3.32</td>
<td>24</td>
</tr>
</tbody>
</table>

*Not estimated for West Africa because only two sequences of CO1 were obtained.

\(N\), number of sequences; \(H\), number of haplotypes; \(N_d\), nucleotide diversity; \(H_d\), haplotype diversity; \(PD\), pairwise difference; \(S\), number of sites with substitutions.

Cyt b, cytochrome b; CO1, cytochrome c oxidase, subunit 1; PBP2, pheromone binding protein 2.

Figure 6. Spatial distribution of the three major cytochrome c oxidase, subunit 1, haplotypes in Europe.

The Palearctic population suffered from severe demographic bottlenecks, which resulted in highly reduced genetic diversity compared to the African populations and led to the fixation of a mitochondrial genome from Central Ethiopia. The higher
Table 3. Spatial analysis of molecular variance

<table>
<thead>
<tr>
<th>Number of groups</th>
<th>CO1</th>
<th>PBP2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Groups</td>
<td>$F_{CT}$</td>
<td>Groups</td>
</tr>
<tr>
<td>2</td>
<td>I F, Sp</td>
<td>0.59*</td>
<td>I F–SM, It</td>
</tr>
<tr>
<td></td>
<td>II It, Gr, Tur, Ir</td>
<td>0.21*</td>
<td>II F–C, F–P, Sp, Gr, Tur, Ir</td>
</tr>
<tr>
<td>3</td>
<td>I F, Sp</td>
<td>0.63*</td>
<td>I F–SM, It</td>
</tr>
<tr>
<td></td>
<td>II It, Gr, Tur</td>
<td>0.26*</td>
<td>II F–C, F–P, Sp</td>
</tr>
<tr>
<td></td>
<td>III Ir</td>
<td></td>
<td>III Gr, Tur, Ir</td>
</tr>
<tr>
<td>4</td>
<td>I F–SM</td>
<td>0.60*</td>
<td>I F–SM, It</td>
</tr>
<tr>
<td></td>
<td>II F–C, F–P, Sp</td>
<td>0.29*</td>
<td>II F–C, F–P</td>
</tr>
<tr>
<td></td>
<td>III It, Gr, Tur</td>
<td></td>
<td>III Sp</td>
</tr>
<tr>
<td></td>
<td>IV Ir</td>
<td></td>
<td>IV Gr, Tur, Ir</td>
</tr>
</tbody>
</table>

*Significant at 0.01 level.

$F_{CT}$: Proportion of genetic variance as a result of differences between groups of populations.

F, France; F–SM, France, Saint-Martin-de-Hinx; F–C, France, Carcassonne; F–P, France, Pierrelatte; Sp, Spain; It, Italy; Gr, Greece; Tur, Turkey; Ir, Iran.

Table 4. Number of migrants per generation between populations

<table>
<thead>
<tr>
<th></th>
<th>CO1</th>
<th>PBP2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>France</td>
<td>Spain</td>
<td>Italy</td>
</tr>
<tr>
<td>France</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Spain</td>
<td>*</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Italy</td>
<td>0.28</td>
<td>0.07</td>
<td>–</td>
</tr>
<tr>
<td>Greece</td>
<td>0.39</td>
<td>0.39</td>
<td>5.24</td>
</tr>
<tr>
<td>Turkey</td>
<td>0.35</td>
<td>0.33</td>
<td>2.10</td>
</tr>
<tr>
<td>Iran</td>
<td>0.17</td>
<td>0.06</td>
<td>0</td>
</tr>
</tbody>
</table>

*Infinite.

Cyt b, cytochrome b; CO1, cytochrome c oxidase, subunit 1; PBP2, pheromone binding protein 2.

effective population size for the nuclear gene enabled more genetic diversity to be preserved, providing us with evidence that the Palearctic population originated also from West Africa. The Palearctic population is thus an admixture of populations, which explains why its gene PBP2, despite the reduction in allele number following the bottlenecks, had nucleotide diversity and pairwise difference higher than that of the West African population. This is accounted for by the multiple geographic origins of the Palearctic individuals. The number of alleles was low, although their high genetic diversity resulted in high values of diversity indices. Mitochondrial genes are linked and, logically, their polymorphism was identical in Africa; however, in the Palearctic, where diversification was much lower, CO1 was more polymorphic than cyt b, showing the importance of using long sequences when studying recent diversification.

TIME OF BIRTH OF THE PALEARCTIC POPULATION

From the obtained results, it is possible to estimate which one among the two hypotheses mentioned above (i.e. human introduction into Europe or natural colonization) is the most plausible.

The Palearctic population has genetic characteristics that appear contradictory. The nuclear gene proved to originate both from West and East Africa, although only one mitochondrial genome, from Central Ethiopia, was found, which was then fixed after the admixture of both populations. Now, the distribution of the Palearctic alleles shows a clear partition into three populations (West, Central, and East), which suggests past isolation, and the species was found to be rather sedentary. Such results appear incompatible with the fixation of one mitochondrial genome throughout the Palearctic, which would have needed high gene flow and dispersal. This suggests that the admixture of
both populations occurred before colonization of the Palearctic, likely in North Africa because both populations are isolated in sub-Saharan Africa. Therefore, the hypothesis of a direct human introduction into Europe through transportation from sub-Saharan Africa does not appear plausible. The question divides then in two sub-questions: how did the species reach North Africa, and how did the species colonize Europe from North Africa.
The 'natural range expansion' hypothesis

The results are congruent with the hypothesis of a natural and relatively ancient colonization in several respects.

- The main mitochondrial and nuclear alleles were distributed over three different regions in the Palearctic: West, Central, and East. The Alps proved to be the major barrier as was already indicated by De la Poza et al. (2008) using RAPD markers. Our results showed a more precise partitioning in three sets similar to that of species that survived the last glaciation in the three refuges of southern Europe, the Iberian peninsula, southern Italy and southern Greece (Taberlet et al., 1998; Hewitt, 2004), and began to expand at the onset of the Holocene. The highest heterozygote diversity was observed where allele distribution areas overlapped, particularly in eastern France, which is also typical of postglacial colonizations from refuges (Petit et al., 2003). The PBP2 allelic partition as well as the SAMOVA analysis showed that the extant French population

Figure 8. European population genealogy inferred by coalescence. Below branches: posterior probability of main nodes. Above branches: first line, mean of node age in years followed by SEM in parentheses; second line, in square brackets: 95% highest posterior density interval. F, France; G, Greece; Ir, Iran; It, Italy; S, Spain; T, Turkey. The haplotype number is from the cytochrome c oxidase, subunit 1, network.
was an admixture of populations that had expanded from both Spain and east of the Alps. The absence of a typical French population may also be accounted for by the effect of the last glaciation. Indeed, the lethal temperature of diapausing larvae of *S. nonagrioides* is \(-5^\circ\text{C}\) with already high mortality at \(0^\circ\text{C}\) (Hassaine *et al*., 1992), and dramatic reductions in populations are observed during severe winters (e.g. up to 91% in southeastern France in 1981) (Galichet, 1982). Therefore, surviving the last glaciation in France was impossible for this species. Because the genetic distances between the European and African populations were low, the most likely hypothesis is that *S. nonagrioides* crossed the Sahara during the last interglacial, the Eemian, a particularly wet and hot period (Waelbroeck *et al*., 2002; Vermeersch, 2006). The discovery of *S. nonagrioides* in Ethiopia shows that it had colonized regions much more to the north than was known previously. Central Ethiopia, one of the origins of the Palearctic population, is close to the Nile, and a northern emigration along this river appears quite possible during a wet and hot period. In sub-Saharan West Africa, *S. nonagrioides* is distributed up to Senegal northwards, and the species might have reached North Africa along the Atlantic coast, which was most likely well vegetated during the Eemian.

- The coalescence analysis supported the hypothesis of the Eemian colonization, as it estimated the tMRCA of the Palearctic population at 108 000 years. This estimation was based on two prerequisites: the expansion of the Palearctic population and the evolution rate of 1.15% per Myr in Africa for the mitochondrial genes. Evidence for the expansion of the Palearctic population was provided by Fu’s *F*<sub>s</sub> test, which, nevertheless, showed a migration only to the west of the Alps. However, as noted above, the nature of the French population is evidence for an expansion also east of the Alps. The absence of significant *F*<sub>s</sub> east of the Alps may be attributed to the scarcity of sample locations that did not enable comparison of southern versus northern diversity, which was possible only west of the Alps, between Spain and France. However, Margaritiopoulos *et al*. (2007) showed that an expansion occurred also east of the Alps, and that northern populations were less diversified than southern ones. The evolution rate of 1.15% per Myr in Africa is hypothetical, although it is common in insects, and was deduced from the fact that it could explain the similar genetic distances between the infra-specific clades in many species of African Sesamiina by the influence of major paleo-climatic events. There is little doubt that such events, which have influenced most species in Africa (De Menocal, 1995), have also resulted in fragmentations or expansions in Sesamiina. The last major cold and dry period, 1 Mya, probably had a strong influence on species such as *S. nonagrioides* that were adapted to wet environments, and we suspect it to be the cause of the fragmentation between its sub-Saharan populations. The fact that using this rate led to a migration period precisely coincident with the most likely interglacial interval is a novel result that provides independent support for this estimate. In addition, the lower bound of the 95% highest posterior density interval of the tMRCA was 25 644 years, which is incompatible with a recent human introduction, and also the tMRCA of the main East recent haplotype was estimated at 18 545 years, just after the Last Glacial Maximum. All these estimates are consistent with the hypothesis of natural colonization during the Eemian.

- The genealogy inferred by coalescence grouped together some sequences from West Europe with those of eastern populations. These particular West haplotypes were close to the African ones on the CO1 network, indicating they were likely ancestral. This suggests that colonization of Europe probably occurred through only one entrance, in eastern Europe. Other results that support this conclusion are the estimated tMRCA of the East Palearctic clade, which was much older than that of the West clade. Furthermore, neither females nor males succeeded in crossing the Alps eastward during the (assumed postglacial) expansion, whereas males did so in the opposite direction, indicating an easier route of migration. According to this inference, females managed to cross the Alps during the Eemian colonization, and not during their postglacial expansion. If we assume that the expansion occurred mainly during the hot and wet period at the beginning of the Holocene, suitable climatic conditions prevailed for only 3000 years, perhaps too small a window of opportunity for females to cross the Alps. On the other hand, the Eemian, which lasted ten of thousands of years, was much more suitable for long-distance migrations. In addition, the hypothesis of the colonization during the Eemian would make an entrance through Spain unlikely because the Gibraltar Strait was particularly wide at that time (Patarnello, Volckaert & Castilho, 2007).

**The ‘human introduction’ hypothesis**

As discussed above, the direct human introduction of the species into Europe appears unlikely. The first required step would have been introduction into North Africa, which would have then occurred during
the past millennium. At least two introductions must have taken place, from West and East Africa. If transportation by sea appears possible from sub-Saharan West Africa, it is most unlikely from East Africa because it needed a long journey before the Suez canal was built. Another possibility could be transportation across the Sahara desert, which would have also lasted longer than one generation of the insect (approximately 40 days at 25 °C), in extremely dry and hot conditions particularly hard for a species of wet environments. Moreover, in East Africa, *S. nonagrioides* is hardly ever found in crops, and therefore it must have been introduced within its wild host plants, which appears even more difficult to imagine. Our conclusion, therefore, is that the first part of the voyage of *S. nonagrioides*, from sub-Saharan to North Africa, does not appear to have a human cause. If, however, we do consider this possibility, it implies that the North African populations had enough time to widely expand and mix sufficiently to enable the fixation of only one mitochondrial genome. It is questionable whether this was possible because environmental conditions were not favourable for the insect as this period, known in Europe as the little Ice Age, was fairly cold (Crowley, 2000). If we nevertheless consider this possibility, or a natural expansion during the Eemian, not extending beyond North Africa, the insect could have been introduced into Europe from North Africa. This introduction, despite the important trade between France and North-West Africa, would have occurred into several other countries but not into France because this would have likely led to a typical population such as those occurring in neighbouring countries. Also, this introduction, though during the little Ice Age, would have been followed by the expansion of the insects’ distribution area, the diversification of its mitochondrial genome, and the quasi-fixation of different alleles of the nuclear gene in different regions. Moreover, the time to the ancestor of the Palearctic mitochondrial genome should then be at most 500–1000 years if we consider that it was fixed in the North African population when introduced. Such an estimate would imply that the fragmentation between the two sub-Saharan populations distant by 2.3% had occurred 4000–8000 years ago, which appear quite unlikely. If we imagine that the Palearctic population evolved quicker than the African one, whose fragmentation would have occurred 1 Mya, the evolution rate of the Palearctic genome would then have been 100–200 times as fast as the African one, which would perhaps be possible for speciation genes but probably dubious for the mitochondrial genes, which are mostly housekeeping genes usually considered largely divorced from external selective pressures (Coyne & Orr, 2004).

Consequently, although human introduction may be considered a possible hypothesis, it requires many improbable scenarios and leads to estimates of evolution rates that do not appear plausible. By contrast, the hypothesis of natural colonization appear to be consistent with all the data.

**Taxonomic status**

By contrast to previous assumptions, the sub-Saharan sub-species is not homogeneous but comprises two populations that separated long before the birth of the Palearctic population. We have argued that their mitochondrial distance of 2.3% indicated a fragmentation approximately 1 Mya. The issue of the taxonomic status therefore has to be examined not only for the Palearctic population, but also the African populations because a mitochondrial distance of 2–3% indicates possible young species in other Lepidoptera (Hebert et al., 2003; Hajibabaei et al., 2006; Hebert, deWaard & Landry, 2010). The mitochondrial distance between the Palearctic population and the closest East African population was only 0.19%, which is much lower than these values; their nuclear alleles were distant by only 0.6%. Both populations are therefore genetically very close and cannot be considered as different species according to this criterion. The mitochondrial distance between the West and East sub-Saharan populations may suggest that they possibly are young species, although the present study shows that these two populations were still able to interbreed during the past 100 kyr. Indeed, the Palearctic population is the progeny of such crossing as can be inferred from the fact that it not only has a mitochondrial genome from East Africa and nuclear genomes from East or West Africa, but also it includes heterozygotes sharing alleles from both regions. According to the Biological Species Concept, all three populations are then conspecific. The Typological Species Concept leads to the same conclusion because there is no morphological difference between the Palearctic and African taxa, contrary to what was believed by Tams & Bowden (1953). The cornutus was found in all the studied specimens. The genitalia lengths were a little shorter in the Palearctic population, although not significantly. The difference in the size of the genitalia may prevent hybridization (Nagata et al., 2007) and therefore be an argument for two taxa being different species. Such a case was found in two close species of the genus *Sesamia* (i.e. *Sesamia poephaga* and *Sesamia epunctifera*), which are morphologically so similar that they are considered possible synonyms. The differences between these two species were significant and much higher than within *S. nonagrioides*; thus, the valva length difference between the populations of
S. nonagrioides was at most 7%, whereas it was 21% between S. poephaga and S. epunctifera (Moyal et al., 2011b). The differences within S. nonagrioides are then small, and, given the great environmental difference between the Palearctic region and sub-Saharan Africa, might be a mere consequence of phenotypic plasticity. Also, the Palearctic population should not be considered as a different species according to the Phylogenetic Species Concept because the Palearctic alleles are divided up among the African alleles.

Therefore, although the three populations of S. nonagrioides are allopatric, and will eventually become true species if they remain isolated for a sufficient length of time, they still belong to the same species according to the main species concepts. The distinction between two subspecies on each side of the Sahara is no longer valid, and the Iranian population does not belong to the sub-Saharan sub-species but is typically Palearctic and appears to have recently originated from a population of East Europe. Thus, S. nonagrioides is presently a species that has a large fragmented distribution and includes three isolated populations: the Palearctic and the East African and West African populations.

ACKNOWLEDGEMENTS

Financial support was provided by the Institut de Recherche pour le Développement (IRD) and laboratory facilities by the Centre National de la Recherche Scientifique (CNRS).

REFERENCES


Coates BS, Sumerford DV, Hellmich RL, Lewis LC. 2009. Repetitive genome elements in a European corn borer, Ostrinia nubilalis, bacterial artificial chromosome library were indicated by bacterial artificial chromosome end sequencing and development of sequence tag site markers: implications for lepidopteran genomic research. Genome 52: 57–67.


