



## Distribution and hybridization of *Culex pipiens* forms in Greece during the West Nile virus outbreak of 2010

Bruno Gomes<sup>a,b</sup>, Elias Kioulos<sup>c</sup>, Anna Papa<sup>d</sup>, António P.G. Almeida<sup>a,e</sup>, John Vontas<sup>f</sup>, João Pinto<sup>a,b,\*</sup>

<sup>a</sup> Unidade de Parasitologia Médica, Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa, Rua da Junqueira 100, 1349-008 Lisboa, Portugal

<sup>b</sup> Centro de Malária e outras Doenças Tropicais, Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa, Rua da Junqueira 100, 1349-008 Lisboa, Portugal

<sup>c</sup> Pesticide Science, Agricultural University of Athens, 11855 Athens, Greece

<sup>d</sup> Department of Microbiology, Medical School, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece

<sup>e</sup> Unidade de Parasitologia e Microbiologia Médicas, Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa, Rua da Junqueira 100, 1349-008 Lisboa, Portugal

<sup>f</sup> Department of Biology, University of Crete, Voutes, 71409 Heraklio, P.O.Box: 2208, Crete, Greece

### ARTICLE INFO

#### Article history:

Received 4 October 2012

Received in revised form 20 January 2013

Accepted 7 February 2013

Available online 1 March 2013

#### Keywords:

West Nile virus

Greece

*Culex pipiens*

Molestus

Migratory birds

### ABSTRACT

In 2010, an outbreak of West Nile virus (WNV) infections occurred in the region of Thessaloniki, Central Macedonia, in northern Greece. During this period, *Culex pipiens sensu stricto* mosquitoes were found infected by WNV lineage 2. *Cx. pipiens s.s.* presents two distinct biological forms, denoted molestus and pipiens. Hybrids between the two forms may potentiate the accidental transmission of WNV to humans. We have genetically characterized the form composition of *Cx. pipiens s.s.* samples collected during the outbreak from the region of Thessaloniki, where WNV cases occurred, and from the region Schinias-Marathonas, with no reported cases at the time. Information on bird fauna was also obtained for the two regions. Application of the CQ11FL diagnostic marker revealed a 350 bp variant of the pipiens-specific allele. Sympatric pipiens and molestus populations were detected in Thessaloniki, whereas Schinias-Marathonas presented a more genetically homogenous molestus population. A pattern of asymmetric introgression between molestus and pipiens was also observed in Thessaloniki. The presence of hybrids between molestus and pipiens forms suggests a greater receptivity of the Thessaloniki region for the establishment of WNV zoonotic cycles. However, the Schinias-Marathonas region also displayed characteristics to sustain WNV transmission cycles. These observations highlight the importance of maintaining active surveillance systems in selected regions geographically located within the range of major migratory bird flyways.

© 2013 Elsevier B.V. All rights reserved.

### 1. Introduction

*Culex pipiens* Linnaeus, 1758 *sensu stricto* is considered a main vector of West Nile virus (WNV) in Europe (Hubálek, 2008). This mosquito species comprises two distinct forms, denoted pipiens and molestus, which are morphologically indistinguishable but exhibit important behavioral and physiological differences. The molestus form is stenogamous (mates in confined spaces, *i.e.*, <0.1 m<sup>3</sup>; Clements, 1999), autogenous (can oviposit without a blood meal), homodynamic (remains active during winter) and mammophilic (prefers to feed on mammals, including humans). In contrast, the pipiens form is eurygamous (mates in open spaces), anaautogenous (oviposition requires a blood meal), heterodynamic (undergoes winter diapause) and ornithophilic (feeds predominantly on birds) (Harbach *et al.*, 1984, 1985; Vinogradova, 2000).

\* Corresponding author at: Universidade Nova de Lisboa, Unidade de Parasitologia Médica, Instituto de Higiene e Medicina Tropical, Rua da Junqueira 100, 1349-008 Lisboa, Portugal. Tel.: +351 213652666; fax: +351 213632105.

E-mail address: [jpinto@ihmt.unl.pt](mailto:jpinto@ihmt.unl.pt) (J. Pinto).

While in northern latitudes molestus and pipiens forms are physically separated by occupying underground and surface habitats, respectively, populations of both forms have been found at the surface in southern European regions (Chevillon *et al.*, 1995; Vinogradova, 2000; Gomes *et al.*, 2009). This sympatric occurrence in aboveground habitats promotes hybridization between forms (Fonseca *et al.*, 2004; Gomes *et al.*, 2009). Populations with intermediate behavior between the two forms have been described in southern Europe (Callot and Van Ty, 1943; Pasteur *et al.*, 1977; Gomes *et al.*, 2009). Hybrids are considered of great epidemiological importance as they may display a more opportunistic biting behavior. This behavior may potentiate the role of *Cx. pipiens s.s.* as a bridge-vector for the transmission of WNV from their avian amplification hosts to humans (Fonseca *et al.*, 2004; Hamer *et al.*, 2008).

Bird migrations have been associated with the spread of WNV. High infection rates in migratory birds have been described and this has been considered a possible cause for virus introduction in Europe and in North America (Rappole and Hubálek, 2003; Hubálek, 2004; Figuerola *et al.*, 2008). Bird migrations normally

follow a north–south axis, linking breeding regions (arctic and temperate) with non-breeding regions (temperate and tropical). Eight well-established migration routes (flyways) have been identified (Si et al., 2009). Of these, the Mediterranean/Black sea flyway is the largest bird migration system in the world, linking a vast area from Africa to west Siberia. The Bosphorus strait is the main entrance for African bird populations in Europe and it is the major migratory bottleneck of this flyway (Birdlife International, 2012). After the passage of the strait, migratory birds find their first European refuge in Greece and Bulgaria, where they rest and breed. In these locations, migratory birds may be bitten by local *Cx. pipiens* s.s. mosquitoes, or other WNV vectors (e.g. *Culex modestus*), which may lead to the establishment of local WNV transmission cycles.

In 2010, northern Greece experienced one of the largest WNV outbreaks described in Europe, with 262 human cases of WNV infection: 197 with neuroinvasive disease (encephalitis, meningitis, or acute flaccid paralysis) and 65 with West Nile fever; 35 (13.4%) cases were fatal (Danis et al., 2011). The outbreak was restricted to the north of the country and most human cases were observed in Central Macedonia, in wetland areas located between four major rivers, west to the city of Thessaloniki (Valiakos et al., 2011). Molecular analyses identified the WNV lineage 2 strain in birds, sentinel chickens and *Cx. pipiens* s.s. mosquitoes in this region (Papa et al., 2011a,b; Valiakos et al., 2011; Chaskopoulou et al., 2011). However, WNV epidemiologic studies conducted during this period have treated *Cx. pipiens* s.s. as a single entity, without determining the relative composition of molestus and pipiens forms and their relative impact in WNV transmission. Furthermore, it is still not fully understood why the outbreak was largely confined to the region of Central Macedonia in northern Greece.

In this study, Bayesian model-based clustering methods were applied to multi-locus microsatellite genotypes to infer the genetic structure of *Cx. pipiens* s.s. in Thessaloniki during the 2010 WNV outbreak and also in Schinias-Marathonas, southern Greece, a region without WNV transmission. Information on the wild avian fauna was also collected for each region, with particular attention to trans-Saharan migratory birds and species for which WNV infection has been reported in previous studies in Europe and North America. Our objectives were: (i) to assess differences in the molestus/pipiens form composition of *Cx. pipiens* s.s., as well as in hybrid frequency, between northern and southern Greece; (ii) to compare the distribution of migratory bird species and/or species with previous records of WNV infection between the two regions; (iii) to determine if differences in both *Cx. pipiens* s.s. form composition and avian fauna could be consistent with a higher receptivity of northern Greece (Thessaloniki) for the establishment of a WNV transmission cycle when compared to the region of Schinias-Marathonas in southern Greece.

## 2. Material and methods

### 2.1. Study regions and mosquito collections

Mosquito collections in the region of Thessaloniki (northern Greece) were performed between 20th August and 15th September 2010 by CDC light traps baited with CO<sub>2</sub> (Sudia and Chamberlain, 1962). Traps were hung outdoors at ca. 1.5 m height and approximately 20 m away from human dwellings. Sampling was carried out in the villages of Chalastra, Anatoliko, Kimina, Malgara, Adendro, Brachia, Vathilakos, Eleousa and Nea Xalkidona, located ca. 10–15 km west of Thessaloniki city (Fig. 1). These villages lie in the region where most human cases of WNV were reported during the outbreak and also where both mosquitoes and avian hosts were found infected (Papa et al., 2011b; Valiakos et al., 2011). This region has a warm temperate climate with hot dry summers and mild winters (class Csa, Köppen Classification System; Peel et al.,

2007). The villages are located close to the delta of rivers Axios and Aliakmonas. Irrigation channels derived from these rivers feed ca. 20,000 hectares of rice fields, the main crop in the area, and provide suitable breeding sites for mosquito larvae. Additional breeding sites such as open sewages and cesspits are found in or close to the villages. A population of ca. 70 wild horses is present in an isolated part of the river delta. Cattle, sheep and domestic birds are common in most villages of the region and domestic horses are found in several horse-riding schools.

Mosquito collections in Schinias-Marathonas region (southern Greece) were performed in the same time period as in Thessaloniki. Two collection methods were used. CDC light traps baited with CO<sub>2</sub> were placed outdoors at an average height of 1.5 m and approximately 20 m away from human dwellings in the wetland area of Marathonas-Schinias. Collections of resting mosquitoes were performed with mouth aspirators inside and around houses at the village of Marathonas (Fig. 1). The region has the same warm temperate climate with hot dry summers and mild winters as in Thessaloniki (class Csa; Peel et al., 2007). A large number of annual vegetable crops and greenhouses are cultivated in the area. The extensive wetland of Schinias-Marathonas, as well as neighboring streams and cesspits in villages constitute the major mosquito breeding sites of the area. No cases of WNV infection during 2010 were reported in the Schinias-Marathonas area, as well as in the whole district of Attiki, to which the area belongs. Sheep, chickens and a few horses are the main domestic animals found in this region.

### 2.2. Migratory birds and WNV avian hosts

Information on bird species diversity for each study region was obtained from the OrnithoTopos database, hosted at the Hellenic Ornithological Society website (OrnithoTopos, 2012). This database displays bird species observation records from birdwatchers, organized by geo-referenced locations. For each species recorded, the maximum number of individuals counted at a single observation is also given. Information from location summary reports, between January 2008 and December 2010, was collected from three locations in Thessaloniki (Axios, Loudias and Aliakmon estuaries) and two locations in Schinias-Marathonas (Schinias and Schinias marsh). Species without information on maximum bird count (i.e., corresponding to non-visual records) were excluded from the analysis. Recorded species from each region were classified as trans-Saharan migratory birds according to the list of Walther (2005). Information about records of WNV infection in bird populations of Europe and North America was also obtained for each species based on published reports (Figueroa et al., 2007, 2008, 2009; Formosinho et al., 2006; Hubálek, 2004, 2008; Jourdain et al., 2008; Malkinson et al., 2002; Rappole and Hubálek 2003; Valiakos et al., 2012).

### 2.3. Molecular analysis

DNA extraction from individual females was performed using the DNAzol method (Invitrogen). Each specimen was identified to species by multiplex PCR assay targeting species-specific polymorphisms at the intron-2 of the *acetylcholinesterase-2* (*ace-2*) gene using primers specific for *Cx. pipiens* s.s., *Cx. quinquefasciatus* and *Cx. torrentium* (Smith and Fonseca, 2004).

Fourteen microsatellites (Fonseca et al., 1998; Keyghobadi et al., 2004; Smith et al., 2005) were analyzed following the procedures described in Gomes et al. (2009). Amplified products were separated by capillary electrophoresis in a genetic analyzer ABI3730 (Applied Biosystems) at Yale DNA Analysis Facility (USA). Fragment sizes were scored using the software GeneMarker 1.4. (Softgenetics, USA).

Download English Version:

<https://daneshyari.com/en/article/5910566>

Download Persian Version:

<https://daneshyari.com/article/5910566>

[Daneshyari.com](https://daneshyari.com)