May climate change allow the spread of the Asian tiger mosquito, vector of viruses, in central Europe?

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Abstract

Aedes albopictus, also known as the Asian tiger mosquito, is a native species of tropical and subtropical areas of Southeast Asia, recently spread also to Europe. *Ae. albopictus* represents a serious threat for human health because it is an important vector of many viruses. The geographical distribution of the mosquito's populations is limited by cold climate. However, according to projections, due to the climate change the species could spread in many regions where it is now not established. The key factor that allowed *Ae. albopictus* to colonize new territories in higher latitudes may be the production of cold-tolerant diapausing eggs. The aim of this study is to determine the parameters for the production of diapausing eggs by *Ae. albopictus* and for the eggs hatching. According to our data, the diapausing eggs are induced by a 11 hours of light and 13 of dark photoperiod that, in central Italy, is reached during the middle of October. Thank to this mechanism, the mosquito can avoid the exposition to low temperature of the most susceptible stage of development, the first instar larva, that cannot survive under 10°C. The results are discussed in relation to the scenario of the global warming.

Introduction

Aedes albopictus (Skuse, 1894) (Diptera Culicidae), also known as the Asian tiger mosquito, is a native species of tropical and subtropical areas of Southeast Asia (Caminade et al. 2012). In the past few decades, this species has spread to many areas of North, Central and South America, Africa, Australasia and Europe (Gubler 2003). *Ae. albopictus* represents a serious threat for human health because its typical daytime flight and blood-feeding and because it is a vector of many pathogens, including the yellow fever, Dengue, Zika and Chikungunya viruses (Hochedez et al. 2006, Bedini et al. 2018). In the only first six months of 2019, in Italy, 46 cases of Dengue, 11 cases of Chikungunya and 2 of Zika, have been reported (Italian National Institute of Health 2019).

Due to its climatic suitability, the range of *Ae. albopictus*' populations is limited by cold climate and to date, in Europe, it is well established only in the southern regions (Medlock et al. 2012). However, projections by modelling methodologies indicate that due to the climate change, in the near future, the species will spread in many regions where it is not established yet. Regions currently characterized by a rather low or moderate suitability have the potential for invasion by mid-century, due to the raise of temperature caused by the global warming. In particular, according to studies at the continental scale, it can be inferred that, within the next fifty years, suitable climatic conditions will be achieved in western Europe (Belgium, France, Luxembourg and the Netherlands), central Europe (Germany) and southern United Kingdom. Furthermore, the climatic projections indicate that, while suitability for *Ae. albopictus* will continue to be high in southern France and most of Italy, it will decrease in the western Mediterranean coast of Spain, as a consequence of the increased dry conditions during Summer (Caminade et al. 2012).
According to several studies, the key factor that allows *Ae. albopictus* to colonize new territories and to establish in higher latitudes (Mitchell 1995) is the production of cold and dry-tolerant diapausing eggs (Toma et al. 2003).
The aim of this study is to determine the parameters for the production of diapausing eggs by *Ae. albopictus* and those for the eggs hatching. The results are discussed in relation to the scenario of the global warming expected by climate changes previsions.

Materials and methods
To collect the eggs of *Aedes albopictus*, 20 plastic black pots were put in a garden (about 3000 m^2) of the entomology laboratory at the Department of Agriculture, Food and Environment, University of Pisa (Italy). The experiments were conducted from May 2017 to March 2018. To allow the wild females to lay the eggs, 4 Masonite sticks were put in each pot. The Masonite sticks with the eggs on the surface were then collected and kept under laboratory conditions (natural photoperiod, 23.0 ± 2.0°C).

Duration of preimaginal stages. For the first experiment, conducted in June, 10 sticks of Masonite with 10-20 eggs/stick (0-24 h) collected as above reported were kept in laboratory conditions (natural photoperiod, 23.0 ± 2.0°C) in 50 mL glass tubes each, at the surface of the water, to allow the eggs' hatching. Each of the newly emerged larvae was then transferred in a 50 mL glass tube with water and a small amount of cat food. The hatching of the eggs and the different preimaginal stages' moults were recorded to evaluate the duration of the different instars and the duration of the complete ontogenetic cycle.

Influence of photoperiod on the production of diapausing eggs. For the second experiment, Masonite sticks with the eggs were collected in alternate days from June to October and kept singularly in laboratory in 50 mL glass tubes, at the surface of the water, until hatching. The exact time of hatching and the number of larvae obtained were recorded.

Influence of photoperiod on the hatchability of eggs. For the last experiment, Masonite sticks with the eggs were collected in alternate days from June to October and kept in laboratory for 150 days in dry conditions (R.H. ≅ 60%). After the dry period, the Masonite sticks were submerged in water allowing the hatching and the number of the newly emerged larvae was recorded.

Results and Discussion

The embryonic duration of the non-diapausing eggs and the duration of the preimaginal instars of *Aedes albopictus*, under laboratory conditions, is shown in Tab. 1. The embryonic development required about 6 days. We observed four larval stages for a total of about seven days of growth. Pupation took place in about four days after the fourth larval's age. The complete cycle, therefore, lasted about sixteen days. The observed times of development are in line with the ones reported by Delatte et al. (2009) who obtained, in controlled conditions, an optimum intrinsic rate of larval growth of *Ae. albopictus* between 25 and 30°C, with the shortest period for immature development at 30°C, corresponding to 8.8 days. In line with our results, Delatte et al. (2009) reported, a duration of the complete life cycle of *Ae. albopictus* of 10.4 ± 0.7 days. The longer time of full development observed in this experiment, comparing with the one of Delatte et al. (2009), should be due to the different temperature at which the two experiments have been conducted (25 vs 23°C).

The hatching percentage of our eggs, let in the water, showed a large variability with significant differences in relation to the month of oviposition ($F = 61.177$; df = 4; $P < 0.001$). The percentage of hatching, if the eggs are maintained in water after the oviposition, varied from 84% in June to 3% in October (Tab. 2). In particular, eggs laid in June showed the highest percentage of hatching, while those laid in October the lowest one (Tab. 2). These differences indicate that summer eggs are not diapausing, while the very low percentage of hatching of the ones laid in October suggests that those are diapausing eggs. Such observation was confirmed by the behaviour of the overwintering eggs. After 150 days, significant differences were observed in the hatching of the eggs ($F = 61.918$; df = 3; $P < 0.001$). In fact, the percentages of eggs' hatching varied from to 15 to 76% for the eggs laid, respectively, in July and October. In particular, the post-hoc test showed that the eggs laid in October had a significant higher percentage of hatching than the ones laid during Summer (Tab. 3).

The production of diapausing eggs has been reported as one of the main adaptations of *Ae. albopictus* responsible for the huge spread of the species, because it allows overwintering in temperate regions (Hawley 1988). According to our data, the production of diapausing eggs is induced by a photoperiod of 11 hours of light and 13 hours of dark that, in central Italy, is reached during the middle of October. By the production of diapausing eggs, the mosquito can avoid the exposition to the winter low temperatures of the most susceptible stage of development, represented by the first instar (Delatte et al. 2009) In fact, temperature is one of the main determinants of the *Ae. albopictus* mosquitoes' survival that limits their geographical distribution, together with photoperiod (Brady et al. 2013). The minimal threshold for first instar was found at 10.4 °C (Delatte et al. 2009). In our laboratory, diapausing eggs (laid in October) start hatching 125 days after the oviposition, so at the end of February, when the photoperiod in central Italy corresponds to 10 hours of light and 14 hours of dark.

In Europe, current temperatures allowed the species to settle in the southern coast of Spain, France, Greece and in the whole countries of Italy, Slovenia, Croatia, Albania and Montenegro. Global warming, despite the effort of reducing the raise of temperature to maximum 2 °C by many industrial country governments during the summit of Paris 2015 (King and Karoly 2017, Dosio and Fischer 2018), will enable the species to establish in countries where, at the moment, it is not able to survive because of the cold climate. In most of Switzerland, Belgium, Luxembourg, Netherlands, Czech Republic, South United Kingdom and Central France and Germany, the photoperiod suitable for the hatching is reached when the temperatures are too low. However, the raising of few degrees will be enough for allowing *Ae. albopictus*' development and spread. For example, a photoperiod of 10 hours light and 14 hours dark is reached in Brussels in mid-February, where the daily medium temperature is 9 °C. Depending of the level of greenhouse gasses emission, by the end of the century (2071-2100), the temperature over Europe is expected to increase between 0.3-1.7 °C for the lowest emissions scenario (lowest Representative Concentration Pathway, RCP 2.6) and between 2.6-4.8 °C for the highest emissions scenario (RCP 8.5) (IPCC 2013). According to this data, in fifty years the medium temperature in Brussels could reach the minimal threshold of 10.4 °C, required by the species for its development.

Since *Ae. albopictus* is the primary vector of many pathogenic viruses, its spread as a consequence of global warming may represent a serious health threat for humans. The increasingly tourism from and to countries in which the viruses are endemic and the increasing migration of people in Europe will provide the reservoir for diseases that may be spread by *Ae. albopictus* to the South and Central European countries. In this scenario, surveillance and control are essential for limiting the density and geographical spread of these mosquitoes, to minimize the risk of a viraemic person coming in contact with them and the outbreak of serious infective diseases.

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Tables

Table 1. Duration of preimaginal stages of *Aedes albopictus* under laboratory conditions.

Stage	Hatching	L1	L2	L3	L4	Pupae	Total
Time[a]	5.6 ± 1.3	1.9 ± 0.7	1.6 ± 0.8	1.6 ± 0.8	2.1 ± 1.0	3.5 ± 1.0	16.3 ± 2.2

[a], days. Data are expressed as mean ± standard error.

Table 2. Percentage of hatching of eggs of *Aedes albopictus* laid in different period of the Summer and Autumn.

Month of spawning	N. of eggs laid	N. of hatched eggs	% hatching
June	179.50 ± 21.04	152.00 ± 18.06	83.73 ± 3.14a
July	314.25 ± 5.27	206.00 ± 10.50	64.73 ± 2.85b
August	286.00 ± 25.47	198.50 ± 37.76	69.77 ± 7.5ab
September	567.00 ± 12.89	335.75 ± 22.27	59.74 ± 2.84b
October	741.75 ± 38.26	24.75 ± 2.72	3.38 ± 0.33c

Table 3. Percentage of hatching of eggs of *Aedes albopictus* laid in different period of the Summer and Autumn after dry treatment.

Month of spawning	N. of eggs laid	N. of eggs hatched	% hatching
July	796.50 ± 75.99	127.50 ± 11.15	15.17 ± 1.53a
August	812.75 ± 66.18	130.75 ± 28.10	16.82 ± 3.20a
September	568.50 ± 23.06	90.25 ± 7.49	15.37 ± 1.13a
October	509.50 ± 45.34	406.25 ± 53.38	75.68 ± 6.51b