High Resolution Spatial Analysis of Habitat Preference of *Aedes Albopictus* (Diptera: Culicidae) in an Urban Environment

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ABSTRACT Over the past decades, the Asian tiger mosquito (*Aedes albopictus* (Skuse, 1895)) has emerged in many countries, and it has colonized new environments, including urban areas. The species is a nuisance and a potential vector of several human pathogens, and a better understanding of the habitat preferences of the species is needed for help in successful prevention and control. So far, the habitat preference in urban environments has not been studied in Southern European cities. In this paper, spatial statistical models were used to evaluate the relationship between egg abundances and land cover types on the campus of Sapienza University in Rome, which is taken as an example of a European urban habitat. Predictor variables included land cover types, classified in detail on a high resolution image, as well as solar radiation and month of capture. The models account for repeated measures in the same trap and are adjusted for meteorological circumstances. Vegetation and solar radiation were found to be positively related to the number of eggs. More specifically, trees were positively related to the number of eggs and the relationship with grass was negative. These findings are consistent with the species' known preference for shaded areas. The unexpected positive relationship with solar radiation is amply discussed in the paper. This study represents a first step toward a better understanding of the spatial distribution of *Ae. albopictus* in urban environments.

KEY WORDS Asian tiger mosquito, urban habitat preference, ovitrap, spatial analysis, vector-borne disease

The mosquito *Aedes albopictus* (Skuse, 1895), commonly known as the "Asian tiger mosquito," originated in Southeast Asia, but has spread in temperate Asia, Europe, North America, as well as in South Africa and in several locations in the Pacific and Indian Ocean regions (Knudsen 1995, Benedict et al. 2007, Paupy et al. 2009). The geographical spread of *Ae. albopictus* has mostly occurred during the past few decades, largely through the international trade of used tires (Reiter and Sprenger 1987, Lounibos 2002, Tatem et al. 2006), but also via transport of Lucky Bamboo plants from China (Madon et al. 2002). In Europe, *Ae. albopictus* was reported for the first time in Albania in 1979 (Adhami and Murati 1987) and in Italy in 1990, in Genoa, and from there it has gradually spread to several Italian regions (Sabatini et al. 1990, Dalla Pozza and Majori 1992, Della Torre et al. 1992, Romi and Majori 2008). In Rome, *Ae. albopictus* was detected in 1997 (Romi et al. 1999), and this led to the first example of complete colonization of an urban area in Italy (Di Luca et al. 2001, Toma et al. 2003, Severini et al. 2008).

The efficient spread of *Ae. albopictus* to urban areas in temperate regions is associated to two major characteristics of this species: the capacity to produce hibernating eggs (Hawley 1988) and the ability to shift from natural breeding sites (e.g., bamboo stumps, tree holes) to anthropogenic ones (e.g., manholes, water storage containers, used tires, flower pots, cemetery urns; Hawley 1988, Knudsen 1995, Tsuda et al. 2006, Vazeille et al. 2008, Paupy et al. 2009).

Ae. albopictus is a vector for many arboviral infections, including dengue and chikungunya (Knudsen 1995, Gratz 2004, Angelini et al. 2007, Rezza et al. 2007, Thenmozhi et al. 2007, de Lamballerie et al. 2008) and was responsible for a large chikungunya epidemics in the Indian Ocean in 2005–2006 (Enserink 2006), for a small outbreak in Italy (Angelini et al. 2007, Rezza et al. 2007), and for two human cases of dengue in South of France in 2010 (La Ruche et al. 2010). These latest cases in Europe highlight the potential risk of arbovirus outbreaks in the European cities where the species is now well established. Habitats of

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Ae. albopictus in urban environment have been investigated in the United States (Barker et al. 2003a,b, Braks et al. 2003; Bartlett-Healy et al. 2012, Unlu et al. 2013), South America (Braks et al. 2003), and Asia (Gilotra et al. 1967). However, there is only little information on the habitat preferences of *Ae. albopictus* in urban areas in Europe. Knowledge on the abundance of *Ae. albopictus* (Cianci et al. 2013) and on its spatial distribution may help in designing control measures, such as removing favourable breeding sites or making favourable habitats less attractive or accessible. Also, identifying areas of higher *Ae. albopictus* abundance is important for constructing risk maps that indicate the risk for an outbreak after an introduction (Hartemink et al. 2009, 2011). Although vector presence and abundance are not the only factors determining whether or not a pathogen can spread in an area, determining the distribution of the vector is an essential step in studying the risk of transmission of a pathogen.

In this paper, we model the relationship between the abundance of eggs and different types of land cover, solar radiation, and the month of capture, adjusting for the repeated measures in the same trap and the meteorological circumstances. Data were collected in a small area within Rome, the campus of Sapienza University, which is taken as an example of a European urban habitat. This paper aims at improving the understanding of habitat preferences of *Ae. albopictus* in urban areas, in order to help in the prevention and control of diseases that this mosquito could spread.

Materials and Methods

Study Area. The campus of Sapienza University in Rome is situated in an urbanized area in the center of Rome (Supp Fig. 1 [online only]) and is mainly characterized by buildings, green areas with grass, trees, bushes and hedges, a botanical garden, car parks, and roads. With its mixture of built-up areas and vegetation, the campus may be considered as representative of many areas of the city. A high resolution classification of land cover was produced by hand digitization of an aerial picture ("Rome, Sapienza." 41° 54′10.51″ N and 12° 30′53.24″ E. Google Earth. July 2007; 25 April 2012, Supp Fig. 2 [online only]) in Quantum GIS (QGIS Development Team 2012; Fig. 1).

Data Collection. The number of eggs was monitored weekly using ovitraps, starting on the 8th of July until the 21st of October 2011. Fifty-five ovitraps were located at ground level in shaded sites (e.g., under

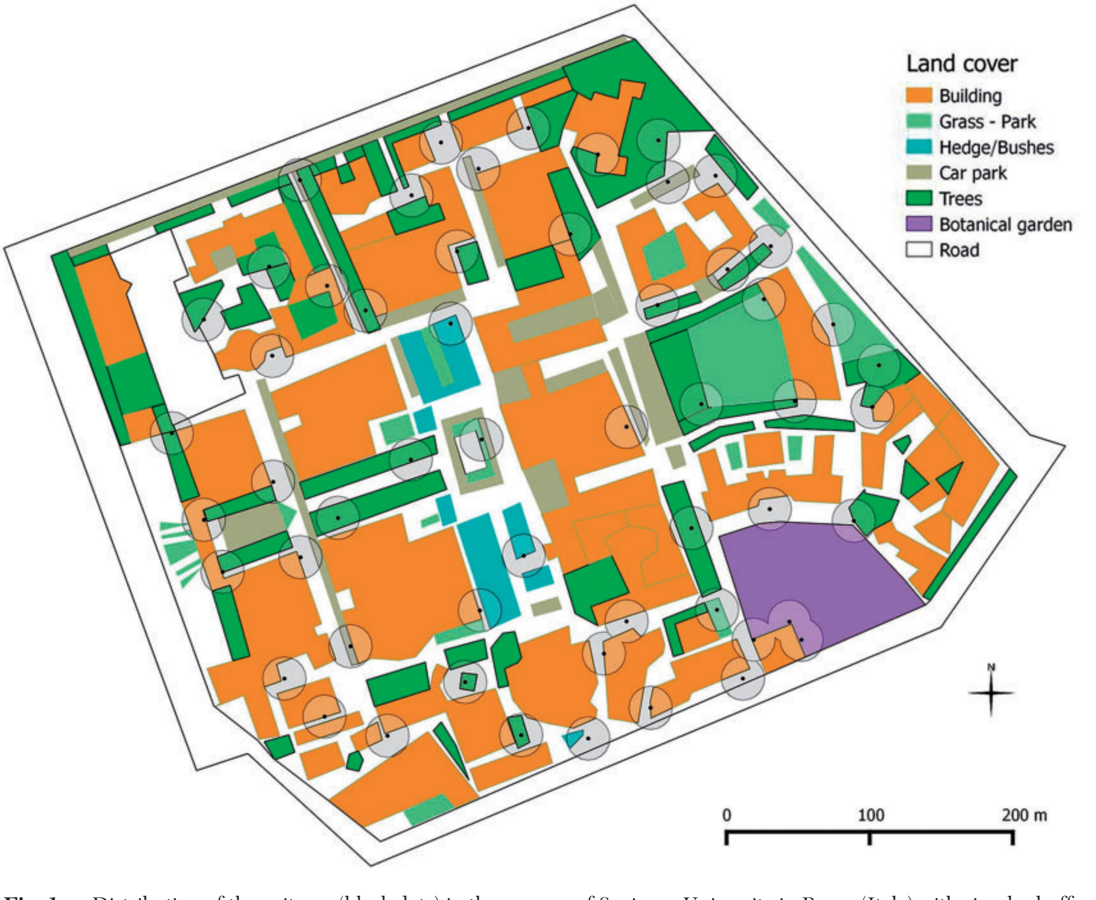

Fig. 1. Distribution of the ovitraps (black dots) in the campus of Sapienza University in Rome (Italy) with circular buffers of 15-m radius around each trap. Different colors indicate different land cover classes.

bushes or trees) and distributed over an area of approximately 22 hectares (Fig. 1), as measured by Google Earth (Google Earth Beta 5.2.1, 2010). Ovitraps consisted of black acrylonitrile butadiene styrene (ABS) truncated cones (diameter at base 8.5 cm, diameter at top 12 cm, height 13.5 cm) filled with 500 ml of tap water and equipped with a hole about 2 cm from the top edge to prevent complete filling in the case of rain. The ovitraps were lined with germination paper for egg laying. The germination paper and water were replaced and ovitraps were cleaned to remove possible remaining eggs on a weekly basis. Only the eggs adhering to the germination paper (even if already hatched) were taken into consideration, whereas larvae found occasionally in the ovitrap were killed and discarded. Each germination paper was kept in a numbered plastic bag and brought to the lab for egg identification and counting under a stereomicroscope.

Statistical Analysis. Regression models for count data were applied to model the relationship between the number of eggs in the traps and the characteristics of the area surrounding the traps. A Poisson model and a negative binomial model were compared, and the best model was chosen based on the Akaike information criterion (AIC). The best model was defined according to the commonly used rule-of-thumb that a model is better than another model when its AIC is at least 2 units smaller (Burnham and Anderson 2004).

The dependent variable was the weekly number of eggs collected in the ovitraps. The independent variables were the land cover categories, the solar radiation, and the month of capture. A random effect for the trap was included in the model to account for the fact that the observations taken from the same trap are correlated (Verbeke and Molenberghs 2009). Meteorological variables were included to adjust for the weather effect. Models with all possible combinations of variables were compared and the best model was selected based on the AIC values. The variables selection was done with the R package `MuMIn` (R Development Core Team 2013).

The land cover was classified into buildings, car parks, roads, grass, trees, hedges, and botanical garden. The vegetation data were grouped in two different ways: in model 1 all vegetation classes were merged into a single vegetation class, whereas in model 2 all land cover classes were used as separate variables. The land cover classes were incorporated into the model using a circular buffer around each ovitrap location, as described and applied in Vanwambeke et al. (2011). The area (expressed in m^2) of each land cover class was calculated within the buffers. Buffers with radius of 10, 15, 20 m were tested, in order to consider the surrounding landscape characteristics at different distances from the traps and the model with buffer size of 15 m was selected because it had the smallest AIC (Table S1). Further discussions about the buffer size can be found in the Supplemental Material (Supp Fig. 3 [online only]).

Solar radiation was used as a proxy for the amount of sunshine and the local temperature in the areas around the traps, which were put in shaded sites and never exposed directly to sunlight. Solar radiation represents the expected amount of sunlight in a specific season, based on factors such as altitude, latitude, aspect of a slope, and day length. It is a very general measure, basically reflecting whether a trap was placed at the sunny or shadowed side of a building or a wall. This variable was calculated as the sum of the direct, diffuse, and global insolation calculated at every location on an elevation map (solar radiation tool of ArcGIS version 10.0, Environmental Systems Resource Institute [ESRI] 2011, Rich et al. 1994), and meteorological conditions were not taken into account. The elevation map combined information on the height of the buildings, to account for the shadow they produced, with a map of the ground elevation (Google Earth). The ground elevation map was interpolated by kriging from a set of altitude points collected in Google Earth. For each buffer, the value of the solar radiation was calculated excluding areas of the buffers covered by buildings. This ensured that the solar radiations were calculated only at the ground level, where the traps were located, and not on the roof of the buildings. The solar radiation is calculated for the area around the traps, meaning that it reflects the amount of sunshine and warmth in the proximity of the trap and not necessarily at the site of the trap itself.

The meteorological data were included in the model in order to adjust for the weather effect, which is known to affect the dynamics of mosquitoes and eggs (Bentley and Day 1989, Deichmeister and Telang 2011). Further investigations on the weather effect were not possible, as no micro climate data at the ovitrap sites were available. Meteorological variables were maximum temperature, average humidity and wind speed, and the cumulative value of the precipitation; all were measured for a period of 5 d before each collection of eggs (Supp Fig. 4 [online only]). The meteorological data were recorded at the nearest weather station, i.e., "Prenestina – Malatesta" in Rome (approximately 2.5 km from the campus; source: http://www.wunderground.com) (accessed 6 March 2015).

Since the ovitraps were located within a small area and checked repeatedly (weekly), the data could be spatially and temporally autocorrelated. The presence of autocorrelation was graphically assessed by looking at the residuals of the model: the autocorrelation function (ACF) plot of residuals was used for the dependence over time (R package `stats`, R Core Team 2013) and the variogram was used for dependence in space (Ribeiro Jr and Diggle 2001). It was also tested whether including a random effect for the time of the capture would improve the model.

Results

An overview of the spatial distribution of number of eggs collected in the campus is presented in Fig. 2, where the average per month of the weekly counts of eggs has been calculated for each trap and plotted in the map. The mean number of eggs per trap with the standard error and the temporal trend of the number

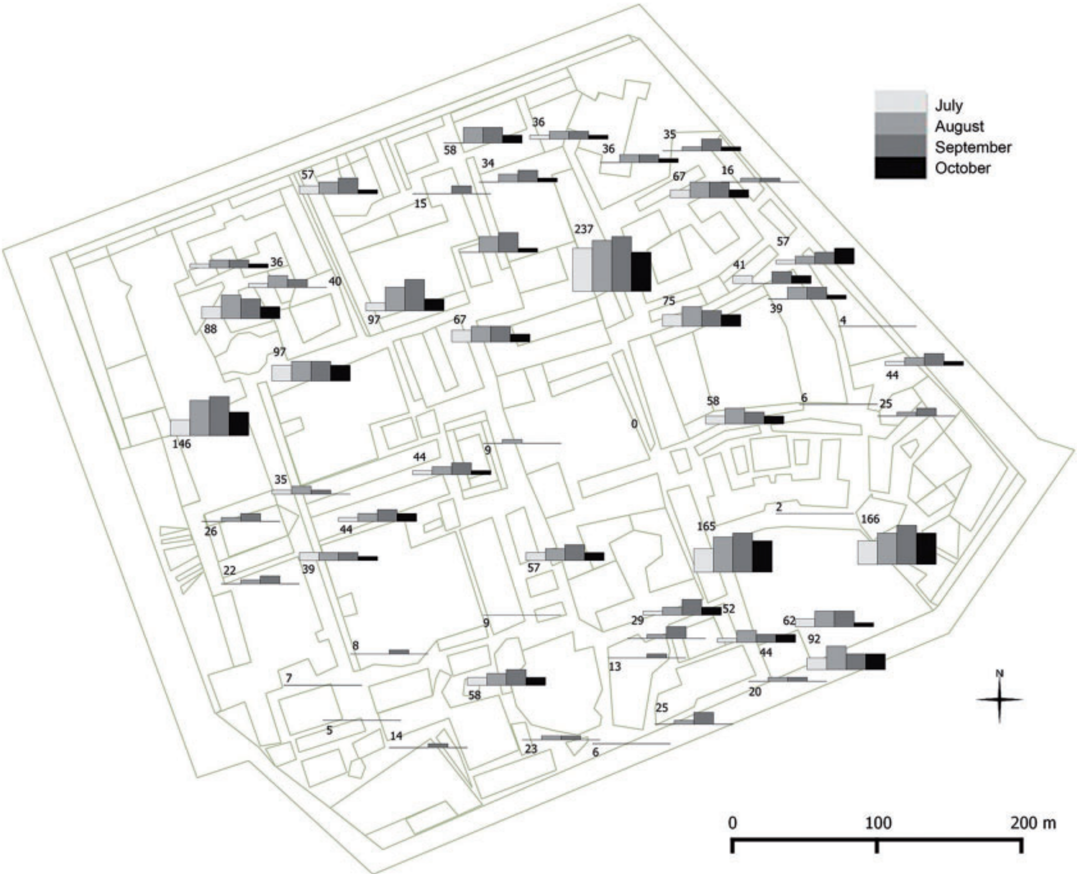

Fig. 2 Map of the campus of Sapienza University in Rome (Italy) with bar charts showing the average per month of the weekly counts of eggs for each ovitrap. The numbers indicate the total number of eggs found in each trap.

of eggs collected in the campus are presented respectively in Supp Fig. 5 and 6 (online only).

The negative binomial regression model was preferred to the Poisson regression model, because it had a lower AIC (Supp Table 1 [online only]). The negative binomial model coefficients were interpreted as incidence rate ratios (IRR), which means that when IRR > 1 the association between the variable and the number of eggs is positive and when IRR < 1 the association is negative. No spatial and temporal autocorrelation was found.

In model 1, where the vegetated land cover classes (i.e., grass, trees, hedges, and botanical garden) were grouped, vegetation, solar radiation, and months of collections were statistically significant at 0.05 level (Table 1). Vegetation and solar radiation were positively associated with the number of eggs.

In model 2, where the vegetation was subdivided into different classes, grass, trees, solar radiation, and months were statistically significant at 0.05 level, with trees and solar radiation having a positive effect and grass having a negative effect (Table 2). The best model selected included the botanical garden, but this variable was not statistically significant at 0.05 level.

Discussion

Spatial statistical models were applied to ovitrap data to identify ecological factors shaping the distribution of *Ae. albopictus* eggs in the campus of Sapienza University in Rome. Vegetation, solar radiation, and month of capture turned out to be significant to explain the differences in egg abundance. Areas with trees were statistically significantly associated with higher numbers of eggs as already shown in its original range (Hawley 1988) and in Thailand and Hawaii (Vanwambeke et al. 2007, 2011), probably because their shadow provides a favourable habitat for mosquitoes. Grass was negatively associated with the number of eggs, indicating that an open area without high vegetation and shelter is less attractive for mosquitoes, as already shown in Rey et al. (2006) and Honório et al. (2009). The positive effect of solar radiation suggests that areas with more exposure to sunshine are favorable for oviposition. It should be noted that the calculated solar radiation basically reflects whether the area around the trap is on the sunny or shadowed side of a building or a wall and, thus, may be taken as a proxy of the amount of sunshine and warmth in the proximity of the ovitrap. Also, it is important to stress that the ovitraps were put in

Table 1. Model 1: Negative binomial regression model with number of eggs as outcome and the best subset of predictor variables, chosen among vegetation, buildings, car parks, roads, solar radiation, month, maximum temperature, average humidity, wind speed, and precipitation

Variable	IRR[a]	*P* value
Vegetation[b]	**1.153**	**0.0440**
Solar radiation[c]	**1.047**	**<0.0001**
August[d]	**1.954**	**<0.0001**
September[d]	**2.801**	**<0.0001**
October[d]	**1.811**	**0.0008**
Temperature Max	0.980	0.0887
Humidity Max	0.948	<0.0001
Precipitation	0.999	0.774

[a] IRR, incident rate ratio; IRR > 1 positive effect, IRR < 1 negative effect.
[b] For the land cover classes, the IRR is calculated for an increase of 100 m^2.
[c] For the solar radiation, the IRR is calculated for an increase of 1000 WH/km^2.
[d] July is the reference category for the month.
In bold variables significant at 0.05 level.

Table 2. Model 2: Negative binomial regression model with number of eggs as outcome and the best subset of predictor variables, chosen among grass, trees, hedges, botanical garden, buildings, car parks, roads, solar radiation, month, maximum temperature, average humidity, wind speed, and precipitation

Variable	IRR[a]	*P* value
Botanical garden[b]	1.221	0.1003
Grass[b]	**0.726**	**0.0396**
Trees[b]	**1.241**	**0.0017**
Solar radiation[c]	**1.049**	**<0.0001**
August[d]	**1.992**	**<0.0001**
September[d]	**2.858**	**<0.0001**
October[d]	**1.885**	**<0.0001**
Temperature Max	0.982	0.0480
Humidity Max	0.946	0.0285

[a] IRR, incident rate ratio; IRR > 1 positive effect, IRR < 1 negative effect.
[b] For the land cover classes, the IRR is calculated for an increase of 100 m^2.
[c] For the solar radiation, the IRR is calculated for an increase of 1000 WH/km^2.
[d] July is the reference category for the month.
In bold variables significant at 0.05 level.

shaded sites and never exposed directly to sunlight and that the landscape analysis could not take in specific consideration the determinants of the shade (e.g., small bushes, benches, low walls, etc.). Therefore, the results obtained suggest that solar radiation influences the attractivity of a location for gravid mosquitoes, either by (indirectly) creating a better temperature at the times at which the mosquitoes search for an oviposition site or, for instance, by increasing the water evaporation from the ovitrap and consequently the humidity around the traps, which is known to attract the mosquitoes (Wan-Norafikah et al. 2009, Rohani et al. 2011). The number of eggs increased in August and September, in agreement with the known population dynamics of *Ae. albopictus* in Rome (Toma et al. 2003).

It should be kept in mind that all collection methods have a bias related to the fraction of the population they target. Strictly speaking results based on ovitrap data indicate suitability of a habitat for oviposition, not necessarily for other activities, such as resting or host-seeking. However, correlation was found between adult mosquitoes collected by sticky traps and number of eggs collected by ovitraps (Facchinelli et al. 2007), and ovitraps are widely used to monitor and survey the species distribution and relative densities (Toma et al. 2003, Severini et al. 2008, Becker et al. 2010) as well as to study habitat preference (Barker et al. 2003b, Rey et al. 2006). The presence of alternative potential breeding sites close to the traps could affect the results, in the sense that they would attract gravid female mosquitoes that would otherwise have laid their eggs in the traps. In our study area, alternative breeding sites could be expected in the botanical garden, where water could accumulate in plant pots and dishes, after watering of the plants. This means that the positive effect (close to significance at the 0.10 level) of the botanical garden may actually have been underestimated by the model and that in reality, the effect is even stronger.

The aim of the study was to identify favorable habitats for *Ae. albopictus* in a small urban area. If we want to generalize the results, we have to consider that, although the campus is quite representative of an urban area (with a mixture of buildings, streets, and some green areas), it is still a small area, which does not include all possible land cover classes. In further studies it would be interesting to include also information that was not available in this study, such as other land cover types (e.g., rivers, ponds, trees species, flowerbeds, distinguish between footpaths and busy roads), the presence of water (e.g., monitoring the water in the manholes and in other containers), and human density. Also, the effect of the coexistence of different land cover categories could be tested.

To our knowledge, this study is the first to investigate the habitat preferences of *Ae. albopictus* in a European urban area. In Europe, and particularly in Italy, the tiger mosquito is established especially in urban areas. Given the nuisance caused by this species and especially the risk of transmission of pathogens if these pathogens would be introduced, it is important to have information on habitat preferences to plan carefully monitoring surveys and control measures. This study represents a first step toward a better understanding of the spatial distribution of *Ae. albopictus* in urban environments. Although this is not the only factor determining whether or not a pathogen can spread in an area, determining the distribution of the vector is an essential step in studying the risk of *Ae. albopictus*-transmitted pathogens.

Supplementary Data

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

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