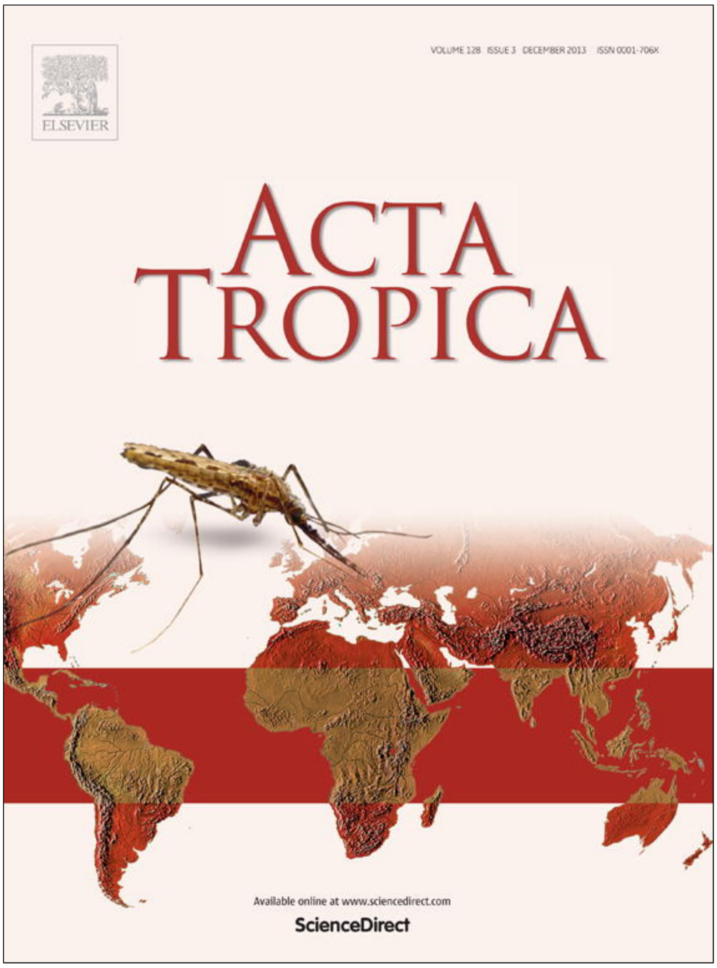
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Imperviousness as a predictor for infestation levels of container-breeding mosquitoes in a focus of dengue and Saint Louis encephalitis in Argentina

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ABSTRACT

Dengue and Saint Louis encephalitis virus are among the most important emerging viruses transmitted by mosquitoes at the global scale, and from 2009 onward both diseases have reached temperate Argentina. To test whether the urbanization level can be used as a predictor for the infestation levels of container-breeding mosquito vectors, we searched for *Aedes aegypti* and *Culex pipiens* in 8400 water-filled containers from 14 cemeteries of Buenos Aires Province and we used generalized linear models to relate positive containers with the impervious area quantified inside (internal PIA) and outside (external PIA) cemeteries. The best model for *Ae. aegypti* explained 91% of the variability and included the season, the internal PIA and the external PIA at 1 km as a quadratic function, showing a parabolic response peaking in ~75%. Regarding the infestation levels of *Cx. pipiens*, the final model explained 75% of the variability and included only the season. In view of these results, the percentage of impervious area efficiently predicted the infestation levels of *Ae. aegypti* but not of *Cx. pipiens*. Considering the worldwide relevance of the former in dengue transmission, the simple quantification of imperviousness proposed herein provides a helpful basis for vector surveillance and control in urbanized areas.

1. Introduction

Dengue and Saint Louis encephalitis virus (SLEV) are among the most important emerging virus transmitted by mosquitoes at the global scale (Solomon and Mallewa, 2001). The incidence of dengue has grown dramatically around the world in recent decades, with over 40% of the world's population at risk and around 50–100 million dengue infections every year (WHO, 2012). On the contrary, SLEV cases are usually detected in small numbers; in the USA, since the outbreak of >2500 cases in 1975–1977, approximately 55 cases are reported per year (Ciota et al., 2011).

In Argentina, dengue reemergence occurred in 1997 transmitted by the mosquito *Aedes aegypti*. During the following decade, at least 4700 cases were registered (Vezzani and Carbajo, 2008) and in the 2009 epidemic more than 25,000 cases were recorded (MSN, 2009). Regarding SLEV, human cases have been sporadically reported since the first detection in 1964 (Spinsanti et al., 2003) until a large human encephalitis outbreak including nine deaths was recorded in 2005 in Cordoba Province (Spinsanti et al., 2008). Argentine strains of the *Culex pipiens* complex were demonstrated to be an efficient vector of SLEV in experimental studies (Mitchell et al., 1980) and were also found infected with the virus in nature (Mitchell et al., 1985). More recently, two genotypes of SLEV were isolated from members of the complex in the center of the country (Díaz et al., 2006).

During the last years, the transmission of dengue and SLEV has extended toward the south in South America. In the summer of 2009, the first dengue outbreak occurred in the Federal District of Argentina (latitude 34°36′ S) and its surrounding municipalities in Buenos Aires Province, accounting for 105 autochthonous confirmed cases (Seijo et al., 2009). Also during 2009–2010, the first autochthonous cases of human SLE were confirmed in the area (López et al., 2010). The vectors of these viruses, *Ae. aegypti* and *Cx. pipiens* complex, have been recorded as the most abundant mosquito species in urbanized areas of the region, both in surveys of immatures from different types of containers (Rubio et al., 2011; Vezzani and Albicócco, 2009) and in adult collections (Vezzani et al., 2006).

Human activities and their impact on local ecology have been highlighted as one of the main drivers of the prevalence or

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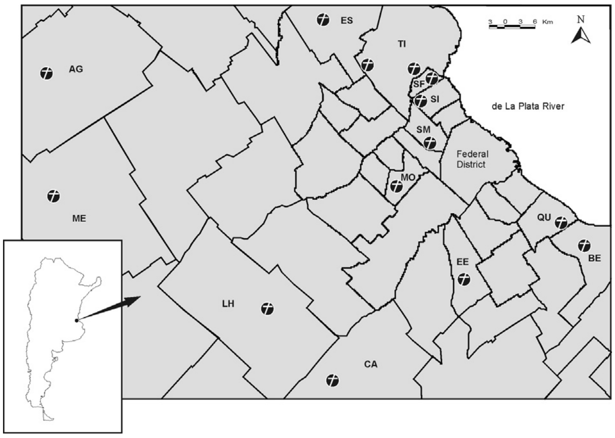

Fig. 1. Geographic location of the studied cemeteries in Buenos Aires Province (Argentina). The districts involved in the study and their total populations (INDEC, 2010) were AG (San Andrés de Giles, 22,257 inh), ME (Mercedes, 62,807), LH (Las Heras, 14,889), CA (Cañuelas, 50,526), EE (Esteban Echeverría, 298,814), ES (Escobar, 210,084), TI (Tigre, 380,709), SF (San Fernando, 163,462), SI (San Isidro, 291,608), SM (San Martín, 422,830), MO (Morón, 319,934), QU (Quilmes, 580,829), and BE (Berazategui, 320,224).

distribution range of dengue and other mosquito-borne diseases (Gubler, 2010; Mackenzie et al., 2004; Wilder-Smith and Gubler, 2008). Imperviousness is one of the primary characteristics of the urbanization process, affecting urban climate and landscape patterns. The percentage of impervious area, represented by paved roads and concrete structures, has been widely used to quantify imperviousness in urban landscape gradients (McDonnell and Hahs, 2008). Impervious areas within cities could be unsuitable for mosquitoes due to the scarcity of vegetation, high temperature and low humidity. In contrast, intermediate levels of imperviousness could be related with a high density of water-filled containers, promoting the proliferation of many urban mosquito species. Although there is no consensus about the effect of different degrees or types of urbanization on dengue transmission [e.g. Barbazan et al. (2000) versus Honório et al. (2009)], the effects of urbanization on the distribution and abundance of dengue vectors have been previously recognized (e.g. Braks et al., 2003; Rubio et al., 2011). Here, we investigated whether the urbanization level can be used as a predictor for the infestation levels of *Ae. aegypti* and *Cx. pipiens* immatures. With this aim, we used generalized linear models to assess the association between flower vases harboring mosquitoes in cemeteries and the impervious area quantified inside and up to 3 km surrounding these urban patches.

2. Materials and methods

2.1. Study area

Buenos Aires Province has a temperate climate with annual mean temperature averaging 14–17 °C and annual precipitation ranging from 600 to 1200 mm. The study area embraces the greatest megalopolis of Argentina, namely Greater Buenos Aires (GBA), and four neighboring rural districts located approximately at 100 km, namely Cañuelas (CA), San Andres de Giles (AG), General Las Heras (LH) and Mercedes (ME) (Fig. 1). The GBA covers 3827 km^2 and has the greater population density of Argentina (335 inh/ha); the four rural districts have less than 1 inhabitant per hectare (INDEC, 2010).

The surveys were conducted in 14 municipal cemeteries with a surface between 3.8 and 25.8 ha (mean 10), located at least 3-km apart (Fig. 1). Internally, cemeteries have two main patch types related to burial traditions, i.e. graves and mausoleums. The former are located in open and vegetated areas whereas the latter are characterized by high coverage of impervious surface and scarce or no vegetation cover (Vezzani, 2007).

2.2. Data collection

Mosquito samples were collected during spring (October 2007), summer (January 2008) and autumn (April 2008). In each cemetery and sampling period, 200 water-filled flower vases were inspected (8400 in total). The number of containers surveyed in grave and mausoleum areas in each cemetery was proportional to the area occupied by each burial type. Containers random selection was based on aleatory points over a grid map of each cemetery and the inspection of up to ten contiguous water-filled flower vases from each point. To collect all immature mosquitoes present in each container, water was filtered with a fine mesh strainer. Larvae were fixed in 70% ethanol and pupae were reared until adult emergence. Third and fourth instar larvae and adults were identified using the dichotomical key of mosquitoes from Buenos Aires Province (Rossi et al., 2002). Two members of the *Cx. pipiens* complex, *Cx. pipiens s.s.* and *Cx. quinquefasciatus*, are sympatric in Buenos Aires (Diez et al., 2012); these species were not distinguished from one another and are here referred to as *Cx. pipiens*.

2.3. Data analysis

Infestation levels of *Ae. aegypti* and *Cx. pipiens* were characterized by means of the number of water-filled containers harboring third and fourth instar larvae and/or pupae. As the sampling effort was equal in each cemetery and month, these values are representative of the Container Index, one of the most widely used measurements in *Ae. aegypti* infestation surveys (Silver, 2008). To verify whether the infestation level reflected mosquito abundance per cemetery, the Spearman rank correlation coefficient between the number of containers with mosquitoes and the number of immatures collected was calculated. Such coefficient makes no assumptions about linearity in the relationship between the two variables (Daniel, 1990).

The percentage of impervious area (PIA) was estimated within cemeteries (internal: I-PIA) and in their surroundings (external: E-PIA). The I-PIA was quantified in each cemetery using Google Earth software 4.3 and further checked by ground proofing. All areas occupied by mausoleums and other edifications (e.g. administrative areas) were considered impervious areas. The E-PIA of each cemetery was quantified using a Landsat 5 TM satellite image (30 m × 30 m resolution) captured in January 22, 2010. A non-supervised classification was performed to identify impervious areas (paved roads and concrete structures) using Erdas Imagine 8.4 software. The E-PIA was calculated in a circle of 1, 2, and 3 km radius (E-PIA$_1$, E-PIA$_2$, and E-PIA$_3$, respectively) around the geometric center of each cemetery using GIS-ArcView 3.2. We assumed that the surface occupied by each cemetery within this circle did not affect the estimation of PIA because it was lower than 8.5% in all cases. In addition, the size of each cemetery and the total population of each district (log transformed, $\log_{pop}$) (INDEC, 2010) were included in the analysis.

Generalized linear mixed models (GLMM) were used to model the infestation levels of both mosquito species. These models allow the use of error distributions other than normal, and the inclusion of random terms (grouping variables) to control for correlations that arise from grouped observations (Paterson and Lello, 2003). In order to model the occurrence of *Ae. aegypti* and *Cx. pipiens* separately, we first tested independency between the presence of both species per cemetery by means of a Chi-squared test and the C_8 coefficient of interspecific association (Hurlbert, 1969). Both analyses showed no association between mosquito species (Yates corrected $X^2 = 0.016$, $p = 0.9$; $C_8 = 0.54$).

Table 1
Univariate statistics for the explanatory variables used to model the infestation levels of *Ae. aegypti* and *Cx. pipiens* in water-filled containers from cemeteries of Buenos Aires Province, Argentina. Generalized linear models parameter and standard error (B ± SE) and the percentage of explained deviance are shown. For continuous variables, only the best fit among the variable (A), its square (B), or the sum of both (C) is reported. For the categorical variable (season), a χ^2 test on the change of deviance with 2 degrees of freedom is informed.

Variable	*Aedes aegypti*		*Culex pipiens*	
	B ± SE	% Explained	B ± SE	% Explained
E-PIA$_1$	$0.057 \pm 0.016^{**}$ $-0.0008 \pm 0.0004^{*C}$	44.0	NS	–
E-PIA$_2$	$0.056 \pm 0.017^{**}$ $-0.0008 \pm 0.0004^{*C}$	37.3	NS	–
E-PIA$_3$	$0.063 \pm 0.020^{**}$ $-0.0011 \pm 0.0005^{*C}$	36.3	$-0.0008 \pm 0.0004^{*B}$	13.6
I-PIA	NS	–	$-0.0223 \pm 0.0093^{*}$ 0.0006 ± 0.000^{C}	12.5
Size	NS	–	$0.048 \pm 0.020^{*A}$	11.8
log$_{pop}$	$1.090 \pm 0.474^{*A}$	23.7	NS	–
Season	***	44.9	***	49.9

NS, not significant.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

The infestation level of each species was modeled using the 'number of containers harboring mosquitoes' as the response variable, quasi-Poisson error distribution to account for potential overdispersion, and log function as a link between the response variable and the linear predictor (McCullagh and Nelder, 1989). Explanatory variables included in the modeling were: season (three levels: spring, summer and autumn), I-PIA, E-PIA$_1$, E-PIA$_2$, E-PIA$_3$, cemetery size and log$_{pop}$. All continuous variables were centered, squared and included as fixed terms in the full model along with the season; the identity of the cemeteries ('Cem') was included as a random term.

Prior to multivariate modeling, preliminary univariate analysis without random terms were run for each explanatory variable. We then tested for colinearity among continuous explanatory variables by generating a matrix of pairwise correlations using the Spearman rank correlation coefficient. If two variables were correlated >±0.75, the one that explained less in the univariate was excluded from further modeling. To check for colinearity among continuous variables, the categorical variable (season), and interactions, we calculated the variance inflation factors (VIFs) (Davis et al., 1986). If any of the VIF values indicated multicolinearity, i.e. VIF > 5 (Zuur et al., 2009a), we dropped the variable with the highest VIF, recalculated the VIFs and repeated the process until all values were lower than 5.

Given that the combination of two variables can result in a significant explanation of the response variable even if each variable separately is not informative, all non correlated variables (whether significant in the univariate analysis or not) were included in the multivariate full model. Once the largest set of explanatory variables without colinearity was identified, a backward stepwise manual procedure followed to evaluate which variables to keep, following the protocol of Zuur et al. (2009b). Finally, to simplify the models, the levels in the variable 'season' that were not significantly different were merged together. Decision rules for dropping variables and joining season levels were based on an *F* test comparing the nested models at each step.

To assess the accuracy of the selected models, we calculated the percentage of explained deviance, which is the equivalent of variance in maximum likelihood estimation techniques. All analyses were performed using the open-source software R 2.13.0 (R Development Core Team, 2011) with lme4 (Bates et al., 2011) and Design (Harrell, 2009) packages.

3. Results

The 14 cemeteries surveyed registered a mean value of internal percentage of impervious surface (I-PIA) of 25.6% (min. 0 – max. 54.8), whereas the external PIAs were around 30% [E-PIA$_1$ 31.3% (1.2–86.5), E-PIA$_2$ 29.9% (1.1–85.5), E-PIA$_3$ 29.5% (1.6–79.2)]. A total of 3857 immatures of *Ae. aegypti* and 74,139 of *Cx. pipiens* were collected in 258 and 538 water-filled containers, respectively. The former was recorded in 12 cemeteries, the latter in all sites, and both species during the three collection seasons. For both mosquito species, the number of infested containers and the number of immatures collected per cemetery and month were significantly correlated (Fig. 2).

Univariate analyses showed that all external PIAs were associated substantially with the infestation levels of *Ae. aegypti*. On the contrary, only the E-PIA at the largest radius considered (3 km) was weakly related with *Cx. pipiens*. The population of the district was associated with the number of containers harboring *Ae. aegypti*, whereas the internal PIA and the size of the cemetery were related with those of *Cx. pipiens* (Table 1). The sampling season explained the highest percentages of deviance for both mosquito species. Correlation analysis revealed that all considered E-PIAs and log$_{pop}$ were highly associated among each other, whereas size and I-PIA were not correlated with any variable (Table 2). Therefore, according to univariate results, multivariate modeling was performed considering the variables E-PIA$_1$, size, I-PIA and season for *Ae. aegypti*, and E-PIA$_3$, size, I-PIA and season for *Cx. pipiens*.

The final GLMM for *Ae. aegypti* explained 91% of the deviance and included the season, the E-PIA$_1$ as a quadratic function and the I-PIA (Table 3). The random factor 'Cem' explained only 6.7% of the variation. According to the model, the infestation levels were similar in October and January, and both lower than in April. The

Table 2
Colinearity testing of continuous variables using the Spearman rank correlation coefficient (r_s). Two variables were considered correlated if r_s > ±0.75 (in bold).

	E-PIA$_2$	E-PIA$_3$	Size	log$_{pop}$	I-PIA
E-PIA$_1$	**0.943**	**0.952**	0.538	**0.812**	−0.174
E-PIA$_2$		**0.956**	0.618	**0.785**	−0.257
E-PIA$_3$			0.684	**0.783**	−0.196
Size				0.614	−0.349
log$_{pop}$					−0.429

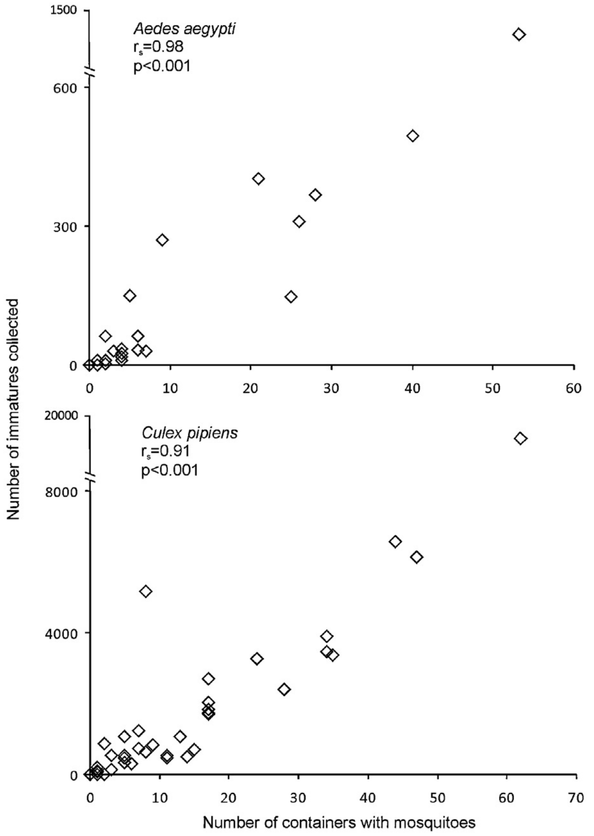

Fig. 2. Correlations between the infestation level and the abundance of *Ae. aegypti* and *Cx. pipiens* per cemetery and month. Spearman rank correlation coefficients (r_s) are informed.

number of containers with *Ae. aegypti* increased with the urbanization level up to a value of E-PIA$_1$ around 75% and then fell at higher urbanization levels (Fig. 3). On the contrary, mosquito infestation decreased slightly as the I-PIA increased.

Regarding the infestation levels of *Cx. pipiens*, the final model explained 75% of the deviance and included only the season (Table 4); the random factor ‘Cem’ explained 25% of the variation. The number of infested containers increased as the reproduction season proceeded; i.e. October < January < April.

Table 3
Final model for the number of containers harboring *Ae. aegypti* per cemetery.

	B ± SE	*t* value
Intercept [April]	2.825 ± 0.099	28.591***
E-PIA$_1$	0.047 ± 3.147e−03	14.975***
[E-PIA$_1$]^2	−5.359e−04 ± 8.995e−05	−5.958***
[I-PIA]^2	−1.131e−03 ± 2.525e−04	−4.477***
October–January	−2.303 ± 0.081	−28.542***

*** $p < 0.001$.

Table 4
Final model for the number of containers harboring *Cx. pipiens* per cemetery.

	B ± SE	*t* value
Intercept [April]	2.934 ± 0.497	5.908***
January	−0.430 ± 0.272	−1.580 NS
October	−2.608 ± 0.652	−4.001***

NS, not significant.
*** $p < 0.001$.

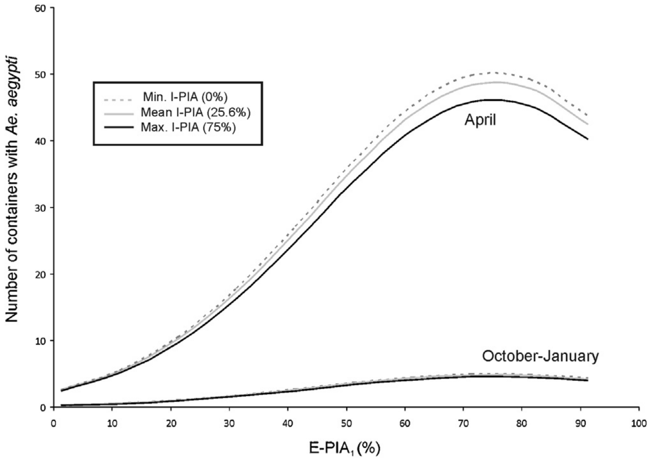

Fig. 3. Infestation levels of *Ae. aegypti* as a function of the external percentage of impervious surface (E-PIA$_1$) depending on the internal percentage of impervious surface (I-PIA), according to the best GLMM obtained.

4. Discussion

Imperviousness is recognized as an accurate predictor for urbanization (Powell et al., 2008) and is frequently used in ecological studies to assess the impact of the urbanization process on biodiversity (Morse et al., 2003; Paul and Meyer, 2001). In the present paper we postulated and tried a quick and simple quantitative estimation of imperviousness to predict the infestation levels of container mosquitoes along an urbanization gradient. Although the external percentage of impervious area at the three radiuses considered was highly correlated with the total population of each district and its calculation requires a little extra work, we consider it is better than directly using population density for a number of reasons. First, it can be computed at any desired radius around the study site, taking into consideration the dispersal range of the mosquito under study in opposition to population data which is typically available only at a district scale. Also, in many countries population data bases are not available, not reliable or not sufficiently updated; in Argentina, for example, information is gathered during national censuses on a decadal basis. In contrast, satellite images bring updated information of easy access. Furthermore, univariate analyses revealed that the percentage of impervious area explained a higher percentage of the variability in the infestation levels of both species than the total population. This might be due to the fact that the calculated variables were quantified at a more detailed scale, and are possibly a better representation of the inherent heterogeneity of the urban landscape.

When trying to predict the infestation levels of the two most abundant container mosquitoes in Buenos Aires, the percentage of the impervious area was efficient for *Ae. aegypti* but not for *Cx. pipiens*. As expected for temperate regions, seasonality was an essential component of the selected multivariate model for both species. Notwithstanding, the lack of interaction between the season and the variables quantifying imperviousness validates their use not only during the peak of abundance (autumn) but throughout the year.

The percentage of impervious areas, both inside and outside the cemetery, were the main factors driving the infestation levels of *Ae. aegypti* after the season, revealing a complex response pattern to each of these urbanization variables. Regarding the PIA surrounding the cemeteries, the lowest infestation levels of *Ae. aegypti* were observed in less urbanized areas, increasing toward the more urbanized ones. Nevertheless, at more extremely urbanized areas (E-PIA$_1$ > 75%) this pattern was reversed, although the infestation levels reached were still higher than in rural areas. Previous studies along urban–rural gradients have shown that the abundance of *Ae. aegypti* is positively associated with increasing urbanization (reviewed by Higa, 2011), although the opposite response pattern has also been registered (Carbajo et al., 2004, 2006; Rubio et al., 2011). These studies should be compared cautiously due to substantial differences in the extent of the gradient covered, as well as in the scale and the general methodology applied. In spite of this, in all cases general explanations for the observed pattern rely on the basic requirements of *Ae. aegypti*. Areas characterized by high availability of water-filled containers for immature development, nectar and blood as energy source for the adults, human blood for egg development and shady habitats for resting and ovipositing have been highlighted as preferred by the mosquito (Clements, 1992; Muir et al., 1992; PAHO, 1994; Scott et al., 1993). The availability of such resources has been associated with different urbanization levels (Carbajo et al., 2006; Higa, 2011). Rural areas are characterized by high vegetation cover and low population density, and therefore, the availability of both human blood and artificial containers are scarce. In contrast, urban areas show a mixture of green spaces, high population density and a wide variety and number of artificial containers. Nevertheless, the city center concentrates commercial and administrative activities and, although the residents and the employees that arrive from the periphery to work daily constitute an abundant blood source, the area presents high density of tall buildings without space for container accumulation and is subjected to intense waste management. Thus, the decrease in infestation levels of *Ae. aegypti* in the urban extreme of the gradient is probably due to limiting container resources (Carbajo et al., 2006).

Regarding the internal structure of the sites, the infestation levels of *Ae. aegypti* increased in cemeteries with less proportion of impervious areas or, equivalently, with higher proportion of vegetated areas. A similar situation has been previously documented in cemeteries of Buenos Aires city, in which *Ae. aegypti* infestation was positively associated with the vegetation cover but not with the container density or the cemetery area (Vezzani et al., 2001). Extensive evidence of the influence of vegetation and shade availability on the suitability of the containers as breeding sites have been reported elsewhere (e.g. Barrera et al., 1979; Tun-Lin et al., 1995; Vezzani and Albicócco, 2009). So, it is reasonable to infer that the preimaginal stages of mosquitoes are benefited in cemeteries with a higher availability of containers in shaded conditions, independently of the total number of containers, probably because cemeteries are settings of an extremely high container density per se (Vezzani, 2007). This result is really relevant for the planning of mosquito control strategies, since cemeteries have been identified as sources of these vectors around the world (reviewed by Vezzani, 2007). Any management decision toward this goal should consider the proportion of the impervious (or vegetated) area as a weight for the selection of priority control sites.

The fact that we did not obtain a model for *Cx. pipiens* as a function of the environmental variables evaluated other than the season could be due to a number of reasons. First, we may have not selected the environmental variables relevant to its distribution or the appropriate scale to detect an association. Second, the two main forms within the complex, i.e. *Cx. pipiens s.s.* and *Cx. quinquefasciatus*, may present different associations with the environment and may be therefore compensating their effects. The urbanization level has been associated with the presence of different forms of the complex in other regions (Vinogradova, 2000). Differences in the proportion of forms between urban and rural environments were detected in the USA (Savage et al., 2006) and Mexico (Díaz-Badillo et al., 2011); no studies have assessed so far this issue in Argentina. Third, *Cx. pipiens* can occupy practically any type of breeding habitat, including ground habitats and artificial containers (Vinogradova, 2000). In view of this, the model may have failed to capture the variation along the gradient because our study was limited to flower vases. Last, and simplest, it may be possible that *Cx. pipiens* does not present an association with urbanization within cemeteries at the studied scale. Whatever the reason, an efficient tool for the prediction of the infestation levels of *Cx. pipiens* in urban and rural environments is still needed.

Contrasting patterns were obtained when comparing the present results with those reported by Rubio et al. (2011) for both mosquito species in used vehicle tires along the urbanization gradient. Opposite to current findings in cemeteries, the Container Index of both species in tires increased steadily toward the rural end of the gradient. This may be due to used tires presenting a higher rate of exchange in the most urbanized settings, which deters adult oviposition and immature development. In addition, rural settings provide scarce artificial containers while used tires are usually of greater water capacity (trucks and tractors), presenting less risk of desiccation and higher difficulty to discard. Therefore, the observed opposite patterns could be due to a shift in the use of container habitats by mosquitoes in different environmental conditions.

The high correlation between the number of containers harboring mosquitoes and the number of immatures collected per site suggests that the measurement of the infestation level considered herein was a good proxy for mosquito abundance, similar to that observed in another cemetery within the study area (Vezzani and Albicócco, 2009). However, the observed association could be specific for artificial containers within cemeteries due to a homogeneous emptying and refilling of water. Mosquito productivity could differ in private premises because people store water in vessels with different refilling frequency depending on climatic conditions and water supply (Padmanabha et al., 2010). The fact that our study design excluded other containers typically found in premises could be considered as a limitation in the scope of our findings. However, it is also a good opportunity to assess the effect of the urbanization ruling out the diversity of containers offered as a confounding factor.

In Argentina, *Ae. aegypti* has been reported from the subtropic to temperate–cold areas up to ~40° S (Grech et al., 2012). This broad distribution through different climatic regions provides an opportunity for further validation of our findings in subtropical and cold urbanized areas. Our results support the need to further disentangle the influence of the urbanization gradient on local mosquito dynamics, which constitutes essential baseline information for vector-borne diseases prevention. In view of the worldwide relevance of *Ae. aegypti* in dengue transmission, the simple quantification of imperviousness proposed herein provides a helpful basis for vector surveillance and control in urbanized regions.

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