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Aedes aegypti and *Aedes albopictus* in Bermuda: extinction, invasion, invasion and extinction

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Abstract We provide an analysis of the invasion and spread of the container inhabiting mosquitoes *Aedes aegypti* and *Aedes albopictus* in the Bermuda Islands. Considered eradicated in the mid-1960s, *A. aegypti* was redetected in 1997, and *A. albopictus* was first detected in 2000. Based on weekly ovitrap data collected during the early stages of the invasion, we mapped the spread of *Aedes* throughout the islands. We analyzed the effects of buildings and roads on mosquito density and found a significant association between density and distance to roads, but not to buildings. We discuss the potential role of human transport in the rapid spread in the islands. The temporal correlation in ovitrap collection values decreased progressively, suggesting that habitat degradation due to control efforts were responsible for local shifts in mosquito densities. We report a sharp decrease in *A. aegypti* presence and abundance after the arrival of *A. albopictus* in the year 2000. Possible mechanisms for this rapid decline at relatively low density of the second invader are discussed in the context of classical competition theory and earlier experimental results from Florida, as well as alternative explanations. We suggest that support for the competition hypothesis to account for the decline of *A. aegypti* is ambiguous and likely to be an incomplete explanation.

Keywords *Aedes albopictus* · *Aedes aegypti* · Vector invasion · Bermuda Islands · Competition

Introduction

Aedes albopictus and *Aedes aegypti* are mosquitoes (Diptera: Culicidae) that inhabit small containers as larvae, invasive to the Bermuda Islands (UK). *A. albopictus* originates from southeast Asia but has dramatically expanded its range to many tropical, subtropical and temperate regions, both in urban and rural areas around the world (e.g., Benedict et al. 2007; Hawley 1988; Lounibos 2002; Novak 1992; Urbanelli et al. 2000). *A. aegypti* originates from Africa and spread to many areas in the tropics, especially in concurrence with the slave trade increase in the 16th and 17th century (Lounibos 2002); currently, both species co-occur in many

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regions of the world (e.g., Braks et al. 2003; Fontenille and Rodhain 1989; Tabachnick 1991). These two mosquito species have prompted considerable interest in multiple research areas, including vector competence (e.g., Boromisa et al. 1987; Diallo et al. 2008; Moore et al. 2007), insecticide resistance (e.g., Hidayati et al. 2005; Stasiak et al. 1969; Wesson 1990), spatial, temporal and geographical analyses (e.g., Benedict et al. 2007; Castro Gomes et al. 2005; Francy et al. 1990; Kobayashi et al. 2002), and ecological and evolutionary studies (e.g., Juliano et al. 2002; Pumpuni et al. 1992). Both species have been involved in historic and current transmission of dengue and yellow fever throughout their range. *A. albopictus* is a laboratory vector of more than 30 viruses, although only a few of these affect humans, including dengue, Chikungunya, St. Louis, and La Cross encephalitis viruses (Mitchell 1995). *A. aegypti* is the historical vector for yellow fever virus (e.g., Beaty and Aitken 1979) and has been reported as the most competent vector of the dengue virus (e.g., Gubler 1998).

The Bermuda Islands (UK), located in the Atlantic Ocean off the east coast of the USA (32°18′N 64°47′W), have a subtropical climate, with mild winters and hot, humid summers, providing suitable conditions for both species of mosquitoes. Historically, *A. aegypti* was responsible for extensive outbreaks of yellow fever and dengue fever during the past few centuries. In the 1940s, a large control campaign was undertaken and *A. aegypti* was considered eradicated by the 1960s (Camargo 1967). However, *A. aegypti* was rediscovered in 1997 and subsequently, in 2000, *A. albopictus* was also found in the islands by health officers. Before the rediscovery of *Aedes* in the islands, the Health Department of Bermuda maintained a mosquito-monitoring program only at ports of entry, but after the rediscovery of *A. aegypti*, the program was expanded. Ovitraps were installed to monitor mosquito prevalence and spread across the islands, comprising, by 2005, 582 weekly collected ovitraps distributed throughout the limited Bermuda surface (<54 km^2). The ovitrap program is paired with a permanent, media and field based resident consciousness program in conjunction with crews deployed in the ground cleaning and surveying containers and other breeding areas. Monitoring mosquito populations by ovitrap collections rather than larval surveys

has been suggested as more efficient by Rawlins et al. (1998) and allowed egg collections to be sent out for analysis and species identification. This monitoring methodology can be somewhat limited, as it only detects the presence of mosquitoes within close proximity, but it could be used as a proxy estimator of presence and size of mosquito populations in the trap's vicinity. The Bermuda Islands provide an interesting opportunity to study the nearly coincidental invasions of these two ecologically similar species, taking into consideration the islands' isolation, small size, and the extensive ovitrap sampling program that allows tracking the mosquitoes' invasion in the islands.

Despite ecological similarities of *A. aegypti* and *A. albopictus* in breeding habitat and host selection, Hornby et al. (1994) suggested that they occupy different niches. In Florida (USA), Brazil, and many regions of Asia, *A. albopictus* generally prefers rural and suburban, underdeveloped or lightly developed areas, whereas *A. aegypti* prefers urban and suburban highly developed areas (Braks et al. 2003; Hornby et al. 1994; Gilotra et al. 1967; O'Meara et al. 1992, 1995). *A. aegypti*, which had been found throughout Florida for many years prior to the arrival of *A. albopictus* in the early 1990s, has since declined in abundance and distribution (Hornby et al. 1994; O'Meara et al. 1995). This decline in *A. aegypti's* abundance occurred soon after the establishment of *A. albopictus* in locations such as scrap tire sites and rural areas where the species' distributions overlapped (O'Meara et al. 1995). Lounibos (2002) suggests that the decline has been due to a combination of several factors, including *A. albopictus* superiority in larval resource competition. In the United States, a correlation was found between the introduction and increase of *A. albopictus* and a more restricted distribution and declining abundance in *A. aegypti* (O'Meara et al. 1995).

Juliano (1998) examined competition between the two species. *A. albopictus* was the dominant competitor in experimental field containers, with positive population growth in treatments with low resource availability and treatments with a high combined species density. Overall, the experiments pointed to *A. albopictus* as the superior competitor, considering its higher rate of survival to adulthood. *A. aegypti* was only able to complete its development in treatments with the lowest density or in treatments

with leaf litter added to the filtered tire water medium (Juliano 1998). A similar experiment performed in Brazil also supports *A. albopictus*' competitive advantage under field situations. *A. albopictus* was suggested as the dominant larval competitor considering that populations maintained positive growth rates in the field at higher combined densities and lower per capita resource availability than *A. aegypti* (Braks et al. 2004). Several studies have reported that detritus may affect competitive outcome, with varying results; *A. albopictus* outcompetes *A. aegypti* in many detritus types, but some food sources, such as liver powder, may favor *A. aegypti* (Black et al. 1989), or allow stable coexistence of the two species (Murrell and Juliano 2008).

The coincident decline in several *A. aegypti* populations that occurred after *A. albopictus* invasions may have resulted from competitive displacement by exploitation, considering the apparent *A. albopictus* superiority in field and laboratory conditions (e.g., Braks et al. 2004; Juliano 1998). Nevertheless, alternative scenarios are possible, including interference between species or some other types of interactions (reviewed by Lounibos 2002, 2007) that could result in similar outcomes: mating interference, microparasite infections, and hatching inhibition. In addition, it has been reported that predatory species such as *Corethrella appendiculata* or *Toxorhynchites rutilus* could change species interaction outcomes in small containers in the US (e.g., Griswold and Lounibos 2006; Juliano et al. 2009; Kesavaraju et al. 2008). However, these predator species are not present in Bermuda, and there is no other predator occupying small containers in the islands (D. Kendell, pers. obs.).

Various approaches have been taken to model competition, most of which trace to the Lotka-Volterra model, which is among the simplest descriptions of two species sharing some common resource base. The combination of conditions necessary for competitive superiority to explain the displacement of one species by another by the Lotka-Volterra model are depicted in Fig. 1. The species whose isocline is higher is able to increase in number, driving the other species down to extinction when reaching its carrying capacity. This analysis has been used in similar circumstances to model the interaction between *A. albopictus* and a North American native container inhabiting, *A. triseriatus* (Livdahl and

Willey 1991). The critical parameters to estimate for quantitative prediction of this interaction are competition coefficients (α) and carrying capacities (K). Although a number of studies of *A. albopictus* and *A. aegypti* have been conducted yielding data that could have provided them, none of the studies of *A. albopictus* and *A. aegypti* have estimated these parameters.

Considering the habitat-specificity of these parameters, it is remarkably difficult to obtain estimates of carrying capacity or competition coefficients in non-experimental field conditions. Nevertheless, it seems likely that those parameters would be similar in ecologically similar species. If this is the situation, the isoclines of the two species would be close together, as shown in Fig. 1a, and the inferior competitor would begin to decline only when the better competitor is near its equilibrium density. Exclusion should be a slow process in such a case (Fig. 1b). A much more rapid exclusion is possible if carrying capacities differ markedly, or if competition coefficients yield a dramatic asymmetry in the impact of one species on the other. An illustration of two species with quite different carrying capacities appears in Fig. 1c, d. The *A. albopictus* invasion of the Bermuda Islands, previously occupied by *A. aegypti*, provides a novel opportunity to examine this possibility, taking advantage of the extensive ovitrap program, the small area of Bermuda, and the fairly homogeneous, urbanized environment.

We report here the invasion, progress, spread, and the contrasting outcomes of *A. aegypti* and *A. albopictus* invasions in the Bermudas Islands. In addition, we aim to evaluate the *A. albopictus* competitive superiority hypothesis by examining indirect indicators of competitive exclusion.

Methods

Sampling

Ovitraps consisted of wide-mouthed amber glass jars (15.2 × 7.6 cm), half-filled with rain water, with a masonite paddle (2 × 16 cm) inserted as an oviposition substrate. All ovitraps in the sampling program were monitored weekly by the Bermuda Ministry of Health, starting with 135 ovitraps by 2000, expanded
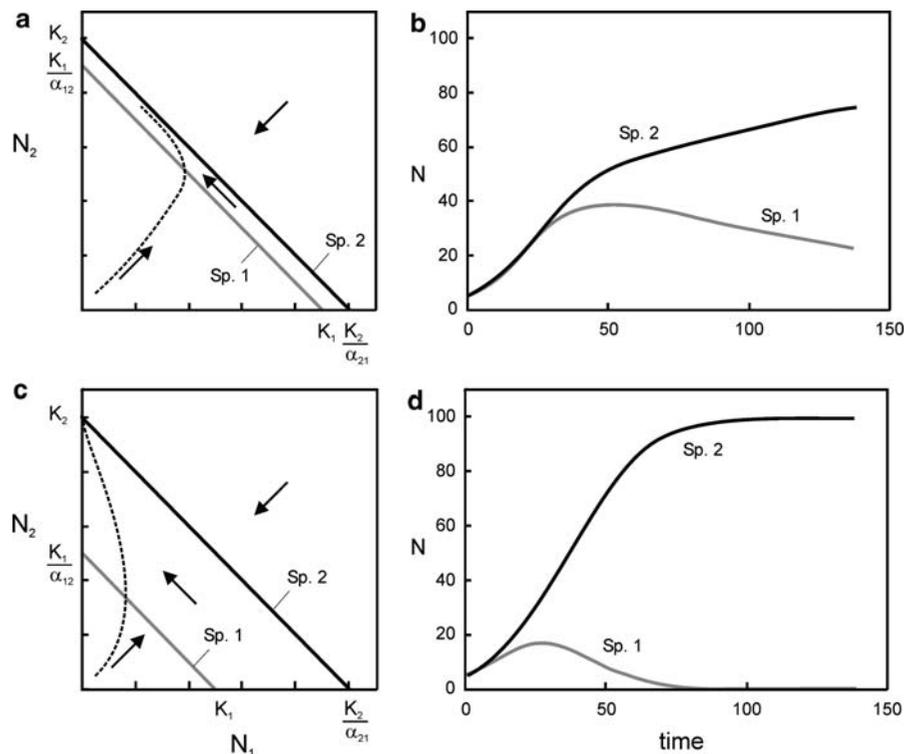
Fig. 1 General representation of the Lotka-Volterra competition model for competitive exclusion, represented in **a** and **c** with a phase diagram of population size of species 1 (N_1, *x*-axis) versus population size of species 2 (N_2, *y*-axis). Zero growth isocline for species 2 is located above the corresponding isocline of species 1; species 2 is a superior competitor and eventually drives species 1 to extinction when reaching its carrying capacity. Specific location of isoclines depend on carrying capacities for both species (K_1, K_2) and the relative competitive coefficients (α_{12}, α_{21}). *Arrows* depict general directions of change within each region, and a trajectory is shown with a dashed line. **b**, **d** Illustrate the densities of each species through time for the trajectories shown in the phase diagrams on the *left*. For clarity, α_{12}, $\alpha_{21} = 1$ in both simulations, but $K_1 = 90$ in the *top* simulation and 50 in the *bottom* case, with $K_2 = 100$ in both cases. Note that the situation in which the species have the most different carrying capacities results in a much more rapid displacement, at a much lower density of the dominant species

to 300 ovitraps by 2003, and expanded again in 2005 to 582 ovitraps.

Species identification

To identify the mosquito species present in the samples, masonite paddles containing eggs from the ovitraps collected in Bermuda from 2003 to 2005 were sent to Clark University. All paddles were sorted by collection site and year. Using a toothpick wet with tap water, eggs were removed from paddles and placed into 0.6 ml tubes. Each individual paddle constituted one sample, and 3–15 eggs were used per sample. If more than 15 eggs were found on a paddle, a random sample was taken. For this study, six to eight samples were taken from each site ($n = 39$) on

the island for each year. DNA was extracted using the E.Z.N.A. Forensic DNA Kit (Omega Bio-tek) in 350 µl of STL buffer solution and eggs were disrupted by mechanical agitation for 4 min in the presence of 3.2 mm chrome-steel balls (Mini Bead-beater 8, BioSpec Products, Inc.). Genomic DNA was extracted following the manufacturer's recommendations, except that HB buffer was not used and DNA was eluted in 55 µl of elution buffer.

The ITS2 region of each species was amplified using 28S (5′-TCACACATTATTTGAGGCCTAC-3′) and 5.8S (5′-TGTGAACTGCAGGACACATG-3′) primers previously shown to produce different sized products from *A. aegypti* and *A. albopictus* (Wesson et al. 1992). PCR reactions (50 µl) consisted of 15 µl of the extracted DNA solution, 2.5 µl of each primer

(10 μM stock concentration), 25 μl Promega PCR Master Mix and 5 μl of sterile, distilled water. After an initial denaturing step at 94°C for 3.5 min, the following cycling parameters (30 cycles) were used: 94°C for 1 min, 50°C for 1 min, and 72°C for 1 min with a final extension at 72°C for 5 min. PCR products were analyzed on a 1% agarose gel using standard electrophoretic procedures. DNA from previously identified eggs from both species was used as standards to verify that PCR products from both species were obtained from mixed-species samples. Samples were scored as positive for a given species if an appropriately-sized PCR product (*A. aegypti* 324 bp; *A. albopictus* 518 bp) was visible on the agarose gels.

Analysis

To map the *Aedes* spread across the island, continuous surfaces were interpolated from the yearly totals for each ovitrap using Idrisi v.15.0 Los Andes Edition (Clark Labs 2007) INTERPOL module with a distance weight of 2.0 and six points search radio option. The values obtained using this procedure allow a relative estimation of oviposition activity in the ovitrap's vicinity. Due to the low number of ovitraps on the ground during the years 2000–2002, caution should be taken when reading those surfaces as the interpolation algorithm is likely to produce artifacts. All spatial analyses were conducted using the same 300 georeferenced ovitraps present during the years 2003–2004; for years 2000–2002 those ovitraps were considered missing data.

Layers containing the georeferenced location of buildings and roads were used to produce continuous surfaces with distances to features using Idrisi's DISTANCE module. The resulting raster layers were imported into ArcMap 9.2 (ESRI 2006) and demographic variable values for the ovitraps locations were extracted using the intersect point tool from Hawth's analysis tool (Beyer 2004). The resulting distance values failed the Shapiro–Wilk test for normality (untransformed distance to buildings $W = 0.76$, untransformed distance to roads $W = 0.49$, both $P < 0.001$) and several attempted transformations failed. The distance values were categorized in quartiles and the effect of variables over the total number of eggs for years 2003–2007 was evaluated with a Van der Waerden non-parametric ANOVA. To assess the temporal correlation in number of eggs per ovitrap per year, a modified *t*-test for correlation was performed using the Clifford, Richardson, and Hémon (CRH) procedure, which corrects for spatial autocorrelation. The CRH test was implemented in Passage v.1.0 (Rosenberg 2001), using scaled euclidian distances and 50 equal observation classes. Significant correlation values after Dutilleul's correction were regressed against time interval.

Taking into consideration the progressive increase in the weekly number of ovitraps deployed in the islands, we chose two statistics to estimate habitat use and crowding levels, and to describe the trajectory of *Aedes* populations in the islands: the average number of eggs per ovitrap, and the proportion of positive ovitraps. No satisfactory transformation for normality was found for these statistics (untransformed average number of eggs $W = 0.67$, untransformed proportion of positive ovitraps $W = 0.81$, both $P < 0.001$), so the data were analyzed using non-parametric ANOVA. For pairwise comparisons, bootstrap procedures were utilized to produce 95% confidence intervals after 1,000 replicates using S-plus v.8.0.4 (Insightful Corp 2007). Nonoverlapping confidence intervals were considered statistically different.

An ordinal logistic regression ($R^2 = 0.45$, $P < 0.05$, lack of fit $P = 0.64$) was used to estimate *A. aegypti* decline based on PCR detection for the years 2003–2005 and the known presence of the species as the only container inhabiting mosquito in 1999. Unless stated otherwise, all statistical analyses were performed using JMP 7.0 (SAS Institute Inc. 2007).

Results

Spatial patterns

The spread of unidentified *Aedes* in the Bermuda Islands was extremely fast. *Aedes* were detected on all populated islands as early as 2003, only 5 years after the rediscovery of *Aedes aegypti* in 1998 and 3 years after the first detection of *Aedes albopictus* in 2000 (Fig. 2). A consistent pattern of high egg counts in collections in the southern area was observed across the different years, even though the specific locations and relative values vary to some degree. In addition, ovitraps located near roads, in the first distance quartile (from 0 to 4.47 m), had higher egg
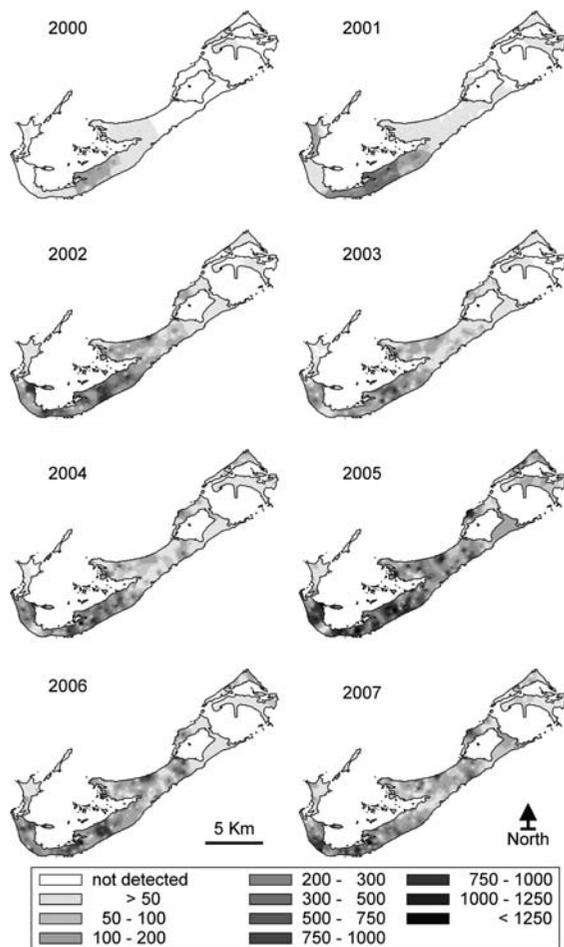
Fig. 2 Estimation of oviposition activity by area for years 2000–2007 based on yearly oviposition totals in ovitraps. Values should be considered as relative indicators for both *Aedes* species (see "Methods")

numbers than the other quartiles (Van der Waerden $\chi^2_{\mathrm{appr}} = 29.32$, $P < 0.001$), while distance to the nearest building was not associated with the number of eggs collected by ovitraps (Van der Waerden $\chi^2_{\mathrm{appr}} = 4.7$, $P < 0.05$).

Temporal patterns

Egg counts per week reveal a distinct seasonal pattern of abundance (Fig. 3). This pattern suggests an early generation, with major reproduction in late June, followed by at least two more distinct peaks, in August and September. These latter peaks may represent subsequent generations. Although activity declines markedly during the cooler months, eggs

were found in traps during every week for at least one of the years of the study period, and larvae can be found in containers throughout the year (D. Kendell, pers. obs.).

The temporal correlation in number of eggs per ovitrap per year reported 22 significant (over 28 possible combinations) correlations between years considering all possible year combinations. The correlation analysis shows a significant decline in the correlation as the time interval increases ($R^2 = 0.5$, $P > 0.001$, Fig. 4).

Community composition

PCR-based species identification revealed an abrupt decline in *A. aegypti* populations on Bermuda egg samples collected in ovitraps during 2003–2005. The percentage of samples positive for *A. aegypti* decreased from 4.35 and 9.09% in 2003 and 2004, respectively, to 3.06% by 2005. In addition, *A. aegypti* disappeared from several sites by 2005, where it was detected in previous years. *A. aegypti* not only decreased in prevalence on ovitraps from 2003 to 2005, but also its presence across the islands. Interestingly, *A. aegypti* was rarely present as the sole species in a sample; in those where *A. aegypti* was detected, at least 98% of the time the traps were also positive for *A. albopictus*. Considering the total positive samples processed for all 3 years, just 7.08% contained *A. aegypti* occurring with *A. albopictus*, and 1.67% of the total positive samples contained *A. aegypti* only; all remaining samples were positive for *A. albopictus* only (see Table 1). These results indicate that the two species were overlapping throughout the island, possibly occupying similar habitats.

The expected decline in the frequency of *A. aegypti* suggests a rapid, abrupt decline in its presence in the islands during the years 2000–2002, consistent with the observed data points (Fig. 5) and observations of vector control officers in the field (David Kendell pers. obs.). This decline occurred during the same years when the average number of eggs per ovitrap sharply increased (Kruskal–Wallis $\chi^2_{\mathrm{appr}} = 106.26$, $P < 0.001$). A similar trend can be observed in the proportion of positive ovitraps, as they increased in the years 2000–2002, to reach stable levels in the years 2002–2007. This increase was not detectable with the nonparametric ANOVA (Kruskal–Wallis

Fig. 3 Weekly egg production, pooled across years (2002–2007). Mean number of eggs per trap, ±SE (based on residual mean square from 1-way ANOVA)
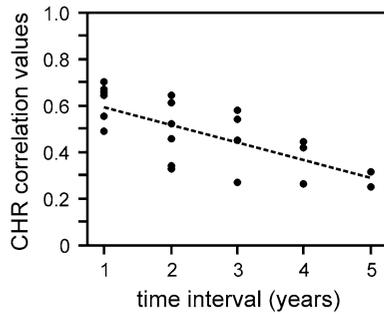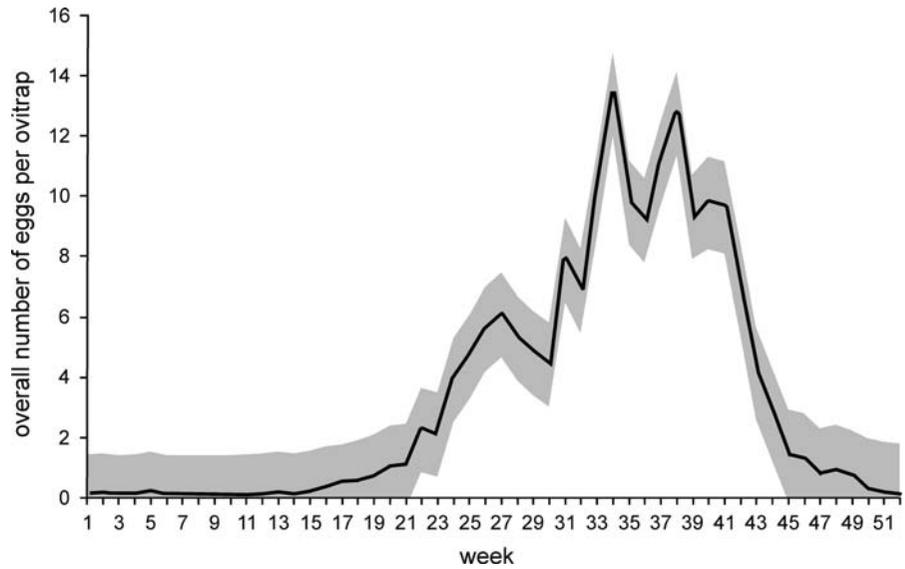

Fig. 4 Temporal correlation in the number of eggs per ovitrap in function of yearly time interval. Only CRH significant correlation values after Dutilleul's correction are used in the correlation analysis ($R^2 = 0.5$, $P < 0.001$)

$\chi^2_{appr} = 8.64$, $P > 0.05$), but it could be detected with non-overlapping confidence intervals after a bootstrap procedure. This overall trend presented in Fig. 4a, b suggests that the decline in *A. aegypti* occurs at the same time that *A. albopictus* increased dramatically in the years 2002–2007.

Discussion

The key issue that we sought to address in this study was the importance of competition in the interaction between two invading species. Our interpretation of these data raises some concerns that larval competition alone may not account for the extremely rapid disappearance of *A. aegypti*. We base this view on (1)

Table 1 Summary of samples processed, successfully amplified, and corresponding species identification from Bermuda samples for years 2003–2005

Year	N_t	Success		*A. albopictus*		*A. aegypti*		Both species	
		N_s	$\%_s$	N_{al}	$\%_{al}$	N_{ae}	$\%_{ae}$	N_b	$\%_b$
2003	138	56	40.6	49	87.5	1	1.79	5	8.93
2004	154	102	66.2	88	86.3	2	1.96	12	11.80
2005	98	82	83.7	79	96.3	1	1.22	2	2.44
All years	390	240	61.5	216	90.0	4	1.67	19	7.08

N_t, total number of samples processed; Success, samples successfully amplified; N_s, number of samples successfully amplified; $\%_s$, percentage of samples successfully amplified. N_{al}, number of samples only positive for *A. albopictus*; $\%_{al}$, percentage of samples only positive for *A. albopictus*; N_{ae}, number of samples only positive for *A. aegypti*; $\%_{ae}$, percentage of samples only positive for *A. aegypti*; N_b, number of samples positive for both species; $\%_b$, percentage of samples positive for both species

evidence that suggests a high rate of habitat turnover (Fig. 4), which would reduce the impact of competition, (2) collapse of *A. aegypti* well in advance of any indication that the two mosquito populations had saturated their environment (Fig. 5), and (3) evidence that the two species do not display substantial asymmetry in their resource use or in their competitive impacts on one another (Table 2). We argue below that additional factors are well worth considering, including differential susceptibility to parasitism, and differential colonizing ability by the two

Fig. 5 *Left axis* predicted decline of A. aegypti (*dotted line*) based on a fitted ordinal logistic function regression for years 1999–2007, *solid triangles* depict observed frequency values. **a** *Right axis* average number of eggs per ovitrap for years 2000–2007 (*solid line*). *Error bars* represent standard errors. *Letters* indicate overlapping 95% confidence intervals after 1,000 bootstrap replicates. **b** *Right axis* frequency of positive ovitraps over totals for years 2000–2007 (*solid line*), *error bars* represent standard errors. *Similar letters* indicate overlapping 95% confidence interval after 1,000 bootstrap replicates
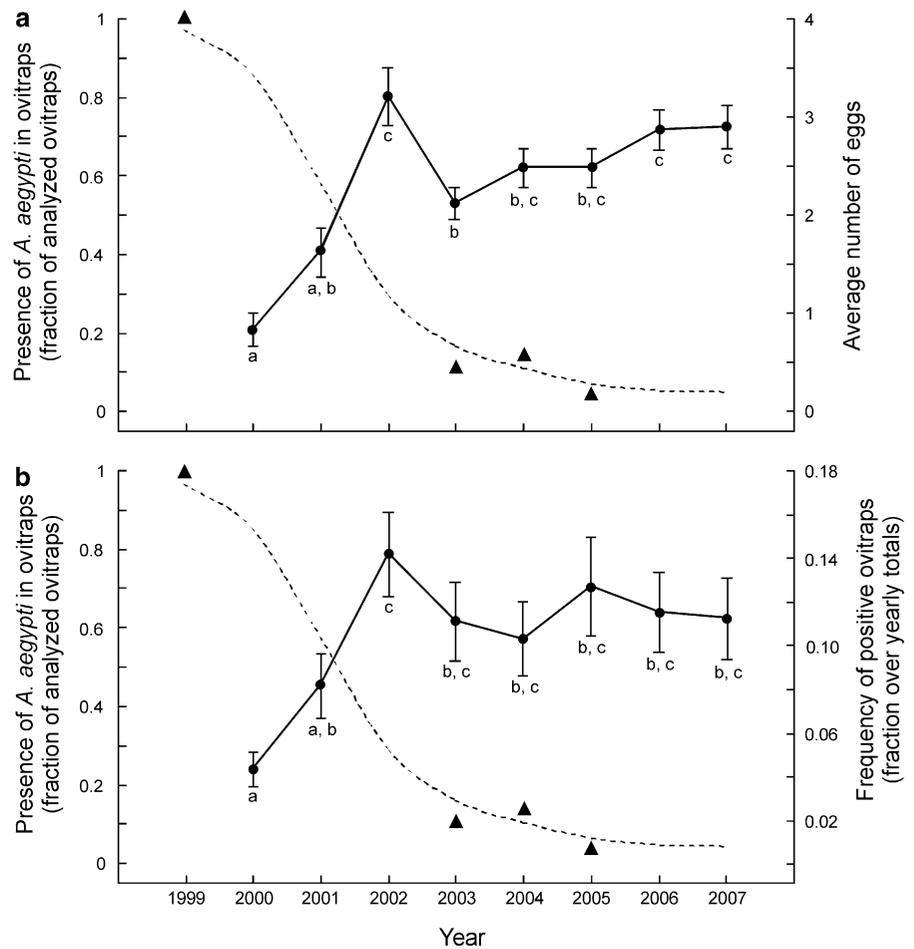

Table 2 Responses of per capita growth rate estimates for each species to the initial density of *Aedes aegypti* and *A. albopictus* larval cohorts in tire fluid, grown under field conditions in Vero Beach, FL (Livdahl 1993)

Species	n	r_{mi}	b_1	b_2	K_j	α_{ij}
A. aegypti	49	0.0299 ± 0.0072	-0.0004 ± 0.0002	-0.00005 ± 0.00007	299	0.120
A. albopictus	50	0.0348 ± 0.0069	-0.0005 ± 0.0001	-0.00006 ± 0.00008	278	0.125

Regressions are based on the Lotka-Volterra growth equation for each species, $r'_i \approx \frac{dN_i}{N_i dt} = r_{mi} + b_i N_i + b_j N_j$ in which each species has a maximum per capita rate at minimal density, r_{mi}, and the coefficients b_i and b_j correspond to the response to conspecific ($-r_{mi}/K_i$) and heterospecific ($-r_{mi}\alpha_{ij}/K_i$) densities, respectively. This model assumes linear decline in r'_i as density of either species increases. Per capita growth rate estimates r'_i were obtained for each species within each replicate tire segment by the method of Livdahl and Sugihara (1984). Standard errors for each regression term are shown. Only the conspecific regression coefficients (b_1) are significantly different from zero ($P < 0.001$). Units are as follows: r_m, days^{-1}; b_1 and b_2, days^{-1} × (individuals/250 ml)$^{-1}$. The number of r' values obtained for each regression (n) differ because some cohorts produced no survivors and those replicates were randomly conjoined with replicates that did yield survivors. Carrying capacities are estimated by $K_i = -b_i/r_{mi}$, shown in individuals per litre, and competition coefficients by $\alpha_{ij} = b_2/b_1$. Competition coefficients are dimensionless

species in the face of a dynamic landscape of mostly domestic habitats.

The rapid spread of *Aedes* mosquitoes across the islands (Fig. 2) is consistent with Bermuda health officers' observation that human activities could be helping the spread of *Aedes* across the islands. Movement and exchange are commonplace for various containers, including potential habitats

ranging from ornamental bromeliads to construction-related water drums, domestic trash, moored or landed boats, and other elements suitable for *Aedes* breeding. The lack of an association between oviposition activity and distance to buildings differentiates the existing mosquito-road association from a mere human density factor, considering that both are interrelated. The mosquito-road association suggests that disposal of domestic elements such as cups, cans, or bottles could be helping mosquitoes, providing both new breeding habitats and opportunities for dispersal.

Local variation in abundance and frequent shifts in abundance peaks (darker pixels in maps, Fig. 2) among years could be attributed to variation in habitat availability and quality. The decrease in temporal correlation through time (Fig. 4), supports this interpretation and suggests a dynamic spatial and temporal variation in local mosquito populations. Constant, fine scale extinction and colonization events likely occur across the islands, as breeding habitats are continuously created and destroyed. Control officers' responses and citizens' awareness of high mosquito densities are most likely a major factor reducing local mosquito populations and producing the observed displacements of high density peaks.

Considering the previous success of *A. aegypti* in the Bermuda Islands, both in historic times (during which it was responsible for numerous yellow fever epidemics and a dengue outbreaks), and in the period 1997–2000, intrinsic environmental variables can probably be disregarded as causes for its disappearance; most likely, the decline relates to the *A. albopictus* introduction in 2000. At first glance, the observed decline in *A. aegypti,* concurrent with the increase in *A. albopictus* numbers, appears consistent with a competitive exclusion scenario. The decrease in *A. aegypti* occurred at the same time that the total numbers of eggs sharply increased during the period 2000–2002. The total number of eggs leveled off in the period 2002–2007, which could suggest that *A. albopictus* had taken over all breeding habitats, reaching carrying capacity (Fig. 5). However, if competitive exclusion between two similar species takes place following the classical competition theory, it could be expected to occur when the superior competitor is close to its equilibrium density (see Fig. 1a, b).

An abrupt decline of one species while its competitor is at low densities is possible under classical theory if the carrying capacities of the two competitors are markedly different (asymmetric competition, *sensu* Lawton and Hassell 1981). Juliano (1998) reared these species at very high densities of larvae within plastic and nylon enclosures (approximately 200 and 600 initial larvae per litre) in filtered tire water with or without fresh leaf litter, and observed superior success by *A. albopictus* at high density with no leaf litter added. The results suggest a disparity of K values, although K was not estimated. The larval enclosures for this experiment were vertically oriented, i.e., relatively deep and narrow.

However, Livdahl (1993) grew the same species in tire segments under field conditions, using unfiltered tire water with its debris. Lower densities were used than in Juliano's study (120–480 larvae per litre*),* and larvae were free to browse throughout the 250 ml of fluid contained within each tire segment. Larval habitats in this experiment were horizontal in orientation i.e., relatively shallow and broad. Regressions of per capita growth rate estimates for these species (summarized in Table 2) yielded rather similar carrying capacities for the two species, and neither species had a significant impact on the other's success. Estimates of competition coefficients (α) were similar, although the error in the parameter estimates needed to calculate α around these estimates is sufficient to leave much room for doubt. Perhaps the physical heterogeneity of unmodified tire water enabled the growth of various kinds of microbial resources that the two species could exploit differentially. If they do exploit different resources as larvae (and Juliano's results do show a much greater need for leaf litter by *A. aegypti* than by *A. albopictus*), then we would not expect such a rapid decline of *A. aegypti* as we have seen in the present study (Fig. 5).

The disparity between the results of those two competition studies, which were conducted concurrently in the same locality, illustrates both the potential variability in competitive intensity that could be faced by larvae occupying a wide variety of habitat conditions, as well as the difficulty in interpreting population dynamics from the restricted perspective of a larval competition experiment. Certainly, the conditions faced by larvae of both species would vary much more in field populations,

which may occupy containers ranging in size from bottle caps to cisterns.

In sum, we consider that a rapid competitive exclusion scenario such as represented in Fig. 1c, d is unlikely after the following considerations. The per capita growth rate and K estimations for both species are similar (Table 2), which should not allow the observed fast decline of *A. aegypti* (Fig. 5). In addition, *A. albopictus* population only appears to have leveled off after *A. aegypti*'s acute decrease, reaching much higher levels in later years, suggesting that it was not close to its equilibrium density (K), a condition for competitive exclusion of species that have similar competitive parameters (see Fig. 1). Furthermore, the intensity of competition between these two species appears to vary substantially with different experimental conditions (e.g., Black et al. 1989; Juliano 1998, 2007; Livdahl 1993) which suggests that in highly heterogeneous urban environments, such as in Bermuda where multiple containers types are available, and with constant turnover of habitats, each species could be able to prevail in certain types. Following these considerations, it appears that an explanation based on larval resource competition alone is incomplete. Perhaps classical theory does not describe accurately the present situation, e.g., nonlinear responses to conspecific or interspecific density could exist, or other processes besides competition should be reconsidered.

Several additional considerations could be made; one possibility is that *A. albopictus* introduced diseases to the islands that compromise *A. aegypti* populations (such as *Ascogregarina*, e.g., Blackmore et al. 1995), causing a reduction in their rate of growth. Juliano (1998) found no evidence to support this idea in his Florida competition study, but he did not manipulate the presence or absence of parasites to examine relative susceptibilities, and did not examine dead larvae from his competition experiment, in which some treatment combinations resulted in total larval mortality. The potential for parasite alteration of a competitive interaction has been shown by Aliabadi and Juliano (2002), who showed a reduced dominance by *A. albopictus* when competing with native *A. triseriatus*. The degree of host specificity and pathogenicity by the two species of *Ascogregarina* involved remains uncertain; some studies indicate that cross-infection between these two mosquitoes and their natural parasites can occur with deleterious effects (Munstermann and Wesson 1990), and others do not (Blackmore et al. 1995 found higher prevalence of *Ascogregarina* spp. among *A. aegypti* in areas that had not yet been invaded by *A. albopictus*), but the *Ascogregarina-Aedes* interactions have still not been thoroughly studied and a number of other parasites, including fungi, flagellates and bacteria could also play a role (Fukuda 1992). Without direct examination of the full array of potential parasites, it is difficult to exclude parasitism as a contributing factor because a parasite may impose effects on its hosts similar to those that could be expected from resource competition.

Mediation of competition by climate has been suggested as a mechanism for coexistence between these species, which has been observed after periods of drought (Britch et al. 2008) and microclimates that favor the more desiccation resistant eggs of *A. aegypti* (Juliano et al. 2002). Climate trends could also accelerate the competitive exclusion process. *A. aegypti* may be near the limits of its ecological range in terms of tolerance to winter conditions in subtropical habitats such as Florida and Bermuda. Figure 3 indicates a dramatic decline in activity during Bermuda's winter, and *A. albopictus*, which most likely has origins in temperate Asia, may have an advantage under such conditions that could accelerate the displacement of *A. aegypti*.

None of these alternatives should be considered as an exclusive explanation; potential synergistic effects with competition need to be taken in consideration. These results fuel interesting questions and invite further studies in this system that could help clarify the mechanism of displacement. Competition experiments between *A. albopictus* and *A. aegypti* performed in the field would allow a test of the competitive exclusion hypothesis and the appropriateness of the classical theory for this system. However, given the variety of different sorts and sizes of containers used by these species, it would be difficult to conduct an experiment that encompasses the full diversity of habitats, and it is always possible to force species to compete in an experimental setting. In addition, the *A. albopictus* population in the islands could be screened with molecular methods for microparasites known to infect *A. aegypti*. The investigation by health department officers of locations of very early detections of *A. albopictus* suggested a single entry in the islands associated

with a single bromeliad shipment (D. Kendell, pers. obs.). If a very limited number of individuals are responsible for *A. albopictus'* invasion in Bermuda, it seems likely that they did not carry the complete load of microparasites that otherwise could be found associated with *A. albopictus* in other regions of the world. We have recently identified *Ascogregarina taiwanensis* in Bermuda *A. albopictus* larvae (J. Sohighian and T. Livdahl, unpublished), so *A. albopictus'* success cannot be attributed to an escape from parasitism, although there could be other parasites, such as microsporidians (Andreadis 2007), that may interact with these species and alter the outcome. It is interesting to note that microsporidians occur in *A. aegypti* host populations but have yet to be discovered in invasive *A. albopictus* populations (T. Andreadis, pers. comm.) associated with *A. albopictus* in other regions of the world.

References

Aliabadi BW, Juliano SA (2002) Escape from gregarine parasites affects the competitive interactions of an invasive mosquito. Biol Inv 4:283–297

Andreadis TG (2007) Microsporidian parasites of mosquitoes. J Am Mosq Control Assoc 23(sp2):3–29

Beaty J, Aitken THG (1979) In vitro transmission of yellow fever virus by geographic strains of *Aedes aegypti*. Mosq News 39:232–238

Benedict MQ, Levine RS, Hawley WA, Lounibos LP (2007) Spread of the tiger: global risk of invasion by the mosquito *Aedes albopictus*. Vector-Borne Zoonot Dis 7:76–85

Beyer HL (2004) Hawth's analysis tools for ArcGIS. Available at http://www.spatialecology.com/htools

Black WC, Rai KS, Turco BJ, Arroyo DC (1989) Laboratory study of competition between United States strains of *Aedes albopictus* and *Aedes aegypti* (Diptera: Culicidae). J Med Entomol 26:260–271

Blackmore MS, Scoles GA, Craig GC Jr (1995) Parasitism of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) by *Ascogregarina* spp. (Apicomplexa: Lecudinidae) in Florida. J Med Entomol 32:847–852

Boromisa RD, Rai KS, Grimstad PR (1987) Variation in the vector competence of geographic strains of *Aedes albopictus* for dengue 1 virus. J Am Mosq Control Assoc 3:378–386

Braks MAH, Honório NA, Lourenço-de-Oliveira SA, Juliano SA, Lounibos LP (2003) Convergent habitat segregation of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) in southeastern Brazil and Florida. J Med Entomol 40:785–794

Braks MAH, Honório NA, Lounibos LP, Lourenço-De-Oliveira R, Juliano SA (2004) Interspecific competition between two invasive species of container mosquitoes, *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae), in Brazil. Ann Entomol Soc Am 97:130–139

Britch SC, Linthicum KJ, Anyamba A, Tucker CJ, Pak EW (2008) Long-term surveillance data and patterns of invasion by *Aedes albopictus* in Florida. J Am Mosq Control Assoc 24:115–120

Camargo S (1967) History of *Aedes aegypti* eradication in the Americas. Bull WHO 36:602–603

Castro Gomes A, Souza JP, Bergamaschia DP, dos Santos JF, Roberto Andrade VR, Leiteb OF, Rangel O, Souza SL, Guimarães NN, Limab VC (2005) Anthropophilic activity of *Aedes aegypti* and of *Aedes albopictus* in area under control and surveillance. Rev Saúde Pública 39:1–5

Clark Labs (2007) Idrisi Los Andes edition v.15.0. Worcester

Diallo M, Ba Y, Faye O, Soumare ML, Dia I, Sall AA (2008) Vector competence of *Aedes aegypti* populations from Senegal for sylvatic and epidemic dengue 2 virus isolated in West Africa. Trans R Soc Trop Med Hyg 102:493–498

ESRI (2006) ArcMAP version 9.2. Redlands

Fontenille D, Rodhain F (1989) Biology and distribution of *Aedes albopictus* and *Aedes aegypti* in Madagascar. J Am Mosq Control Assoc 5:219–225

Francy DB, Moore CG, Eliason DA (1990) Past, present and future of *Aedes albopictus* in the United States. J Am Mosq Control Assoc 6:127–132

Fukuda T (1992) A preliminary report of a survey for parasites and pathogens of *Aedes albopictus* and other container-inhabiting mosquitoes in North Central Florida. J Fla Mosq Control Assoc 63:76–77

Gilotra SK, Rozeboom LE, Bhattacharya NC (1967) Observations on possible competitive displacement between populations of *Aedes aegypti* linnaeus and *Aedes albopictus* skuse in Calcutta. Bull WHO 37:437–446

Griswold MW, Lounibos LP (2006) Predator identity and additive effects in a treehole community. Ecology 87:987–995

Gubler DJ (1998) Dengue and dengue hemorrhagic fever. Clin Micro Rev 11:480–496

Hawley WA (1988) The biology of *Aedes albopictus*. J Am Mosq Control Assoc 4(sp1):1–40

Hidayati H, Mohd SA, Nazni WA, Lee HL (2005) Insecticide resistance development in *Culex quinquefasciatus* (Say), *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse) larvae

against malathion, permethrin and temephos. Trop Bio-med 22:45–52

Hornby JA, Moore DE, Miller TW Jr (1994) *Aedes albopictus* distribution, abundance, and colonization in Lee County, Florida, and its effect on *Aedes aegypti*. J Am Mosq Control Assoc 10:397–402

Insightful Corp (2007) S-plus v.8.0.4. Palo Alto

Juliano SA (1998) Species introduction and replacement among mosquitoes: interspecific resource competition or apparent competition? Ecology 79:255–268

Juliano SA (2007) Species interactions among larval mosquitoes: context dependence across habitat gradients. Annu Rev Entomol 54:37–56

Juliano SA, O'Meara GF, Morrill JR, Cutwa MM (2002) Desiccation and thermal tolerance of eggs and the coexistence of competing mosquitoes. Oecologia 130:458–469

Juliano SA, Lounibos LP, Nishimure N, Greene K (2009) Your worst enemy could be your best friend: predator contributions to invasion resistance and persistence of natives. Oecologia Online publication date: 16 Oct 2009

Kesavaraju B, Damal K, Juliano SA (2008) Do natural container habitats impede invader dominance? Predator-mediated coexistence of invasive and native container-dwelling mosquitoes. Oecologia 155:631–639

Kobayashi M, Nihei N, Kurihara T (2002) Analysis of northern distribution of *Aedes albopictus* in Japan by geographical information system. J Med Entomol 39:4–11

Lawton JH, Hassell MP (1981) Asymmetrical competition in insects. Nature 289:793–795

Livdahl T, Sugihara G (1984) Nonlinear interactions of populations and the importance of estimating per capita rates of change. J Anim Ecol 53:573–580

Livdahl T (1993) Competition and egg-hatch inhibition among tire-breeding mosquitoes. Technical report, Florida Department of Agriculture and Consumer Services, Bureau of Entomology and Pest Control, Jacksonville, FL

Livdahl T, Willey MS (1991) Prospects for an invasion: competition between *Aedes albopictus* and native *Aedes triseriatus*. Science 253:189–191

Lounibos LP (2002) Invasions by insect vectors of human disease. Annu Rev Entomol 47:233–266

Lounibos LP (2007) Competitive displacement and reduction. AMCA Bull 23(sp):276–282

Mitchell CJ (1995) The role of *Aedes albopictus* as an arbovirus vector. Parasitology 37:109–113

Moore PR, Johnson PH, Smith GA, Ritchie SA, Van Den Hurk AF (2007) Infection and dissemination of dengue virus type 2 in *Aedes aegypti*, *Aedes albopictus*, and *Aedes scutellaris* from the Torres Strait, Australia. J Am Mosq Control Assoc 23:383–388

Munstermann LE, Wesson DM (1990) First record of *Ascogregarina taiwanensis* (Apicomplexa: Lecudinidae) in North American *Aedes albopictus*. J Am Mosq Control Assoc 6:235–243

Murrell EG, Juliano SA (2008) Detritus type alters the outcome of interspecific competition between *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae). J Med Entomol 45:375–383

Novak RJ (1992) The Asian tiger mosquito, *Aedes albopictus*. Wing Beats 3:5

O'Meara GF, Gettman AD, Evans LF Jr, Scheel FD (1992) Invasion of cemeteries in Florida by *Aedes albopictus*. J Am Mosq Control Assoc 8:1–10

O'Meara GF, Evans LF Jr, Gettman AD, Cuda JP (1995) Spread of *Aedes albopictus* and decline of *Ae. aegypti* (Diptera: Culicidae) in Florida. J Med Entomol 32:554–562

Pumpuni CB, Kneples J, Craig G (1992) Influence of temperature and larval nutrition on the diapause inducting photoperiod of *Aedes albopictus*. J Am Mosq Control Assoc 8:223–227

Rawlins SC, Martinez R, Wiltshire S, Legall G (1998) A comparison of surveillance systems for the dengue vector *Aedes aegypti* in Port of Spain, Trinidad. J Am Mosq Control Assoc 14:131–136

Rosenberg MS (2001) PASSAGE. Pattern analysis, spatial statistics, and geographic exegesis. Version 1.0. Tempe

SAS Institute Inc (2007) JMP version 7.0. Cary

Stasiak RS, Grothaus RH, Miner WF (1969) Susceptibility of *Aedes albopictus* (skuse) larvae from South Vietnam to five insecticides, 1969. Mosq News 30:246–249

Tabachnick WJ (1991) Evolutionary genetics and arthropod-borne disease. The yellow fever mosquito. Am Entomol 37:14–24

Urbanelli S, Bellini R, Carrieri M, Salicandro P, Celli G (2000) Population structure of *Aedes albopictus* (Skuse): the mosquito which is colonizing Mediterranean countries. Heredity 84:331–337

Wesson DM (1990) Susceptibility to organophosphate insecticides in larval *Aedes albopictus*. J Am Mosq Control Assoc 6:258–264

Wesson DM, Porter CH, Collins FH (1992) Sequence and secondary structure comparisons of ITS rDNA in mosquitoes (Diptera: Culicidae). Mol Phyl Evol 1:253–269