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Community Ecology of Container Mosquitoes (Diptera: Culicidae) in Virginia Following Invasion by *Aedes japonicus*

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Abstract

The success of an invasive species in a new region depends on its interactions with ecologically similar resident species. Invasions by disease vector mosquitoes are important as they may have ecological and epidemiological consequences. Potential interactions of a recent invasive mosquito, *Aedes japonicus* Theobald, with resident species in Virginia were evaluated by sampling larvae from containers and trapping adults. Distinct species compositions were observed for artificial containers and rock pools, with *Ae. albopictus* most abundant in the former and *Ae. japonicus* in the latter. However, these two species were found to co-occur in 21.2% of containers sampled. Among the six mosquito species most common in containers from May through September, 2006, only interspecific associations of *Ae.japonicus* with *Aedesalbopictus*(Skuse) and *Aedestriseriatus*(Say) were significant, and both were negative. In addition to differences in habitat preference, mean crowding estimates suggest that interspecific repulsion may contribute to the significant negative associations observed between these species. High relative abundances of late instars and pupae of *Ae. japonicus* seem to provide this species with a mechanism of evading competition with *Ae. albopictus*, facilitating their coexistence in artificial containers. Although annual fluctuations were observed, trends in adult populations over a 6-yr period provide no evidence of declines. In summary, this survey of diverse container types and all life stages provided only limited evidence for competitive displacements or reductions of resident container species by *Ae. japonicus*, as observed elsewhere in its invasive range.

Keywords

invasive; mosquito; container-inhabiting; interspecific interaction

Biological invasions are occurring at an alarming frequency worldwide, the impacts of which threaten biodiversity, ecosystem functioning, resource availability, national economies, and human health (Simberloff 1996, Pimentel et al. 2005, Vilà et al. 2010). Historically, some of the most important biological invasions have involved disease vectors, particularly mosquitoes (Diptera: Culicidae), a trend that has continued at an increasing rate over the past century (Lounibos 2002, Juliano and Lounibos 2005). The most recent successful invasions of mosquitoes have resulted from human transport of immature stages,

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especially of species that occupy container habitats (Lounibos 2002). Competition, predation, and parasitism among immature stages have been shown to play important roles in structuring mosquito communities of container habitats. In addition to their effects on resident species or ecosystems, invasive mosquitoes are of particular concern because they may also impact human or vertebrate animal health (Juliano and Lounibos 2005).

Native to Japan, Korea, Taiwan, and China (Tanaka et al. 1979), *Aedes*(*Finlaya*) *japonicus* Theobald (Diptera: Culicidae) is one of the most recently recognized mosquito species to invade the United States. Initially collected in the United States in 1998 (Peyton et al. 1999, Andreadis et al. 2001), *Ae. japonicus* has since become established throughout the northeastern United States (Scott 2003) and has expanded west of the Mississippi River (Reeves and Korecki 2004, Gallitano et al. 2005, Dunphy et al. 2009, Neitzel et al. 2009). Separate establishments of *Ae. japonicus* have occurred in Oregon, WA (Roppo et al. 2004, Irish and Pierce 2008) and Hawaii (Larish and Savage 2005). Distinct genetic profiles of *Ae. japonicus* collected from various sites in Japan and throughout its range in the United States suggest multiple introductions of the species in the United States (Fonseca et al. 2001, 2010) via international transport of used tires (Peyton et al. 1999, Lounibos 2002).

In both its native range and the United States, *Ae. japonicus* larvae primarily inhabit a wide variety of natural (treeholes, rock pools) and artificial containers (buckets, tires, and birdbaths), with rock pools being the preferred habitat (Tanaka et al. 1979, Andreadis et al. 2001, Scott 2003). *Ae. japonicus* are known to co-occur with numerous resident species in natural and artificial containers in North America (Andreadis et al. 2001, Scott et al. 2001, Oliver et al. 2003, Thielman and Hunter 2006, Andreadis and Wolfe 2010). The likelihood of *Ae. japonicus* colonizing a new region depends on its ability to contend with new environmental conditions and compete with resident species that occupy a similar ecological niche. In the United States, *Ae. japonicus* is most likely to co-occur with the indigenous rock hole mosquito, *Aedes atropalpus* (Coquillett), the Eastern treehole mosquito, *Aedes triseriatus* (Say) and its sibling species, *Aedes hendersoni* Cockerell, as well as the established invader *Aedes albopictus* (Skuse). Experimental results suggest that *Ae. japonicus* larvae are effective competitors in both natural and artificial containers (Armistead et al. 2008a, b; Alto 2011), and larval surveillance suggests that its presence in these habitats may contribute to population reductions of several species native to northeastern United States (Andreadis and Wolfe 2010).

In addition to ecological effects on other species, *Ae. japonicus* may pose public health risks as a vector. In the laboratory *Ae. japonicus* has demonstrated the ability to transmit Japanese encephalitis (Sucharit et al. 1989, Takashima and Rosen 1989) and Getah (Takashima and Hashimoto 1985) viruses, although it is not considered an important vector of either virus in its native range. *Ae. japonicus* is also a competent laboratory vector of eastern equine encephalitis (Sardelis et al. 2002a), LaCrosse (Sardelis et al. 2002b), St. Louis encephalitis (Sardelis et al. 2003), chikungunya, dengue (Schaffner et al. 2011), and West Nile viruses (WNV) (Sardelis and Turrell 2001, Turell et al. 2001). However, in the United States only WNV has been detected in wild-caught females (Werner 2001, White et al. 2001, Scott 2003, Centers for Disease Control and Prevention [CDC] 2009). The tendency of *Ae. japonicus* to feed primarily on mammalian hosts, including humans (Miyagi 1971, Scott 2003, Apperson et al. 2002, Molaei et al. 2009) illustrates the vector potential for this species. As of yet, however, reports of *Ae. japonicus* as a major nuisance species, especially when compared with *Ae. albopictus,* appear to be limited (Schaffner et al. 2011). Involvement of newly established *Ae. japonicus* in existing disease transmission cycles has the potential to alter the nature of an existing public health threat by changing the transmission rate because of differences in vector competence (Juliano and Lounibos 2005).

To assess potential interactions of *Ae. japonicus* in mosquito container communities and identify ecological processes that may be operating as a result of the invasion of this species, field surveys of larvae and adults were conducted in an area where this species is known to occur. Sampling of natural and artificial larval habitats was used to confirm and quantify co-occurrences and potential interactions of *Ae.japonicus* with resident mosquitoes. Routine trapping of adults over a 6-yr period was used to assess trends in abundance of the species of interest. The current study especially focuses on potential interactions among native and invasive *Aedes* spp. to evaluate the potential for competition resulting from their co-occurrence.

Materials and Methods

Study Site

A broad survey of immature and adult container-dwelling mosquitoes was conducted in Fair-fax County, VA (latitude 38° 50′: N, longitude 77° 7′: W), a suburb of Washington, DC.

Larval Collections

A county-wide field survey of containers was conducted from May through September 2006. Sampling was predominantly limited to public or county property; however, containers located at private residences were sampled when responding to nuisance mosquito complaint calls to the Fairfax County Health Department. A few tree holes and ornamental bromeliads were sampled at different times, but rock pools of the Potomac River (and tributaries) were the only natural containers yielding many observations. Containers were only sampled once, and the entire aquatic contents of each were removed, volume determined, and sieved through a mesh fine enough to retain first instars. Samples were transported to the laboratory in 18-ounce (532.32 ml) *Whirl-Pak* bags (Nasco, Fort Atkinson, WI). Wet containers without mosquitoes were noted. The GPS coordinates of each site, sun exposure (none, partial, or full), and container type were recorded.

In the laboratory, fourth instars from each container were identified to species and counted. All other immatures were counted, sorted and separated by stage, placed in separate 21 × 12 cm cylindrical growth chambers (BioQuip Products, Inc., Rancho Dominguez, CA) containing 400 ml of tap water and food (3:1 mix of ground rabbit chow and brewer's yeast), and reared to fourth instar for identification. Predators, such as *Toxorhynchitesrutilus* (Coquillett), were identified and removed. Mosquito pupae were identified at adult emergence. Mortality at all immature stages was noted.

Data Analysis

Three metrics were used to assess overall mosquito community structure: Kendall's co-efficient of rank correlation, tau (τ) (Ghent 1963); C_8 coefficient of association (Hurlbert 1969); and mean crowding (Lloyd 1976, Rathcke 1976). Significant similarity between the ranked abundance of the six most common species collected (*Ae. albopictus, Ae. japonicus, Culex pipiens, Culex restuans* Theobald, *Ae. triseriatus,* and *Ae. hendersoni*) from small versus large artificial containers, and rock pools versus tires and artificial containers was determined using Kendall's τ. Associations between co-occurring species were quantified from presence-absence data using the C_8 coefficient of association, which ranges from 1 to −1. Positive associations between species may reflect a common habitat preference or interspecific attraction, while negative associations may result from different habitat preferences or interspecific repulsion.

Significance of positive and negative association (C_8 values) was assessed with a corrected χ^2 formula (Pielou 1977) or Fisher exact test in cases with cell totals less than or equal to five. Indices of mean intra-and interspecific crowding per unit resource (container fluid volume), were also calculated. Interspecific mean crowding of species x by y per unit resource a was quantified as $\Sigma(x_i y_i/a_i)/\Sigma x_i$, and intraspecific mean crowding by the same formula but substituting $(x_i - 1)$ for y_i

A subset of analyses was conducted to analyze habitat preferences and population performance of *Ae. Albopictus* and *Ae.japonicus.* Specifically, the effects of container type ($n = 2$), sun exposure ($n = 3$) and interaction of container type X sun exposure on collection frequency, mean crowding, and average instar number were determined. Because of limited samples in May and September, the effects of month were excluded. The effects of these variables on collection frequency was determined using the CATMOD procedure (SAS Institute 1989) with subsequent maximum likelihood contrasts, while two-way ANOVAs were used to assess their effects on average instar number, intra-, and interspecific mean crowding for each species. Finally, a Kolmogorov–Smirnov two-sample test was used to compare the distributions of average instar number, with pupae designated as the number five, for *Ae. japonicus* and *Ae. albopictus.*

Adult Collections

Adult mosquitoes were collected with carbon dioxide (CO_2)-baited CDC light traps (John Hock Co., Gainesville, FL) and gravid traps (Reiter 1987) (BioQuip Products, Inc., Rancho Dominguez, CA) set at the same 70 preselected sites located throughout Fairfax County each May through October (epidemiological weeks 20 – 40) from 2004 to 2009. Gravid traps were baited with a fermented mixture of water, freshly mowed grass clippings, hay, and Brewer's yeast. One of each trap type was set weekly at each site. Traps were set in the early afternoon and retrieved the following morning. All adult female mosquitoes collected were sorted, counted and identified to species. Efforts were made to distinguish between the morphologically similar *Ae. hendersoni* and *Ae. triseriatus;* however, it is likely that data presented here reflect a mixed sample of both species. The mean number of adult female *Ae. albopictus, Ae. japonicus,* and *Ae. triseriatus* per trap night was calculated for each week.

Results

Natural and Artificial Containers

During the study period, 191 containers (131 artificial and 60 natural) were sampled for the presence of larvae and pupae, of which 134 were positive for mosquitoes. These included rock pools, tires, flowerpot saucers, tarpaulins, drainpipes, French drains, birdbaths, cemetery vases, trash cans, drums, and other small miscellaneous artificial containers. The following 10 species were collected, in order of frequency of occurrence: *Ae. albopictus, Ae. japonicus, Culex pipiens, Cx. restuans, Ae. triseriatus, Ae. hendersoni, Anopheles punctipennis* Say, *Tx. rutilus, Ae. atropalpus,* and *Orthopodomyia signifera* (Coquillett). *Ae. atropalpus* and *An. punctipennis* were collected only from rock pools, the former on only four occasions, while *Tx. rutilus* and *Or. signifera* were collected only from artificial containers. These four species have not been included in any subsequent analyses because of limited sample size.

Kendall's coefficient of rank correlation (τ) was positive for all three habitat comparisons, but the rank abundance of species was only significantly similar for small versus large artificial containers (Fig. 1). *Ae. japonicus* was the most abundant species collected from rock pools, while *Ae. albopictus* was the most abundant in artificial containers (Figs. 1 and 2). These species were also the most productive in these habitats, as measured by the total number of pupae collected per container type (Table 1). Greater numbers of *Cx. pipiens, Cx. restuans,* and *Ae. triseriatus* pupae were recovered from artificial containers; however, *Cx. pipiens* was more productive in larger habitats while the opposite was found for *Ae.triseriatus*(Table 1). No difference in productivity was observed for *Ae. albopictus* or *Cx. restuans* collected from artificial containers.

Using the C_8 index of association, significant positive associations included those between *Cx. pipiens* and *Cx. restuans*, *Ae. albopictus* and *Ae. triseriatus*, *Ae. albopictus*, and *Ae. hendersoni*, and *Ae. hendersoni* and *Ae. triseriatus* (Table 2). *Ae. japonicus* was found to have significant negative associations with Ae. triseriatus and *Ae. albopictus*. The exclusion of rock pools had no effect on the statistical significance of any associations (Table 2). It should be noted that a non-significant coefficient of association of −0.04 was calculated for *Ae.atropalpus* and *Ae.japonicus* when only rock pools were included.

Calculations of mean intra- and interspecific crowding for the six most abundant mosquito species collected showed that *Ae. japonicus, Ae. triseriatus,* and *Cx. pipiens* encountered a higher density of *Ae. albopictus* than any other species or conspecifics, while *Ae. hendersoni* most frequently encountered a higher density of *Ae. triseriatus* (Table 3). *Ae. albopictus* and *Cx. restuans* both encountered a higher density of conspecifics than any other mosquito species. Based on these data, further analyses were performed to investigate the co-occurrences of *Ae. japonicus* with *Ae. albopictus.* The limited number of *Ae. triseriatus* sampled and low frequency of its collection prohibited further analyses of co-occurrences of this species with *Ae. japonicus.*

Co-occurrences of *Ae.japonicus* and *Ae.albopictus*

Ae. albopictus and *Ae. japonicus* were identified from 68.9 and 43.9%, respectively, of mosquito-positive containers sampled during the study, and co-occurred together in 21.2% of these containers. Both species were collected most frequently from June through August (Fig. 2A). *Ae.albopictus* was consistently present in higher abundance than *Ae. japonicus* in all months except May (Fig. 2B), even in July when *Ae. japonicus* was found in a greater percentage of containers. Container type was found to have a significant effect on collection frequency of both *Ae. albopictus* and *Ae.japonicus* (Table 4), with the former occurring predominantly in artificial containers and the latter in rock pools. A significant effect of sun exposure on collection frequency was found only for *Ae. japonicus,* with maximum likelihood contrasts suggesting a preference for fully or partly shaded containers (full shade versus full sun, $P = 0.0102$; partial vs. full sun, $P = 0.0007$). The ML ANOVA showed no significant container type X sun exposure interactions for either species.

Intraspecific mean crowding of both *Ae. albopictus* and *Ae. japonicus* followed similar trends throughout the study period, however *Ae. japonicus* encountered the highest density of conspecifics 1 mo before that of *Ae. albopictus* (Fig. 3). A similar pattern of intraspecific mean crowding was observed when only *Ae. japonicus* recovered from rock pools were considered for analysis (data no shown). Interspecific mean crowding of *Ae.japonicus* by *Ae.albopictus* was greater than that of *Ae. albopictus* by *Ae. japonicus* in all months except May, and was highest for both species in August (Fig. 3). No significant effects of container type, sun exposure, or container type X sun exposure on intraspecific mean crowding were observed for *Ae. albopictus* ($F_{5, 75} = 0.55$; $P = 0.7397$) or *Ae. japonicus* ($F_{4, 60} = 0.55$; $P = 0.702$). These variables were not found to have significant effects on interspecific mean crowding of *Ae. japonicus* by *Ae. albopictus* ($F_{4, 23} = 0.65$; $P = 0.6308$) or *Ae. albopictus* by *Ae. japonicus* ($F_{4, 23} = 1.41$; $P = 0.262$) either.

The average instar number, or age, of *Ae. albopictus* larvae from all collections during the study was 2.77, while that of *Ae.japonicus* was 3.86. When considering only those containers in which both species occurred, average instar numbers were similar (2.8 vs. 3.3). Neither container type nor sun exposure was found to have a significant effect on the average instar number of *Ae. albopictus* ($F_{5, 85} = 0.73$; $P = 0.6019$) or *Ae. japonicus*($F_{5, 52} = 1.59$; $P = 0.1788$). The average instar number of *Ae. japonicus* larvae was consistently greater than that of *Ae. albopictus,* with pupae comprising the greatest proportion of *Ae. japonicus* sampled in each month of the study (Fig. 4). A comparison of the overall instar distributions of the two species from May through September by a Kolmogorov–Smirnov two-sample test indicated a significant difference (D = 18.130; $P < 0.001$).

Adult Surveillance

Weekly collections of adult mosquitoes demonstrated that *Ae. albopictus* was consistently collected in greater abundance in CO_2-baited CDC light traps than gravid traps, while the opposite was found for *Ae. japonicus* (Fig. 5). Fluctuations in trapping abundance were observed for both species from 2004 to 2009, although these changes were most pronounced for *Ae.japonicus,* especially in gravid trap collections, which showed a marked decline for this species from 2004 to 2006 followed by an increase from 2007 to 2009. Similar trends were observed from gravid trap collections of *Ae. albopictus,* albeit to a lesser extent, while CDC light trap collections suggest that abundance was consistent across study years.

Discussion

Invasion success of a non-native species in a new region is dependent on its interactions with ecologically similar resident species. A field survey of natural and artificial containers was conducted to assess container-inhabiting mosquito community structure and glean insight into potential species interactions after the invasion of *Ae. japonicus* in Virginia. The mosquito species occupying the containers sampled during this study were consistent with previously published reports from the eastern United States, and the significant positive associations observed (Table 2) were not unexpected in container habitats as co-occurrences of these species in container habitats are well documented (e.g., Andreadis et al. 2001, Hribar et al. 2001, Yee 2008).

Distinct species compositions based on ranked abundances were observed for artificial containers and rock pools, with *Ae. albopictus* most abundant in the former and *Ae. japonicus* in the latter (Fig. 1). However, both species were frequently found to co-occur in containers sampled throughout the study period (Fig. 2). Data indicate a preference of *Ae. japonicus* for shaded habitats, particularly rock pools, while *Ae. albopictus* was more frequently collected from artificial containers (Fig. 1; Table 4). However, development and performance of neither species appeared to be significantly impacted by container type or sun exposure, as measured by average instar number and intraspecific mean crowding.

Differences in habitat preference likely contribute to the significant negative association between *Ae. albopictus* and *Ae. japonicus* (Table 2); however, this finding persisted even when rock pools were excluded from analyses, suggesting that interspecific repulsion may also be occurring. Mean crowding estimates provide some evidence for this, indicating that intraspecific competition is likely more important in constraining population growth of *Ae. albopictus* than competition with *Ae. japonicus,* while interactions with *Ae. albopictus* may be just as important as those with conspecifics for *Ae. japonicus* (Table 3). This notion is supported by recent field competition experiments in which higher survivorship, shorter developmental time, and higher population growth of *Ae. albopictus* under conditions of interspecific larval competition gave this species a competitive advantage over *Ae. japonicus,* suggesting negative effects on growth and development of *Ae. japonicus* in habitats where this species co-occurs with *Ae. albopictus* (Armistead et al. 2008a). However, an investigation of behavior in the presence of predators suggests that more highrisk behaviors exhibited by *Ae. albopictus* may promote coexistence with *Ae. japonicus* (Kesavaraju et al. 2011). It is also possible that selection for interspecific avoidance occurred before invasion, given that *Ae. japonicus* is sympatric with *Ae. albopictus* throughout most of its native range.

The persistence of *Ae. japonicus* in artificial containers in the presence to *Ae. albopictus* may also be facilitated by differences in seasonality and instar distributions. High relative abundances of pupae and older instars of *Ae. japonicus* (Fig. 4) and higher interspecific mean crowding of *Ae. japonicus* on *Ae. albopictus* than that of *Ae. albopictus* on *Ae. japonicus* early in the season (Fig. 3) suggest this species gets a head start over *Ae. albopictus* (Fig. 4). The earlier appearance of *Ae. japonicus* in the season my stem from this species ability to overwinter in the larval stage, an observation that has been made by others in its native range (LaCasse and Yamaguti 1950), New Jersey (Scott 2003), and North Carolina (B. Harrison, personal communication), or may simply be explained by earlier hatching of overwintering eggs. Regardless, it would appear to enable *Ae. japonicus* to avoid *Ae. albopictus* and escape competitive effects when the two species co-occur early in the season. However, it is unclear if *Ae. japonicus* is afforded a competitive advantage over *Ae. albopictus* when the former has a head start. The importance of cohort structure in density-dependent interspecific competition between these two species needs to be explored experimentally to address this question further.

Observations made here on differences in habitat preference and seasonality suggest that the invasion of *Ae. japonicus* in Virginia will ultimately promote its coexistence with *Ae. albopictus.* Population trends gleaned from broad-based surveillance of adult females of these species suggest that this is the case in Fairfax, VA. Although populations of *Ae. japonicus* appeared to be on the decline during the first years of surveillance (2004 –2006), a subsequent rebound was observed from 2007 to 2009. These observations are likely attributable to annual variations in environmental conditions (i.e., temperature and rainfall) and do not reflect a local decline, or suggest an eventual extinction, of *Ae. japonicus* populations after the introduction and initial expansion of this species throughout the area. The varying attractiveness of CO_2-baited CDC light traps and gravid traps to the target species has been well characterized, and collections using these methods clearly reflect different population subsets (host vs. oviposition seeking); however, the size, duration, and consistency of these regular adult collections should sufficiently overcome these inherent biases to provide an accurate depiction of population trends.

The current study focused primarily on populations of *Ae. albopictus* and *Ae. japonicus,* however interesting relationships between other container-inhabiting species were observed. *Ae. triseriatus* and *Cx. pipiens* were found to encounter a greater density of *Ae. albopictus* than any other species or conspecifics (Table 3), suggesting that interspecific interactions are likely to affect mosquito and population performance of these species. This is supported by multiple studies in which *Ae. albopictus* was found to be competitively superior to both *Ae. triseriatus* (Livdahl and Willey 1991, Novak et al. 1993, Teng and Apperson 2000) and *Cx. pipiens* (Costanzo et al. 2005). The frequency of collection and abundance of *Ae.triseriatus* larvae were low during this study, although adult mosquito surveillance suggests that this is a reflection of sampling methods, particularly a paucity of tree hole collections, and does not represent a true decline in *Ae. triseriatus* populations. An evaluation of co-occurrences between *Ae. triseriatus* and *Ae. japonicus* was not possible, although mean crowding estimates indicate that interactions with *Ae. albopictus* are more likely important for both of these species than interactions with each other (Table 3). These findings are consistent with competition experiments that have shown only minimal evidence for competitive asymmetry between *Ae. japonicus* and *Ae. triseriatus* (Alto 2011). However, the decline or displacement of *Ae. triseriatus* in container habitats associated with an increased prevalence of *Ae.japonicus* has been observed in multiple larval surveys (Joy and Sullivan 2005, Burger and Davis 2008, Andreadis and Wolfe 2010), and warrants further investigation.

Finally, the successful establishment of *Ae. japonicus* in Fairfax County, VA, appears to be associated with a population decline and potential displacement of *Ae. atropalpus* in local rock pools. The limited collection of this native rock pool mosquito while surveying these habitats is cause for concern as this species was once abundant throughout the area; in fact its type locality given by Coquillett (1902) is nearby Plummer's Island in Montgomery County, MD. The decline of *Ae. atropalpus* in rock pools has been observed in Connecticut (Andreadis and Wolfe 2010) and North Carolina (B. Byrd, personal communication), and this species is absent from New Jersey rock pools, albeit common in tires (Scott et al. 2001). It is important to note that two major flooding events caused by heavy rains and a hurricane occurred during the larval habitat portion of this study and limited rock pool collections in late June and early July, and again in early September. Although no conclusions can be drawn from the limited co-occurrence of these species during this study, it is possible that interspecific larval resource competition with *Ae. japonicus* in rock pools may have had negative effects on populations of *Ae. atropalpus* in areas where these two species co-occur. These findings are supported by recent laboratory competition experiments in which *Ae. atropalpus* was found to be more sensitive to larval densities than *Ae. japonicus* (Armistead et al. 2008b). However, the opposite has also been demonstrated (Hardstone et al. 2012). Differences in overwintering strategies (eggs vs. larvae) or later hatching of diapausing eggs than *Ae. japonicus* may have played a role in the seeming decline of *Ae. atropalpus* in this region.

In conclusion, the invasion success of *Ae. japonicus* in Fairfax, VA, appears to be associated with both its preference for rock pools and its early seasonal appearance. These data suggest that *Ae. japonicus* is likely to persist in both rock pools and artificial containers, even in the presence of *Ae. albopictus.* Although it is unclear when *Ae. atropalpus* populations began to decline in local rock pools, there is some evidence to suggest that it may have resulted from interactions with *Ae. japonicus.* In contrast, *Ae. japonicus* appears to be able to overcome the superior competitive ability of *Ae.albopictus* through differences in seasonality, specifically with higher relative abundances of older instars early in the season. While this study focused predominantly on the potential inter-specific interactions of *Ae. japonicus* with resident mosquito species in artificial containers and rock pools, other factors, particularly predators, may be influencing the structure of these communities and should be investigated. Furthermore, the tolerance of these species to varying environmental conditions, intraguild predation, or differences in foraging behavior may be important in their interspecific interactions.

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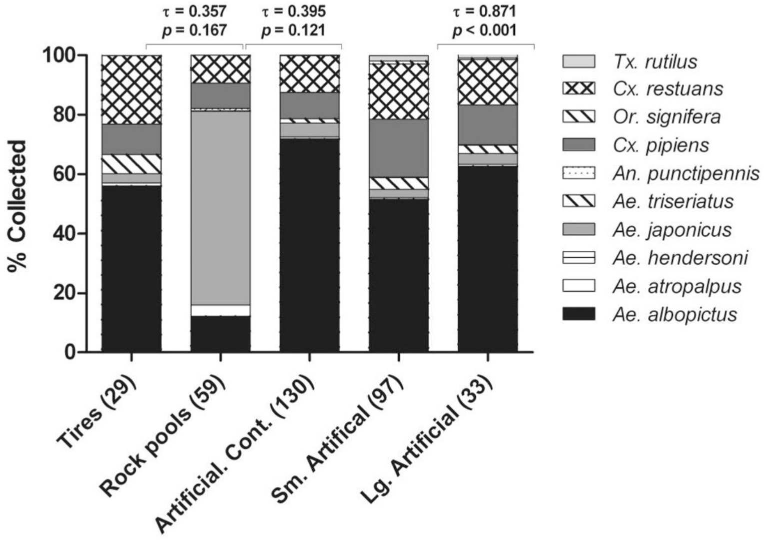

Fig. 1.

Mosquito species composition of container habitats expressed as percentage of total mosquitoes collected in Fairfax County, VA, from May through September 2006. The number of each type of container sample is provided on the x-axis as (*n*). Kendall's coefficient of rank correlation (τ) to test similarity of compositions and associated *P* values for each habitat comparison are indicated above the histogram bars.

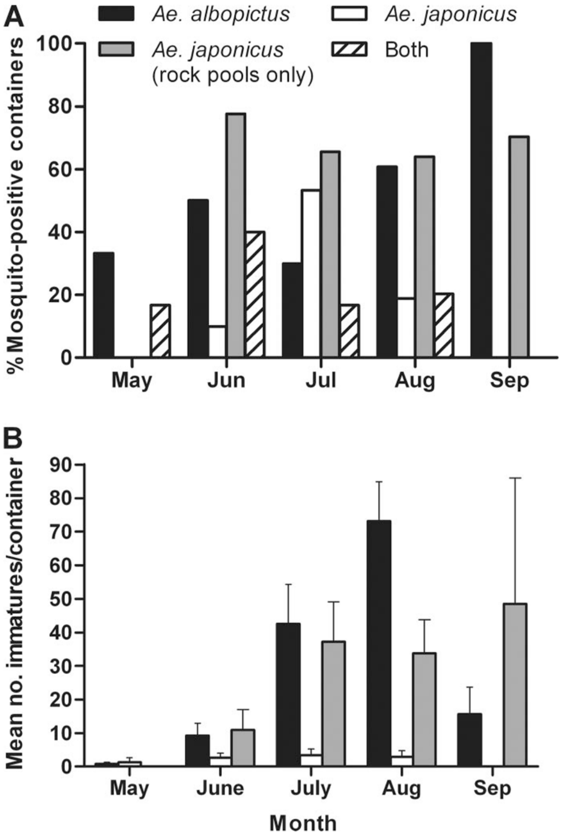

Fig. 2.

Seasonal occurrences of *Ae. albopictus* and *Ae. japonicus* collected from artificial containers May through September, 2006. (A) Proportion of mosquito-positive containers containing *Ae. albopictus*, *Ae. japonicus*, *Ae. japonicus* from rock pools collections only, or both species, by month. (B) Monthly abundance (mean number of mosquitoes collected per container) of *Ae. albopictus* and *Ae. japonicus* (±SE) from 91 mosquito-positive artificial containers.

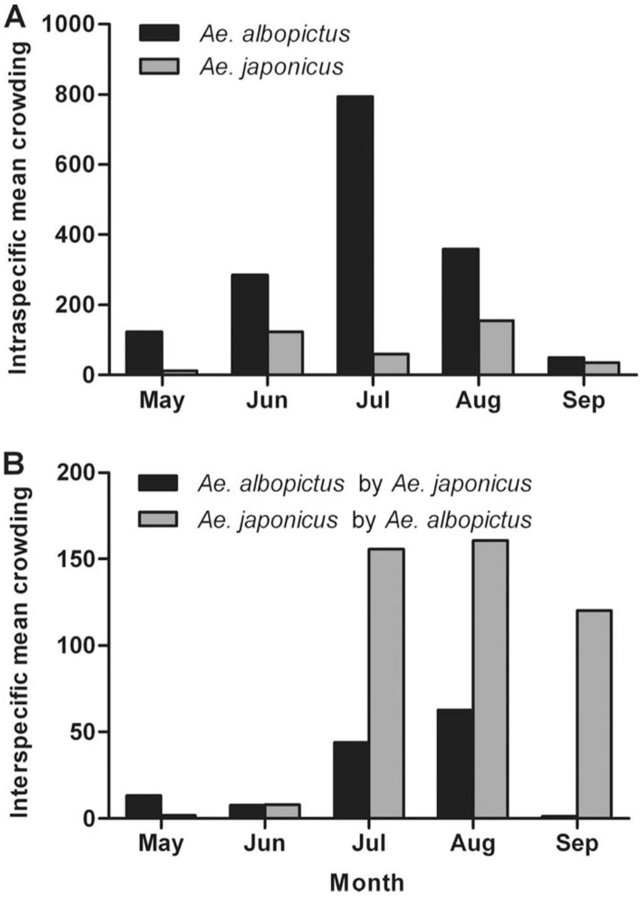

Fig. 3.

Crowding experienced by *Ae. albopictus* and *Ae. japonicus* in artificial containers sampled from May through September 2006. (A) Intraspecific mean crowding (density of conspecifics encountered per unit resource) experienced by *Ae. albopictus* and *Ae. japonicus* by month. (B) Interspecific mean crowding of *Ae.albopictus* by *Ae.japonicus* and *Ae. japonicus* by *Ae. albopictus*.

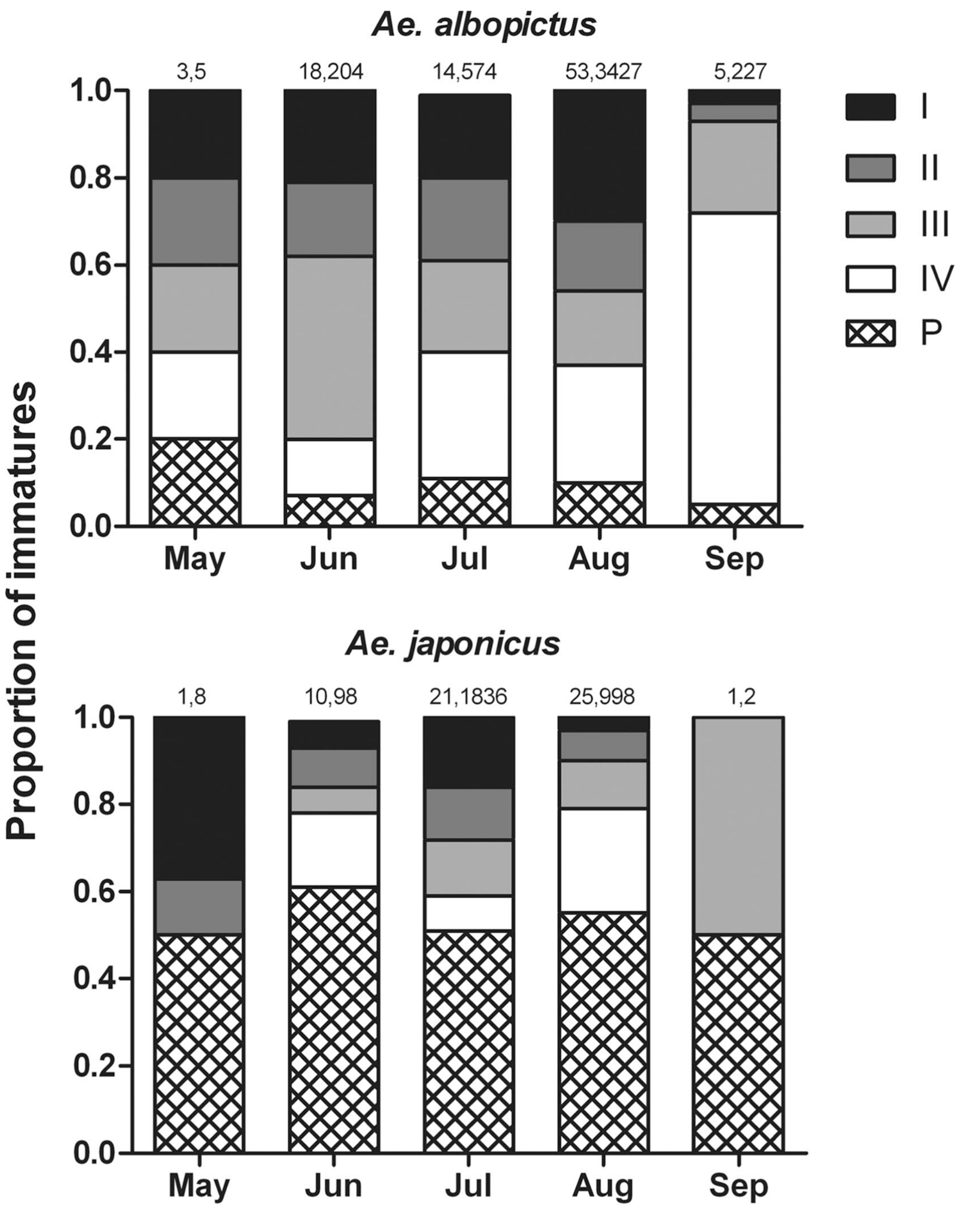

Fig. 4.

Monthly instar distributions of *Ae. albopictus* and *Ae. japonicus* from May through September 2006 in Fairfax, VA. Total numbers of species-positive containers sampled (n_1) and larvae collected (n_2) per month are indicated above each histogram bar (n_1, n_2).

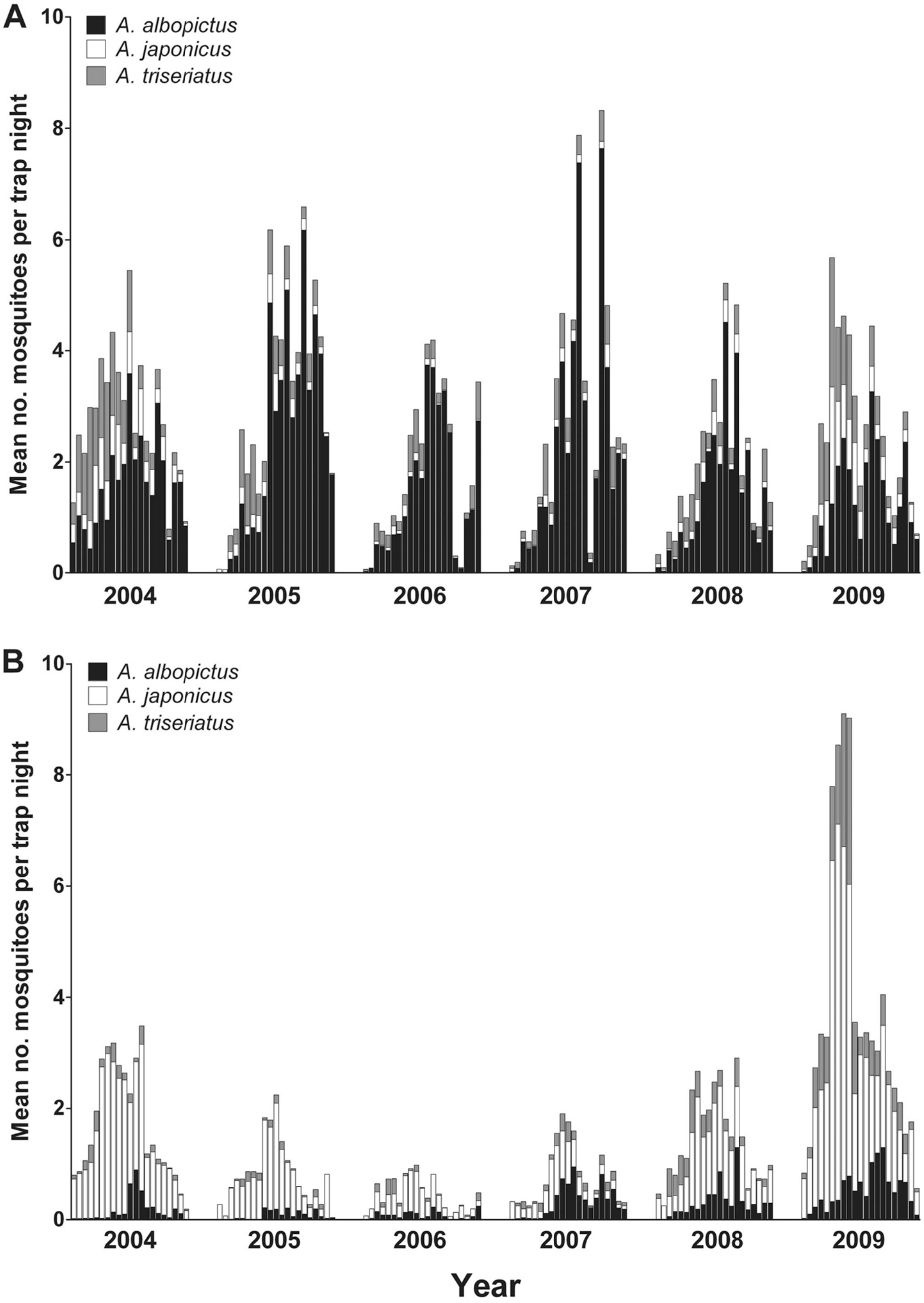

Fig. 5.

Weekly abundance of *Ae. albopictus*, *Ae. japonicus*, and *Ae. triseriatus* calculated as the mean number of female mosquitoes collected per trap night in (A) CO_2-baited light traps and (B) gravid traps (bottom) from epidemiological week 20–40 (May–September), 2004–2009, in Fairfax County, VA.

Table 1

Productivity as measured by the total no. of pupae sampled for the most abundant species collected in Fairfax County, VA, in 2006 by container type

		Total no. pupae collected				
Container type	**No. containers**	***Ae. albopictus***	***Ae. japonicus***	***Ae. triseriatus***	***Cx. pipiens***	***Cx. restuans***
Artificial	130	412	27	18	71	163
Small	97	191	1	17	4	80
Large	33	221	26	1	67	83
Tires	59	52	22	9	3	92
Rock pools	59	13	129	4	5	11

Table 2

Coefficients of association (C_8) for the most abundant species in 191 artificial and natural container samples from Fairfax County, VA, in 2006

	Coefficient of association (C_8)				
Species	***Cx. restuans***	***Cx. pipiens***	***Ae. triseriatus***	***Ae. japonicus***	***Ae. hendersoni***
Ae. albopictus	−0.056	−0.015	0.067^{**}	-0.238^{**} (-0.207^{**})	0.037^{*}
Ae. hendersoni	0	−0.272	0.232^{*}	0 (0)	
Ae. japonicus	0.075 (0.105)	0.042 (0.133)	-0.048^{**} (0)		
Ae. triseriatus	−0.122	−0.140			
Cx. pipiens	0.638^{**}				

Parentheses enclose C_8 values that exclude rock pools.

$^{*}P < 0.01$,

$^{**}P < 0.001$ by χ^2; all other interspecific associations are nonsignificant.

Table 3

Intra- and interspecific mean crowding of the most abundant mosquito species collected from artificial and natural containers in Fairfax County, VA, in 2006

Species, *x*	**Mean crowding[a] (density of species *y* encountered by species *x* per liter vol)**					
	Species, *y*					
	Ae. albopictus	***Ae. hendersoni***	***Ae. japonicus***	***Ae. triseriatus***	***Cx. pipiens***	***Cx. restuans***
Ae. albopictus	395.80	3.013	53.01	31.59	351.88	57.37
Ae. hendersoni	40.73	32.49	18.06	56.27	1.77	5.74
Ae. japonicus	139.3	9.03	127.56	12.85	51.56	83.06
Ae. triseriatus	136.7	11.90	19.21	34.47	25.93	39.26
Cx. pipiens	1, 098.43	2.48	37.56	12.96	126.98	52.33
Cx. restuans	20.16	2.51	57.41	4.33	34.05	116.60

[a] Interspecific mean crowding was determined from only those containers in which both species co-occurred.

Table 4

ML ANOVA for the effects of container type and sun exposure on the occurrence of *Ae. albopictus* and *Ae. japonicus* larvae in Fairfax County, VA, from May through Sept. 2006

Species	**Source**	**df**	χ^2	***P***
Ae. albopictus	Intercept	1	2.84	0.0919
	Container type	1	31.31	30.0001
	Sun exposure	2	1.06	0.5877
	Container type X sun exposure	2	5.89	0.0525
	Likelihood ratio	0		
Ae. japonicus	Intercept	1	14.31	0.0002
	Container type	1	7.32	0.0068
	Sun exposure	2	11.46	0.0033
	Container type X sun exposure	2	5.77	0.0559
	Likelihood ratio	0		