

Do natural container habitats impede invader dominance? Predator-mediated coexistence of invasive and native container-dwelling mosquitoes

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Abstract Predator-mediated coexistence of competitors occurs when a species that is superior in competition is also more vulnerable to a shared predator compared to a poorer competitor. The invasive mosquito *Aedes albopictus* is usually competitively superior to *Ochlerotatus triseriatus*. Among second instar larvae, *A. albopictus* show a lesser degree of behavioral modification in response to water-borne cues from predation by the larval midge *Corethrella appendiculata* than do *O. triseriatus*, rendering *A. albopictus* more vulnerable to predation by *C. appendiculata* than *O. triseriatus*. The hypothesis that *C. appendiculata* predation favors coexistence of these competitors predicts that *C. appendiculata* abundances will be negatively and positively correlated with *A. albopictus* and *O. triseriatus* abundances, respectively, and that coexistence will occur where *C. appendiculata* are common. Actual abundances of *O. triseriatus*, *A. albopictus*, and *C. appendiculata* in three habitats fit this prediction. In natural container habitats like tree holes, *C. appendiculata* were abundant and competitors co-existed at similar densities. In cemeteries and tires, which occur primarily in non-forested, human-dominated habitats, *A. albopictus* dominated, with abundances twice

those found in tree holes, but *C. appendiculata* and *O. triseriatus* were rare or absent. We also tested for whether antipredatory behavioral responses of *A. albopictus* differed among habitats or populations, or were correlated with local *C. appendiculata* abundances. We could detect no differences in *A. albopictus* antipredatory behavioral responses to water-borne cues from predation. Tree hole habitats appear to promote co-existence of *O. triseriatus* and *A. albopictus* through interactions with predatory *C. appendiculata*, and this predator effect appears to limit invasion success of *A. albopictus* in tree holes. There are many studies on predator-mediated coexistence in natural habitats but to our knowledge this is the first study to suggest differential predator-mediated coexistence between natural and man-made habitats.

Keywords *Aedes albopictus* · *Ochlerotatus triseriatus* · Predator-mediated coexistence · *Corethrella appendiculata* · Deforestation

Introduction

Invasion biology is increasingly perceived as vital for conservation because invasion by exotic species is increasing at an unprecedented rate, and some of these species have enormous economic and ecological impacts (Kolar and Lodge 2001). Native predators could have an effect on the density and population growth of invasive animals, and these effects could impose barriers to successful invasion (Lodge 1993). Predators may preferentially feed on invaders, particularly if invasive species lack appropriate adaptations to escape or to avoid novel predators, thereby impeding invasion and potentially facilitating survival of native species relative to that attained in the absence of predation,

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even when the invader is superior to the native species in resource competition (Garvey et al. 2003). Smith (2006) showed that the native predators, eastern newts (*Notophthalmus viridescens*), promoted co-existence between native toad tadpoles (*Bufo terrestris*) and invasive Cuban tree frog tadpoles (*Osteopilus septentrionalis*), whereas in the absence of the predator the invasive Cuban tree frog tadpoles dominated. DeRivera et al. (2005) showed that the native predator, the blue crab (*Callinectes sapidus*), limits the geographical range of the invasive, European green crab (*Carcinus maenas*).

Behavioral responses to predators appear to be a major mechanism of prey adaptation to predation in aquatic systems (Sih 1984; Lima and Dill 1990). Prey that alter their behavior in response to predation risk, including reducing movement and foraging, refuge use, and startle displays, can be less vulnerable to predation (Kats et al. 1988; Buskirk et al. 1997; Relyea 2002). Information on the role of prey behavior in invasion systems is lacking but it could be important in understanding the process of invasion and barriers to invasion (Holway and Suarez 1999). Consider a system in which a native prey shows adaptive changes in behavior that reduce risk of predation from the native predator. The success of an invading prey that is also a competitor of the native prey will depend upon whether the invader is a superior competitor to the native prey and also whether the invader shows appropriate behavioral or other facultative responses to the novel predators it encounters as it invades a new area. Without such appropriate responses, the presence of the predator, and its maintenance by populations of native prey, may act as a barrier to invasion, or may facilitate coexistence of invader and native (i.e., keystone predation; Leibold 1996). If however, the invader is both a superior competitor and has more effective behavioral avoidance of predation, the invader may eliminate and replace the native prey. In such an invasion system, it is also possible that selection imposed by the predator on the invading population may cause evolution of predator deterrence or avoidance in the invading species (e.g., Phillips and Shine 2005). If abundance and impact of predators varies among invaded locations, such selection could result in differentiation of invader behavioral responses and local variation in the outcome of the invasion. Most of the studies on predator-mediated biotic resistance have investigated the role of predators in natural habitats (e.g., DeRivera et al. 2005) and there have been only a few studies on how different habitats, especially natural and man-made habitats, influence predator-mediated biotic resistance to invasive species.

The habitats that are compared in the study described here are small container habitats. Rainwater that collects in these small containers supports a diverse, specialized invertebrate community (Kitching 2000). These container

systems can be natural (e.g., tree holes) and man made (e.g., cemetery vases and discarded tires). *Aedes albopictus* (order, Diptera; family, Culicidae) is a container-dwelling invasive mosquito that invaded the USA in the 1980s from Asia (Hawley et al. 1987) and has established itself in the southeastern United States (O'Meara et al. 1995). *A. albopictus* is a vector of human diseases including West Nile and dengue (Ibanez-Bernal et al. 1997; Turell et al. 2005). Larval *A. albopictus* are superior competitors to native mosquitoes (e.g., Livdahl and Willey 1991; Novak et al. 1993; Teng and Apperson 2000; Aliabadi and Juliano 2002), and have managed to displace them in some invaded areas (Juliano and Lounibos 2005). *Ochlerotatus triseriatus* (order, Diptera; family, Culicidae) is a container-dwelling mosquito that is native to North America. *A. albopictus* is a superior competitor to *O. triseriatus* under laboratory conditions (Livdahl and Willey 1991; Novak et al. 1993; Teng and Apperson 2000; Aliabadi and Juliano 2002) but populations of *O. triseriatus* in Florida tree holes have not declined since the invasion of *A. albopictus* (Lounibos et al. 2001).

In Florida, *A. albopictus* and *O. triseriatus* co-occur with the larval predator *Corethrella appendiculata* (order, Diptera; family, Corethrellidae). *C. appendiculata* are midges and when they are in the larval form they prey upon early instars of *A. albopictus* and *O. triseriatus* (Kesavaraju et al. 2007a). *C. appendiculata* appear to use mechanoreceptors to detect their prey and predominantly hunt at the bottom of the containers (Kesavaraju et al. 2007a). Moving prey at the bottom of containers are at a greater risk of being captured by *C. appendiculata* compared to motionless prey at the surface of the water (Kesavaraju et al. 2007a). Studies on the antipredatory behavior of *O. triseriatus* in response to other predators, which is similar to their response to *C. appendiculata* predation, have shown that this reduced movement also affects the foraging behavior and results in reduced foraging opportunities (Kesavaraju et al. 2007b). Second instar *O. triseriatus* reduce movement at the bottom of containers in the presence of water-borne cues from *C. appendiculata* predation, and although second instar *A. albopictus* show a qualitatively similar response to such cues, the degree of behavioral change is significantly less than that for *O. triseriatus* (Kesavaraju et al. 2007a). Larvae of *A. albopictus* are more vulnerable to predation by *C. appendiculata* than are *O. triseriatus* (Kesavaraju et al. 2007a; Griswold and Lounibos 2005b). Fourth instar *O. triseriatus* are relatively invulnerable to predation by *C. appendiculata*, but despite this, they also reduce movement at the bottom of containers in the presence of water-borne cues from *C. appendiculata* predation (Kesavaraju et al. 2007a). Behavioral studies comparing antipredator behavior of *A. albopictus* and *O. triseriatus* in response to *C. appendiculata* water-borne cues show that *A. albopictus* is

more vulnerable to predation by *C. appendiculata* than is *O. triseriatus* (Kesavaraju et al. 2007a). These data suggest that in habitats where *C. appendiculata* are abundant they may function as keystone predators (Leibold 1996), facilitating co-existence between competitively dominant, but predator-vulnerable *A. albopictus* and competitively inferior, but predator-resistant *O. triseriatus*, and in particular limiting declines of the competitively inferior native species (Griswold and Lounibos 2006).

Selection caused by novel conditions, and resulting evolutionary changes, can occur in response to species invasions (Lee 2002). For example, several native snake species in Australia have shown a reduction in gape size and increase in body size since the arrival of invasive cane toads. These changes prevent ingestion of toads that are large enough to be toxic (Phillips and Shine 2004). In that same system, there is evidence for evolution of invasive cane toad defenses in response to local variation in predation regimes (Phillips and Shine 2005). Invasive *A. albopictus* have been in both North and South America long enough to evolve modified diapause responses (Lounibos et al. 2003), suggesting that local adaptation to some environmental variables is possible for these introduced populations. Controlled laboratory investigations of selection by predation on *O. triseriatus* have shown that rapid evolution of larval behavior is possible (Juliano and Gravel 2002). Local populations of *O. triseriatus* differ in behavior patterns (Juliano and Reminger 1992; Juliano et al. 1993; Juliano and Gravel 2002), but that variation is not significantly associated with the large-scale geographic range of predators (Juliano et al. 1993), and quantitative associations of behavior of local populations with measured predator abundances have not been tested. Because *A. albopictus* has occupied parts of south Florida for >15 years, if there is local variation in predation, then local populations of *A. albopictus* from habitats where *C. appendiculata* are abundant may have been selected for greater behavioral response to water-borne predation cues than those from habitats of low abundance of *C. appendiculata*. If selection for greater antipredator responses also has the correlated cost of reduced competitive ability, this kind of evolutionary response by *A. albopictus* could contribute to the apparently limited impact of *A. albopictus* on *O. triseriatus* in areas where they co-occur with *C. appendiculata*.

In this research we tested whether: (1) there are any correlations of abundances of the predator *C. appendiculata* with abundances of the prey *A. albopictus* and *O. triseriatus*, and particularly whether coexistence of these mosquitoes is associated with presence of this predator; (2) populations of *A. albopictus* from habitats or sites with different abundances of *C. appendiculata* differ in behavioral responses to *C. appendiculata* water-borne cues from predation.

Materials and methods

Correlation of abundances

Abundances of *A. albopictus*, *O. triseriatus*, and *C. appendiculata* were determined from field samples taken between May and October in both 2004 and 2005 in Florida, USA, from cemeteries (Oak Hill, Bartow; Rose Hill, Tampa; Joshua Creek, Arcadia; White City, Fort Pierce), tire sites (M&K Used Auto Parts, Vero Beach; A & A Auto Salvage, Fort Pierce; Snake Road Auto Salvage, Stuart; Action Auto Salvage, Okeechobee) and tree hole sites (Indrio Road, Fort Pierce; Sherwood Hammock, Fort Pierce; Myakka River State Park, near Sarasota; Highlands Hammock State Park, near Sebring) (see Appendix for map of the locations). The months between May and October are the wet season in Florida and the abundances of mosquitoes are higher at these times (M. H. Reiskind, personal communication). All the contents of containers (e.g., water, detritus etc.) and tree holes from these sites were collected and the number of individuals of the three species in each of the containers were identified and counted. Tree hole sites were located in forested areas, with tree holes exclusively in live oak (*Quercus virginiana*), whereas tire and cemetery vase sites were located in non-forested, human-developed areas ranging from rural to urban. Field collections were conducted during the summers of 2004–2005 and all the sites were sampled at least twice with the exception of two tire sites (A & A Auto Salvage and Action Auto Salvage). From eight to 30 containers were sampled from each site and because the containers from all sites were sampled destructively, we allowed a minimum of 2 months between samples at a given site. A nested multivariate ANOVA (MANOVA) (Scheiner 2001) was used for the analysis with the numbers of *A. albopictus*, *O. triseriatus*, and *C. appendiculata* from a container as dependent variables and habitat types (cemeteries, tires, and tree holes) and sites nested within the habitat types as independent variables. Abundances were $\log_{10}(y+1)$ transformed to meet assumptions of normality and homogeneous variances. Standardized canonical coefficients (SCCs; Scheiner 2001) were used to identify the relative contribution of the dependent variables to significant differences among sites and habitat types, and to interpret correlations of mean abundances among habitat types and among sites. Correlations of abundances of *A. albopictus*, *O. triseriatus*, and *C. appendiculata* were further analyzed by testing Pearson correlations among the \log_{10} values of numbers of each species across containers, across habitat types (pooling all sites within a type).

Behavioral difference among sites

Behavior of second instar *A. albopictus* larvae from different sites was recorded in the absence and presence of cues from

predation. *A. albopictus* used in the experiment originated as field-collected larvae from the four cemetery and the four tire sites, and three of the tree hole sites (Indrio Road, Sherwood Hammock, and Highlands Hammock State Park). Field-collected larvae were reared to adulthood and blood fed using chickens (University of Florida Institutional Animal Care and Use Committee protocol no. VB-17) to obtain eggs. Resulting progeny, one generation removed from the field, were used in behavioral assays.

Following Kesavaraju et al. (2007a), the predation treatment was prepared by holding ten second instar *A. albopictus* with three fourth instar *C. appendiculata* larvae for 5 days in 10 ml deionized water in polystyrene disposable cups, and the control treatment was prepared by holding ten second instar *A. albopictus* alone in similar containers. Dead, eaten, and pupated individuals were replaced daily. The test subjects, F_1 generation *A. albopictus* from the different sites, were hatched and held in 5 ml of water in 4 dram vials and fed with 1 ml of liver powder suspension prepared by stirring 0.3 g of liver powder in 1,000-ml beaker with 1,000 ml water on a stir plate and transferred using an Eppendorf pipette (Juliano and Gravel 2002; Kesavaraju and Juliano 2004). A single feeding was sufficient for *A. albopictus* to develop to second instar.

Test larvae were starved for 24 h in 10-ml cups with 10 ml of water before being transferred to prepared water for behavior recording. All larvae of both predator and prey were removed from the prepared water, leaving behind only cues (e.g., dissolved substances, uneaten body parts, feces, etc.), before adding the test subjects for video recording. One second instar *A. albopictus* larva was placed in each cup of prepared water and its behavior was recorded on a computer using Winfast XP 2000 PCI card for 15 min. Each video clip included four cups (two control and two predation).

Behavior analysis

Behaviors were classified into activities and positions (Juliano and Reminger 1992). Activities were: (1) browsing—mouthparts in contact with the container surfaces, (2) filtering—moving through the water propelled by feeding movements of the mouthparts, (3) thrashing—moving with vigorous lateral flexion of body, (4) resting—not exhibiting any of the above activities. Positions were: (1) surface—siphon in contact with water surface; (2) wall—within 1 mm of the sides; (3) bottom—within 1 mm of the bottom; and (4) middle—more than 1 mm from the sides, bottom, and surface.

Activity and position of the test larvae were recorded every 30 s for 15 min upon playback of the video clips. Frequencies of behaviors were then converted to proportions (total number of observations per replicate = 30) for each

replicate larva. The number of variables per replicate was reduced with principal component analysis (PCA). Principal components (PCs) with eigen values >1 were retained and analyzed by MANOVA, with the sites nested within habitat type (tree hole, tire, and cemetery). SCCs were used to evaluate the relative contribution of the PCs to significant effects (Scheiner 2001). A second analysis tested for any differences among sites, ignoring type.

Because previous studies have shown that resting and surface are the least risky behaviors of mosquito prey compared to other behaviors (Juliano and Reminger 1992), we estimated the difference between the means of PC1 (which primarily quantified proportion of time spent resting at the surface) for control and predation treatments for each site as a means of quantifying the degree of change in behavior in response to predator cues. We tested the correlation of this difference with the mean abundance of *C. appendiculata* for the site. We also tested the correlation between means PC1 for control and predation treatments (i.e., quantification of the frequency of resting at the surface) for each site with *C. appendiculata* abundance.

Results

Correlation of abundances

There was a significant habitat type effect (Pillai's trace = 1.198, $df = 6, 16, P = 0.0125$) indicating that the abundances of the prey and predators differed among tree hole, tire, and cemetery sites. Abundance of *A. albopictus* was lowest in tree hole sites and greater in cemetery and tire sites (Fig. 1). In contrast, abundances of *C. appendiculata* and *O. triseriatus* were greatest in tree hole sites, and lower in tire and cemetery sites (Fig. 1). SCCs indicated that the site type mean abundances of *O. triseriatus* (SCC = -0.376) and *C. appendiculata* (SCC = -0.298) were negatively correlated with the site type mean abundances of *A. albopictus* (SCC = 0.293) (Fig. 1a, b), and were positively correlated with each other (Fig. 1c). There was also a significant site nested within type [site(type)] effect (Pillai's trace = 0.347, $df = 27, 1,173, P < 0.0001$) indicating that sites within the types also differed in their abundances prey and predators. Correlations among the site mean abundances of the three species were generally similar to the correlations observed across site types, with *C. appendiculata* and *O. triseriatus* positively related to each other and both negatively related to *A. albopictus* (Fig. 1a–c).

The abundances of *C. appendiculata* and *O. triseriatus* were greatest in tree hole sites but lower in tire and cemetery sites. Among containers within habitat types, patterns of correlation among species abundances were not consistent with correlations at the type or site level. In cemeteries,

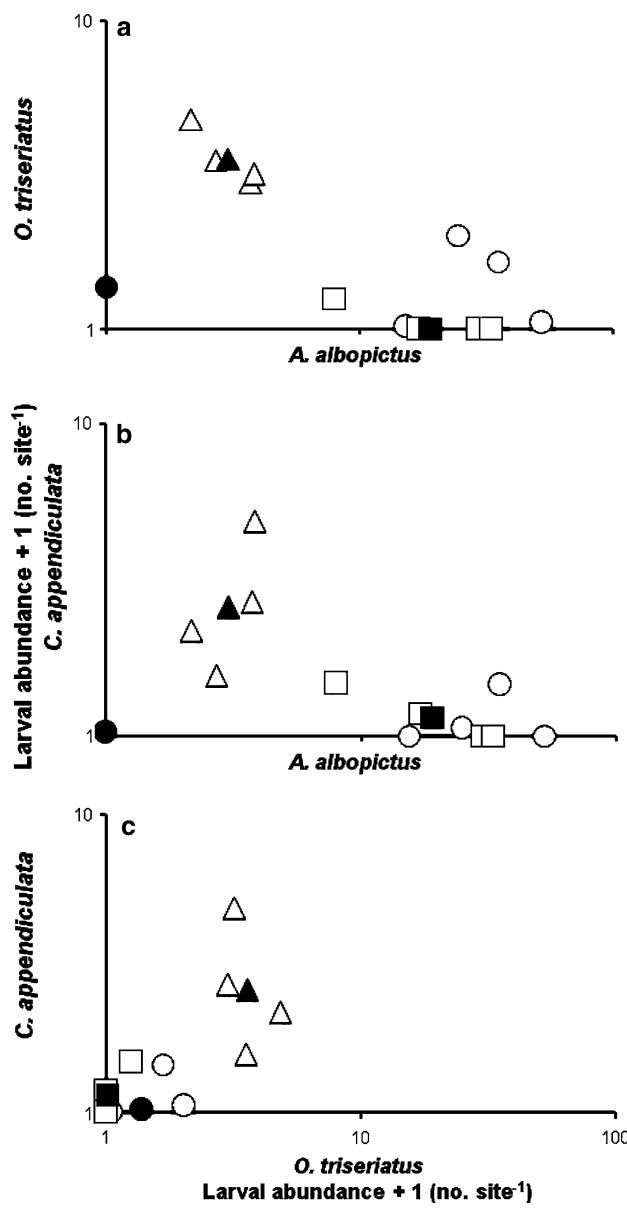


Fig. 1 Abundances (back-transformed means) of three species of mosquito larvae, *Aedes albopictus*, *Ochlerotatus triseriatus* and *Culex appendiculata*, in cemeteries (circles), automobile tires (squares), or tree holes (triangles) at 11 sites in Florida, USA, May–October 2004 and 2005. **a** *O. triseriatus* and *A. albopictus*, **b** *C. appendiculata* and *A. albopictus*, **c** *C. appendiculata* and *O. triseriatus*. Open symbols are least squares means for individual sites within each type of site and closed symbols are least square means for the type

A. albopictus and *O. triseriatus* abundances were significantly positively correlated (Fig. 2a), and neither prey abundance was significantly related to abundance of *C. appendiculata* (Fig. 2b, c). In tires, *A. albopictus* and *C. appendiculata* abundances were significantly negatively correlated (Fig. 2e), whereas abundances of *A. albopictus* and *C. appendiculata* were not significantly related to abundance of *O. triseriatus*, which was very rare in tires

(Fig. 2d, f). In tree holes, *O. triseriatus* and *A. albopictus* abundances were both significantly negatively correlated with *C. appendiculata* abundances (Fig. 2h, i), and were not significantly correlated with each other (Fig. 2g). Ignoring abundances and considering only presence vs. absence, a contingency table test of association between species in tree holes showed that *A. albopictus* and *C. appendiculata* were significantly negatively associated ($\chi^2 = 14.08$, $P = 0.0001$), but *O. triseriatus* and *C. appendiculata* were not significantly associated ($\chi^2 = 2.77$, $P = 0.0960$), though the trend was for a negative association.

Behavioral difference among sites

The correlated response variables were reduced to three uncorrelated PCs with eigen values >1 , which together summarized 89% of the variation in behavior frequencies. A greater positive score on PC1 indicated that larvae spent more time resting at the surface and a negative score indicated they spent more time browsing at the wall and bottom. A greater score on PC2 indicated that larvae spent more time thrashing in the middle and a negative score indicated they spent more time browsing. A greater score on PC3 indicated that larvae spent more time filtering in the middle and a negative score indicated they spent more time in other behaviors (Table 1).

Treatment (control, predation) was significant but habitat type (tree hole, tire, and cemetery), and the treatment by habitat type interaction were not significant (Table 2; Fig. 3). Because habitat type was not significant, we dropped it from the analysis and tested for site effects among all 11 tested sites, in order to detect any differentiation in behavior among sites. The site and the interaction of site and treatment were also not significant (Table 2; Fig. 3). Thus, there were no differences in *A. albopictus*' response to control and predation treatments among the habitat types or sites. Correlation analysis indicated that there was no significant correlation between the magnitude of *A. albopictus*' behavioral response (quantified by difference in PC1 values between control and predation treatments) and *C. appendiculata* mean abundance (Fig. 4b). There were also no significant correlations between means of PC1 for control and predation water treatments for each site and *C. appendiculata* abundance at that site (Fig. 4a).

Discussion

Competitive interactions between prey species can be altered by differential prey responses to predators (Werner and Anholt 1996; Relyea 2000). Classic studies (Morin 1981) and more recent work (Ciros-Perez et al. 2004) show that coexistence of a competitively inferior species with a supe-

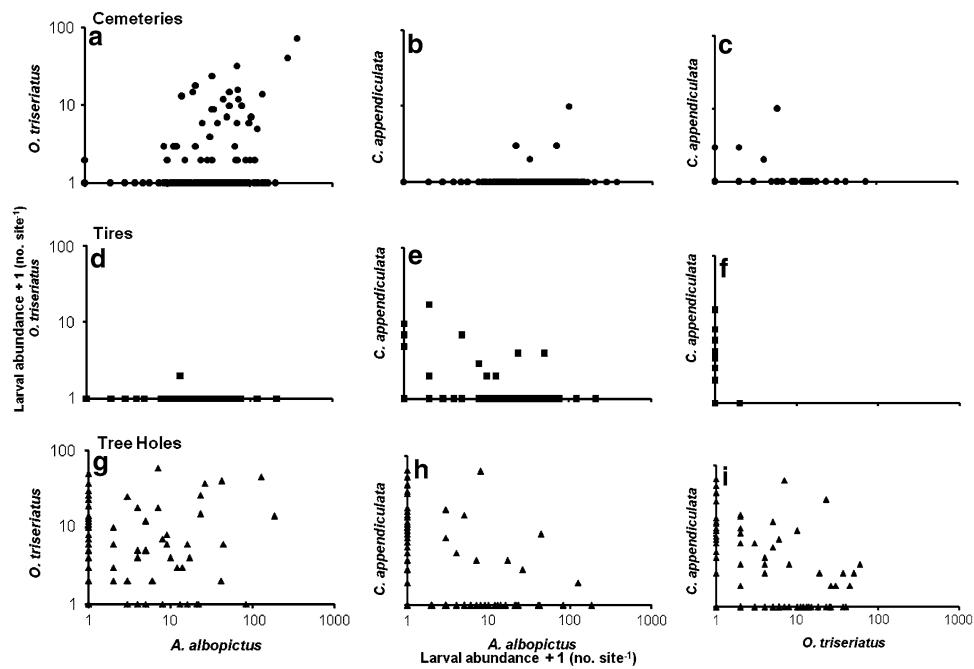


Fig. 2 Correlation of $\log_{10}(y + 1)$ values of total number of each species of mosquito per container from **a–c** cemeteries, **d–f** tires and **g–i** tree holes. Cemeteries **a** *O. triseriatus* and *A. albopictus* [correlation coefficient (CC) = 0.233, $P = 0.0004$], **b** *C. appendiculata* and *A. albopictus* (CC = 0.083, $P = 0.2283$), **c** *C. appendiculata* and *O. triseriatus* (CC = 0.121, $P = 0.0782$); tires **d** *O. triseriatus* and *A. albopictus* (CC = -0.016, $P = 0.8760$), **e** *C. appendiculata* and *A. albopictus*

(CC = -0.314, $P = 0.0021$), **f** *C. appendiculata* and *O. triseriatus* (CC = -0.433, $P < 0.0001$), **g** *O. triseriatus* and *A. albopictus* (CC = 0.190, $P = 0.0669$), **h** *C. appendiculata* and *A. albopictus* (CC = -0.271, $P = 0.0085$). Each point in the graph represents the total number present in a container

Table 1 Rotated factor patterns to test the differences in behavioral responses of *Aedes albopictus* across different sites. The three principal components (PCs) explained 89% of the variation. Values >40 are in bold

Variables	PC1	PC2	PC3
Resting	98	-20	-8
Browsing	-88	-45	-12
Thrashing	24	99	-11
Filtering	2	8	99
Surface	98	19	-1
Wall	-79	-39	-7
Middle	26	93	42
Bottom	-56	-23	-17
Interpretation	Resting, surface vs. browsing, wall, bottom	Thrashing, middle vs. browsing	Filtering, middle vs. other

rior species is aided by selective predation on the competitively superior species. Field studies have shown that *A. albopictus* has displaced the competitively inferior species, *A. aegypti*, in many areas of northern and central Florida, particularly in tire and cemetery vase sites (O'Meara et al. 1995; Juliano and Lounibos 2005). We see in our present data that these classes of sites are largely predator free, and

that displacement is indicative of the simple effect of inter-specific competition in largely predator-free habitats. Both laboratory and field studies have shown that *A. albopictus* is the superior resource competitor to *O. triseriatus*, particularly when food availability is very low (Novak et al. 1993; Lounibos et al. 2001; Aliabadi and Juliano 2002). Despite this, Lounibos et al. (2001) reported that *O. triseriatus* have not been replaced by *A. albopictus* in the field, especially in tree holes in wooded habitat. Behavioral responses of *A. albopictus* to water-borne cues to risk of predation are either absent (Kesavaraju and Juliano 2004) or of much smaller magnitude than those of native *O. triseriatus* (Kesavaraju et al. 2007a). This likely contributes to greater vulnerability of *A. albopictus* to predation by *C. appendiculata* (and to other predators like *Toxorhynchites rutilus* (order, Diptera; family, Culicidae) compared to *O. triseriatus* (Lounibos et al. 2001; Griswold and Lounibos 2005a; Griswold and Lounibos 2005b).

Among habitat types, *C. appendiculata* and *O. triseriatus* abundances were negatively correlated with abundances of *A. albopictus*, with both native species abundant in tree holes compared to cemetery and tire sites (Fig. 1). In cemetery and tire sites, where *C. appendiculata* and *O. triseriatus* abundances were low, *A. albopictus* dominated and their abundances were twice those found in tree hole habitats (Fig. 1). The combination of our distribution data for

Table 2 Multivariate ANOVA table to test the differences in behavioral responses of *A. albopictus* across different sites. Significant effects are in **bold**. *Num* Numerator, *Den* denominator

Variables	Num <i>df</i>	Den <i>df</i>	Pillai's trace	<i>P</i>	Standardized canonical coefficients		
					PC1	PC2	PC3
Part 1^a							
Type	6	14	0.269	0.8905	0.526	-0.726	-0.112
Treatment	3	6	0.977	<0.0001	1.036	0.491	0.064
Type × Treatment	6	14	0.738	0.2941	0.396	0.848	0.818
Part 2^b							
Site	30	1209	0.071	0.5042	-0.023	-0.474	0.941
Treatment	3	401	0.614	<0.0001	1.351	0.749	-0.139
Treatment × Site	30	1209	0.091	0.1641	0.723	0.671	0.715

^a With type effect

^b Without type effect

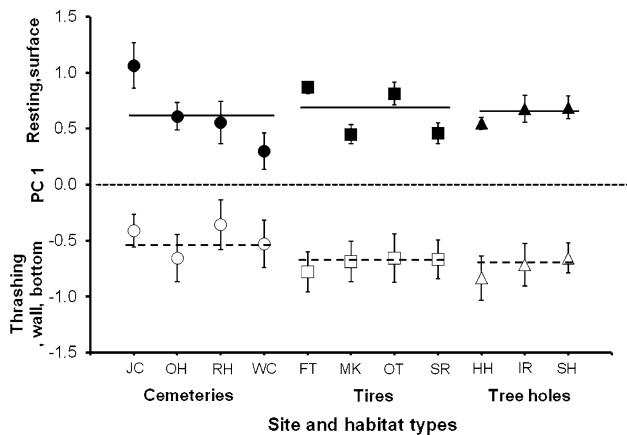


Fig. 3 Principal component 1 (PC1) (means \pm SE) for control (open symbols) and predation (closed symbols) treatments at each site with mean PC1 for cemeteries (circles), tires (squares) and tree holes (triangles) indicated by the horizontal line (dotted lines control, solid lines predation)

these species and previous data on competition (Teng and Apperson 2000; Lounibos et al. 2001; Aliabadi and Juliano 2002), and vulnerability to predation (Lounibos et al. 2001; Griswold and Lounibos 2005a, b; Kesavaraju et al. 2007a) strongly suggest predator-mediated coexistence via predation by *C. appendiculata* (i.e., *C. appendiculata* are keystone predators; Leibold 1996) in the tree hole systems. By limiting the abundances of competitively superior, but predation-vulnerable *A. albopictus* within sites, *C. appendiculata* prevent *O. triseriatus* from being competitively excluded by invading *A. albopictus*. The data on individual containers clearly show that *C. appendiculata* abundance is negatively related to abundances of both prey species, particularly in tree holes (Fig. 2), as would be expected for the local relationship of predator abundance to prey abundance. It is also clear that *A. albopictus* is rarely present in tree holes with *C. appendiculata* (ten of 40 tree holes with *C. appendiculata* had *A. albopictus*; Fig. 3h), whereas *O. triseriatus* is more frequently observed in tree holes with *C. appendiculata* (25 of 40 tree holes with *C. appendiculata* had *O. triseriatus*; Fig. 2i). The contingency table anal-

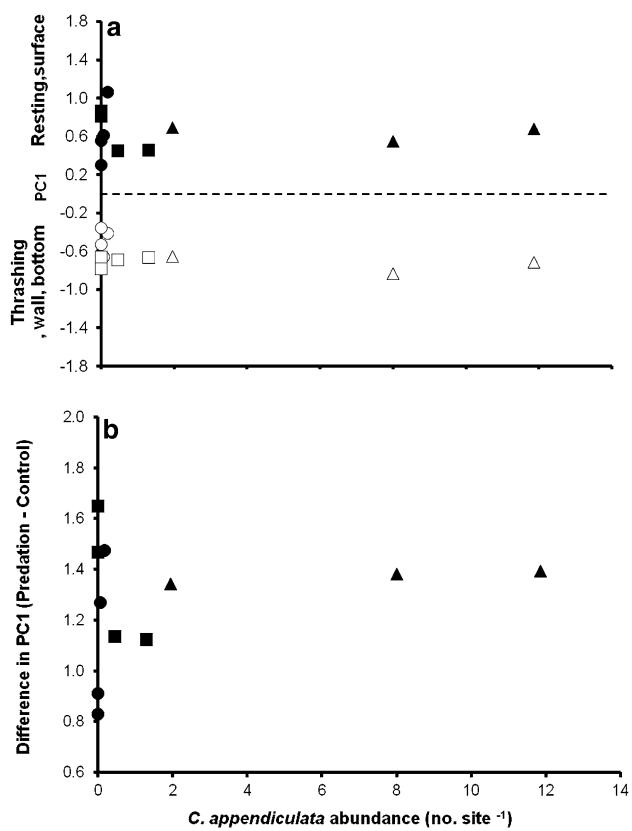


Fig. 4 Correlation between *C. appendiculata* abundance (no. site $^{-1}$) and **a** mean PC1 of control and predation treatments (control, CC = -0.446, *P* = 0.1483; predation, CC = -0.0487, *P* = 0.8870) and **b** difference in PC1 (i.e., change in behavior) for each site (CC = -0.229, *P* = 0.4960) with habitat types identified as cemeteries (circles), tires (squares) or tree holes (triangles). For abbreviations, see Figs. 2 and 3

ysis shows that in tree holes, where *C. appendiculata* are most abundant, *A. albopictus* is significantly and strongly negatively associated with *C. appendiculata* whereas *O. triseriatus* showed no significant association with *C. appendiculata*. These patterns also suggest that *O. triseriatus* finds a refuge from competition with *A. albopictus* in sites and containers where *C. appendiculata* is present.

Adults of species in the genus *Corethrella* take blood meals from frogs, using protein to produce eggs (McKeever 1977; McKeever and Hartberg 1981; Bernal et al. 2006). These flies show species-specific attraction to mating calls of frogs (Bernal et al. 2006). *C. appendiculata* collected during the field survey in Florida, have taken blood meals from *Hyla cinerea* (green tree frog) in the laboratory (B. Kesavaraju, unpublished data). Amphibians are very sensitive to disturbances and their abundances are often lower in urban areas (e.g., cemetery and tire sites) when compared to forested areas (e.g., tree hole sites) (Knutson et al. 1999). Low abundances of *C. appendiculata* in the cemetery/tire sites could be related, in part, to low abundances of frogs upon which *C. appendiculata* depend for blood meals.

It is tempting to infer from the limited behavioral responses of *A. albopictus* to North American predators like *T. rutilus* and *C. appendiculata* that this invader must have had limited evolutionary history with such predators in its native range. Such an inference is likely untrue although quantitative data are lacking. *Toxorhynchites* spp. are distributed worldwide in the tropics and subtropics, and are found in temperate Asia, where North American *A. albopictus* originated (Hawley 1988). Less is known about distribution of the genus *Corethrella*, although members of this genus are found in Japan (Miyagi 1974). Thus it seems probable that *A. albopictus* has an evolutionary history with congeners of the North American predators it encounters most, though specifics of micro-habitat overlap remain undocumented. The limited behavioral response to these predators by *A. albopictus* may thus reflect an alternative suite of antipredator adaptations in this mosquito (e.g., life history adaptations, habitat choice).

Populations that regularly co-occur with predators may evolve adaptations that help alleviate risk of predation, and those adaptations may be limited or absent in populations in predator-free habitats (Downes and Adams 2001). *A. albopictus* populations from tree hole habitats with high *C. appendiculata* abundances would thus be expected to show a greater degree of reduced movement in response to water-borne predator cues compared to populations collected from cemetery and tire habitats that are largely predator free. All the populations of *A. albopictus* in this study showed similar reduced movement and increased resting at the surface when they encountered water-borne cues from predation. Further, there was no difference in baseline patterns of behavior (i.e., no population effect) (Fig. 3). There was also no cross-site correlation in *C. appendiculata* abundance and the behavioral responses of *A. albopictus*, suggesting no local adaptation of *A. albopictus* behavior to predator density (Fig. 4). It is possible that the absence of differentiation indicates lack of sufficient time for evolution of population differentiation, or lack of sufficient isolation between populations for local differentiation in antipredator behavior. However, populations of *A. albopictus* have been in Florida

sufficiently long, with sufficient isolation, for evolution of large-scale geographic differences (e.g., in diapause; Lounibos et al. 2003). In other predator–prey systems, the intensity of antipredator behaviors was dependent on proximity and gene flow between populations sympatric and allopatric with predators (Storfer and Sih 1998). The lack of difference in *A. albopictus* antipredator behavior between populations of high and low predator abundance could arise because these populations are not sufficiently isolated and are experiencing considerable gene flow between them.

Previous studies have shown that *A. albopictus* is more vulnerable to *C. appendiculata* predation than is *O. triseriatus* (Griswold and Lounibos 2005a, b; Kesavaraju et al. 2007a), but follow up studies on how vulnerability affects distribution in the field were lacking. Our study shows that in natural container habitats like tree holes, *C. appendiculata* is more abundant than in artificial container habitats, and may be impeding dominance by *A. albopictus*. In the artificial container habitats like tires and cemeteries, *C. appendiculata* abundance is lower and *A. albopictus* dominates. Recent experiments conducted in tires have indicated that addition of *C. appendiculata* reduces colonization by *A. albopictus*, but that the presence of *O. triseriatus* alone does not (S. A. Juliano and L. P. Lounibos, personal communication). These results point out the importance of multiple species interactions for determining success and impact of invasive species. This conclusion suggests that maintaining a diverse array of relatively undisturbed natural ecosystems (e.g., forests) with their associated fauna at multiple trophic levels may provide valuable protection against invasion by undesirable non-native species.

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Do natural container habitats impede invader dominance? Predator-mediated coexistence of invasive and native container-dwelling mosquitoes

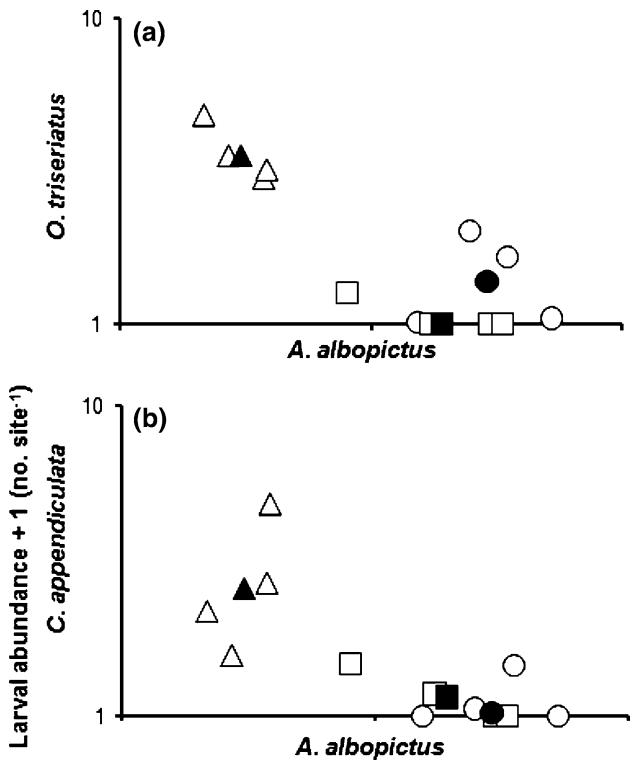
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The pooled least squares means for cemeteries in Fig. 1a and b (filled circles) were plotted incorrectly. Those pooled least squares means should be as follows:

All other points in Fig. 1 are plotted correctly.



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