Short Communication

Interspecific Interactions Between Adult *Aedes albopictus* and *Culex quinquefasciatus* (Diptera: Culicidae)

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Received 20 August 2015; Accepted 4 November 2015

Abstract

Single and mixed species densities of adult *Aedes albopictus* and *Culex quinquefasciatus* were manipulated to determine if different combinations affected their egg laying preference or mortality rates. Oviposition was measured in environments that contained containers of different surface areas (small cups vs. larger bowls), and the number of eggs (*Aedes*) and egg rafts or larvae (*Culex*) deposited by each species was examined with respect to intra- and interspecific density treatment levels. Mixed species densities did not have an effect on survivorship, but single species densities did affect longevity, with higher densities leading to shorter life spans in *Cx. quinquefasciatus*. *Cx. quinquefasciatus* lived longer than *Ae. albopictus* overall. There was no significant effect of density combinations on oviposition patterns in either species, but *Cx. quinquefasciatus* laid more rafts in bowls compared with cups. There is little evidence of adult interactions between these species; however, future experimental work is necessary to more fully characterize the possible effects of adult interactions on these species.

Key words: *Aedes, Culex*, interspecific, oviposition, survival

An important variable affecting mosquito vector populations in nature is larval competition. There has been extensive research conducted on competition between different species of mosquito larvae (*Sunahara and Mogi 1997, Reiskind and Lounibos 2009, Burke et al. 2010, Alto et al. 2015*), with findings often yielding situations of strong asymmetry (*Juliano 2010*) and context dependence (*Costanzo et al. 2005*). Competition can affect specific life-history traits, which in turn may affect viral transmission (*Alto et al. 2005, 2008, 2015*). Although there has been an emphasis on larval interactions, few investigations exist documenting interactions at the adult stage. For example, *Rey and O’Connell (2014)* determined that interactions between adult *Aedes aegypti* and *Aedes albopictus* affected egg-laying patterns at adult oviposition sites. Adult interactions are less likely to occur compared with larval interactions, as larvae are often limited by nutrients. Nutrient limitation, which is often the basis for larval competition, likely is less important for fully formed adults. Larvae are confined to their shared aquatic habitats, thus increasing the opportunities for interactions between individuals, but interactions between adults may still be significant and may help to better understand populations of vectors in nature, especially if such interactions affect densities around shared oviposition sites.

The goal of this research was to test the hypothesis that adult female *Culex quinquefasciatus* (*L.*) and *Aedes albopictus* (*Skuse*) interact in ways that may affect aspects of their ecology. *Aedes albopictus* and *Cx. quinquefasciatus* are abundant in artificial containers in the south (*Yee 2008*), and are abundant in tire habitats (*Qualls and Mullen 2006; Yee et al. 2012, 2015*), where they often are the dominant species (*Yee et al. 2012*) and where larvae are known to compete (*Allgood and Yee 2014*). Both species are also invasive, with the potential to negatively affect other species (*Juliano 2010*). *Aedes albopictus* appeared in the United States in 1995 (*Hawley et al. 1987*), whereas *Cx. quinquefasciatus* likely arrived from Africa over a century ago (*Ross 1964, Vinogradova, 2000*). *Aedes albopictus* routinely lays eggs on the walls of artificial vessels and can spread a single batch among multiple sites (*Hawley 1988*); oviposition is increased by detritus infusions (e.g., grass and leaves; *Obenauer et al. 2010*). Conversely, *Cx. quinquefasciatus* lay their eggs on the surface of water with high surface areas (*Subra 1981, Millar et al. 1994*) and produce eggs in rafts (*Wachira et al. 2010*), and thus all their eggs are laid in a single environment at one time. We investigated if oviposition choices were affected by intra- or interspecific interactions in the same environment that contained aquatic habitats of different sizes, and if these interactions could affect survival patterns. We predicted that egg-laying patterns would be altered under mixed species combinations, especially if adult interactions took place at or near oviposition sites. We also predicted that longevity would be shorter in the mixed species cages owing to increased activity as a result of the presence of the second species. It was also predicted that higher density levels, regardless of species, would increase the mortality rate of both species via a similar mechanism.
Materials and Methods

Oviposition Experiment
Adults (F1) of each species were generated from laboratory colonies established from larvae or eggs collected in Hattiesburg, Mississippi. Eggs were produced from adults that were blood-fed using Japanese quail, Coturnix japonica (IACUC #11092207). Larvae were reared in pans filled with reverse osmosis (RO) water and fed a diet of a 1:1 ratio by weight of lactalbumin-yeast powder (0.15 g on days 1, 4, and every other day thereafter) in a controlled environment incubator until pupation. Pupae were isolated and transferred into a colony cage to develop into adults.

For the oviposition experiment, adult female mosquitoes were blood-fed as stated above. Adult female mosquitoes were left in colony cages with males for about a week to ensure mating. Afterwards, females were blood-fed as stated previously. After approximately 48 h, adult females were anesthetized using CO2 and separated into intra- and interspecific combinations (Rey and O’Connell 2014). We placed a plastic bowl (41 cm in diameter by 5 cm in depth) and cup (28 cm in diameter by 4 cm in depth) at opposite ends of each cage. Bowls and cups were lined with brown paper and filled with 300 or 340 ml of infusion water, respectively. Infusion water was created using plant detritus and dead crickets soaked in tap water for one week to provide an oviposition cue for gravid female mosquitoes. Sugar pads (cotton soaked in 10% sucrose solution) were provided as a source of nutrition for adults. Cages were placed in an incubator set to 27°C, 80% humidity, and a photoperiod of 14:10 (L:D) h. To test the effect of intra- and interspecific adult interactions, the following species combinations were used: 4:0, 0:4, 8:0, 0:8, 4:4, 8:8, 4:8, and 8:4 (Aedes albopictus:Cx. quinquefasciatus). All combinations were replicated four times, except 0:4 and 4:0, which were replicated five times. After 48 h, the number of eggs laid (Aedes) on paper or rafts on the surface (Culex) were recorded. Because some rafts hatched and disassociated before 48 h, we also hatched, counted, and analyzed the total number of Culex larvae in each container.

Survivorship Experiment
Virgin adult female Aedes albopictus and Cx. quinquefasciatus were placed into intra- and interspecific combinations (Aedes:Culex: 1:0, 0:1, 2:0, 0:2, 2:1, and 1:2) with 15 replicates of each combination. Females were separated from males after they eclosed from pans and were not given a bloodmeal. Flight cages (30 by 25 by 28 cm) were placed into intra- and interspecific combinations (PROC LIFETEST, SAS 2004) was used to test differences in longevity across the treatments. Specifically, differences between 1) species, 2) densities, and 3) and their interaction were tested. Based on the outcomes of those tests, we also examined differences between species in low (1 adult) and high (2 adults) combinations, between high and low intraspecific densities, and across interspecific interactions (1:0 vs. 1:1, vs. 1:2, and 0:1 vs. 1:1 vs. 2:1). Wilcoxon tests were used to assess significance in all cases (Sokal et al. 1995).

Results

Oviposition Results
In total, 2,167 Aedes albopictus eggs and 63 Cx. quinquefasciatus egg rafts were laid during the experiment. Based on ANOVA, the number of Aedes albopictus eggs, Cx. quinquefasciatus rafts, and Cx. quinquefasciatus larvae did not significantly differ between intra- and interspecific treatment levels (Table 1). Overall, Aedes albopictus did not show a preference for either cup (1,140 eggs, 51.3%) or bowl (1,027, 48.7%). Culex quinquefasciatus did appear to exhibit a preference for bowls, with 25 rafts (39.6%) laid in cups and 38 (60.3%) laid in bowls. In total, 6,512 Cx. quinquefasciatus larvae were hatched and reflected a similar pattern as rafts (28.1% in cups, 71.9% in bowls).

Survivorship
We found that the effects of species ($\chi^2 = 119.12, df = 1, P < 0.001$; Fig. 1), density ($\chi^2 = 67.58, df = 6, P < 0.001$), and their interaction ($\chi^2 = 132.75, df = 9, P < 0.001$) were all significant. Female Cx. quinquefasciatus in low intraspecific densities survived longer than those in the high density ($\chi^2 = 4.41, df = 1, P = 0.034$; Fig. 2). Additional follow-up tests examining low and high combinations regardless of species ($\chi^2 = 1.88, df = 1, P = 0.180$), between high and low intraspecific densities for Aedes albopictus ($\chi^2 = 0.15, df = 1, P = 0.694$), and across interspecific densities (Ae. albopictus, 1:0 vs. 1:1, vs. 1:2, $\chi^2 = 2.06, df = 2, P = 0.356$ and Cx. quinquefasciatus, 0:1 vs. 1:1 vs. 2:1, $\chi^2 = 3.32, df = 2, P = 0.201$) were all nonsignificant.

Discussion
We found no support for our hypothesis that interspecific combinations of adult Cx. quinquefasciatus and Aedes albopictus would affect survival or oviposition patterns under laboratory conditions. Given the small size of our cages compared with natural conditions and larger cages used in a recent study (Rey and O’Connell 2014), we provided more optimal circumstances for adult interactions to manifest. Adults did not appear to interact in any meaningful way, which

| Table 1. Results of separate one-way analyses of variance on Aedes albopictus egg count, Cx. quinquefasciatus egg rafts, and Cx. quinquefasciatus larval count across intra- and interspecific adult densities |
|------------------|---|---|---|---|---|
| Source            | df | SS  | MS   | F   | P-value |
| Aedes albopictus  |   |    |      |     |         |
| Aedes albopictus (AA) | 1, 24 | 0.1150 | 0.1150 | 0.9900 | 0.3323 |
| Culex quinquefasciatus (CX) | 2, 24 | 0.0930 | 0.0465 | 0.4010 | 0.6754 |
| AA × CX            | 2, 24 | 0.0709 | 0.0355 | 0.3060 | 0.7401 |
| Cx. quinquefasciatus egg raft count |   |    |      |     |         |
| AA                | 2, 24 | 0.5180 | 0.2590 | 0.3180 | 0.7315 |
| CX                | 1, 24 | 0.0011 | 0.00011 | 0.0014 | 0.9706 |
| AA × CX           | 2, 24 | 2.0130 | 1.0070 | 1.2360 | 0.3129 |
| Cx. quinquefasciatus larval count |   |    |      |     |         |
| AA                | 1, 24 | 0.1150 | 0.1150 | 0.9900 | 0.3323 |
| CX                | 2, 24 | 0.0930 | 0.0465 | 0.4010 | 0.6754 |
| AA × CX           | 2, 24 | 0.0709 | 0.0355 | 0.3060 | 0.7401 |
suggests that such interactions may be infrequent or absent under natural conditions, where adults freely move in and out of oviposition sites. However, higher densities of adult mosquitoes, which likely occur around productive container sites, may still produce limited effects. There are several factors that may decrease the likelihood for interactions between these species. *Culex quinquefasciatus* is not container specialist (Siverly 1972) and has existed in North America for well over 100 yr (Ross 1964), whereas *Ae. albopictus* is a container specialist (Hawley 1988) and invaded just 30 yr ago (Hawley et al. 1987). Thus, there has been little opportunity for selection to shape the interactions of these species around containers. In addition, there are differences between species in the timing of oviposition (afternoon for *Ae. albopictus*, dusk for *Cx. quinquefasciatus*) and the specific location where eggs are placed that may minimize opportunities for adults of these species to interact.

Regardless of interspecific interactions, we did find differences in oviposition preferences between species. *Culex quinquefasciatus* have a natural proclivity to oviposit in larger bodies of water with a high surface area (Millar et al. 1994); the majority of egg rafts and larvae in this experiment were found in bowls. Female *Cx. quinquefasciatus* also rely on the egg pheromone 6-acetoxy-5-decanolide, produced as apical droplets on top of egg rafts, to attract gravid females to oviposit (Clemens 1999). In one replicate that contained eight adults of each species, eight egg rafts were found in the bowl. This result illustrates an example of expected *Cx. quinquefasciatus*
oviposition patterns in a container with high surface area and perhaps pheromones. Conversely, Ae. albopictus egg distributions were approximately equal between cups and bowls. Unlike species that share oviposition site selection (Rey and O’Connell 2014), a lack of interspecific interactions could have been owing to differences in egg-laying methods, with Cx. quinquefasciatus laying rafts on the water and Ae. albopictus laying on the wall, thereby decreasing direct competition for oviposition space. Our experiment suggests that inherent differences between species, including preference for certain surface areas, egg pheromone production, and egg location preferences may supersede the presence of other mosquito species, preventing interference in adults of these species.

Strong differences in survival between species also were found, with the average life span for Ae. albopictus at 6.33 d, whereas Cx. quinquefasciatus survived, on average, for 12.41 d (Fig. 1). Aedes albopictus have been shown to have a higher energy demand, which can be seen in their larva’s need for frequent feeding (Barrera 1996). Culex quinquefasciatus are also larger than Ae. albopictus (Allgood and Yee 2014), thus providing them with more stored energy reserves accumulated as larvae. Beyond species differences, intraspecific densities were most detrimental to survival for Cx. quinquefasciatus (Fig. 2). This may be related to several factors, including competition for food and reproduction locations that suit their niche in the environment. This is an unlikely explanation, given the controlled nature of our experiment; however, vibrations from mosquito wings may be one explanation for differences across densities. Wing vibrations, primarily characterized as a way for males to find conspecific females, can also be used as a general means of communication (Roth 1948, Clements 1999). Higher intraspecific densities may mean a higher persistence in frequency, and this may signal others to fly more, increasing overall energetic output. Interactions between females of the same species are also understood, but could be another explanation for the density effects we found here.

Other factors that affect survivorship and oviposition, including oviposition site marking, aggressive behavior by females, and the presence of other individuals (Rey and O’Connell 2014), only add on to the multitude of variables that affect mosquito populations. Future studies that focus on these and other potential adult interactions may lead to better characterization of vector ecology.

Acknowledgments
We thank S. Schelble and W. Glasgow for assistance with these projects and three anonymous reviewers for helpful suggestions on this manuscript. This work was supported by the Department of Biological Sciences at the University of Southern Mississippi, The Honor’s College, and by grants to D. A. Yee from the National Institutes of Health (R15[AI]-92404-01A1) and D.A. Yee from the National Science Foundation (DB B0923063).

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