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Larval competition alters susceptibility of adult *Aedes* mosquitoes to dengue infection

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Dengue, the most important human arboviral disease, is transmitted primarily by *Aedes aegypti* and, to a lesser extent, by *Aedes albopictus*. The current distributions of these invasive species overlap and are affected by interspecific larval competition in their container habitats. Here we report that competition also enhances dengue infection and dissemination rates in one of these two vector species. We determined the effects of competition on adult *A. aegypti* and *A. albopictus*, comparing their susceptibility to infection with a Southeast Asian strain of dengue-2 virus. High levels of intra- or interspecific competition among larvae enhanced the susceptibility of *A. albopictus* to dengue virus infection and potential for transmission, as indicated by disseminated infections. Doubling the number of competing larvae (*A. albopictus* or *A. aegypti*), led to a significant (more than 60%) increase in the proportion of *A. albopictus* with disseminated dengue-2 infection. Competition-enhanced vector competence appears to result from a reduction in 'barriers' (morphological or physiological) to virus infection and dissemination and may contribute to the importance of *A. albopictus* in dengue transmission. Similar results for other unrelated arboviruses suggest that larval competition, common in mosquitoes, should be considered in estimates of vector competence for pathogens that infect humans.

Keywords: *Aedes aegypti*; *Aedes albopictus*; larval competition; dengue virus infection

1. INTRODUCTION

The last few decades have seen a growing appreciation for the role that parasites and pathogens play in determining population dynamics and the structure of ecological communities (Dobson & Hudson 1986; Scott & Dobson 1989). For example, parasites alter reproduction and survival of wild animal populations (Gulland 1995), generate insect population cycles (Anderson & May 1980; Dwyer *et al.* 2000) and can mediate reversals in competitive interactions (Hudson & Greenman 1998). However, less attention has been devoted to understanding how ecological interactions and associated changes in life-history traits among organisms, alter subsequent host–parasite dynamics. There is a growing body of literature aimed at understanding how ecological interactions between two species (e.g. host, pathogen) may be mediated by interactions with a third species (e.g. competitor), termed trait- and density-mediated indirect effects (Wootton 1994). Ecological interactions that alter the life history of insect vectors may be especially important for infectious disease transmission because the outcomes of those interactions (e.g. growth rate, size, lifespan) are important factors in determining the potential for disease transmission (Dye 1986; Anderson & May 1991). Ecological interactions that modify vector–host contact (e.g. vector longevity, host-seeking and blood-feeding behaviour, vector abundance) and vector–virus interactions (e.g. vector competence), such as heterogeneity in susceptibility to infection (Dwyer *et al.* 2000), may dramatically alter disease transmission, a realistic feature often overlooked in modelling efforts of vector-borne diseases.

Vector competence is a laboratory measure of the potential for a vector (e.g. mosquito) to become infected and subsequently to transmit a pathogen after imbibing an infectious blood meal. Typically, mosquito transmission of an arbovirus requires acquisition of the virus from a blood meal, establishment of an initial viral infection in the mosquito midgut, viral dissemination to secondary target organs and subsequent transmission to a vertebrate host in salivary secretions during biting (Hardy *et al.* 1983; Woodring *et al.* 1996). Successful completion of this process requires that the virus overcome numerous morphological (e.g. basement membranes) and physiological (e.g. virus modulating factors) barriers to infection and dissemination within the mosquito (Hardy *et al.* 1983; Hardy 1988; Woodring *et al.* 1996; Ader *et al.* 2004). Midgut and salivary glands are enveloped by basement membranes, consisting of multilayer basal laminae, which may impede virus movement in these organs (entry or exit) as well as to other tissues (Hardy *et al.* 1983). The strength of these barriers may be influenced by environmental conditions experienced by larvae (e.g. Grimstad & Walker 1991). Differences observed in midgut morphology (basement membrane thickness) of different sized *Aedes triseriatus* mosquitoes, attributable to variations in larval nutrition, were associated with altered vector competence for LaCrosse virus (LACV; Grimstad & Walker 1991).

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Competition and other ecological interactions, experienced by mosquito larvae, influence morphological (e.g. size) and physiological characteristics of adult mosquitoes and may, therefore, affect adult vector competence for pathogens such as arboviruses (Grimstad & Haramis 1984; Briegel 1990; Grimstad & Walker 1991; Paulson & Hawley 1991; Sumanochitrapon *et al.* 1998; Briegel & Timmermann 2001). However, the connection between ecological conditions and vector competence has been difficult to make, and little is known how ontogenetic, ecological effects in the larval stages influence disease transmission among arthropods. Heterogeneity in life-history traits of arthropod vectors, and concomitant changes in vector potential, may have profound effects on disease transmission. An ecological perspective will contribute to our understanding of vector–pathogen interactions and may aid in strategies to control vectors and mitigate disease transmission. In this study we address how ecological interactions may influence arthropod competence for dengue virus (DENV) because it is the most important arbovirus affecting humans. DENV is responsible each year for approximately 50–100 million human cases of dengue fever and hundreds of thousands of cases of dengue hemorrhagic fever, a life-threatening form of the disease. We conducted an experiment to test the hypothesis that larval competition affects vector competence of mosquitoes *Aedes albopictus* and *Aedes aegypti* for DENV.

Aedes aegypti and *A. albopictus* are sympatric in Southeast Asia, and now co-occur in various countries of Africa, as well as in many locations in the Americas where DENV is endemic and a serious health risk. The establishment of either of these invasive vector species in new regions increases the risk for DENV transmission to humans, and recent estimates of the spread of *A. albopictus* suggest that a large proportion of the Earth will be at risk from dengue over the next 20–50 years (Juliano & Lounibos 2005; Benedict *et al.* 2007). In the southern United States, the introduction and spread of *A. albopictus* in the 1980s and 1990s was associated with declines in range and abundance of resident *A. aegypti* (O'Meara *et al.* 1995). These related species occupy similar aquatic habitats and interspecific competition among their larval stages in water-filled containers (e.g. tyres, vases) is well documented and a probable contributor to the observed declines of *A. aegypti* (Juliano *et al.* 2004). Based upon previous work from our laboratory and in the literature, we predicted that higher competition would be associated with increases in vector competence.

2. MATERIAL AND METHODS

(a) *Competition study*

We conducted a laboratory larval competition experiment with numbers of *A. albopictus* : *A. aegypti* per container—160 : 0, 320 : 0, 160 : 160, 0 : 320 and 0 : 160—as treatments, reared under standardized conditions (Alto *et al.* 2005). These densities were within the range observed in discarded tyres occupied by *A. albopictus*, *A. aegypti* or both species in field conditions (Alto *et al.* 2005). Mosquitoes were obtained from laboratory colonies (*A. albopictus* Lake Charles strain and *A. aegypti* Rockefeller strain) whose history is described in a previous study with Sindbis virus (SINV; Alto *et al.* 2005). Larval rearing vessels consisted of 5 l plastic containers filled with 4000 ml tap water, 500 ml oak leaf infusion water (O'Meara *et al.* 1989) and 0.2 g larval food (1 : 1 albumin : yeast). Food resources were allowed to incubate for 5 days before newly hatched (less than 24 hours old) mosquitoes were added to experimental containers. Three days after adding the larvae, a supplemental 500 ml oak infusion and 0.2 g larval food were added. Thirteen days later, 50% of the liquid was removed, without larvae, and 0.1 g larval food, 250 ml oak leaf infusion water, and 2250 ml tap water were added. Ten replicates were used for each treatment, except for 320 : 0 and 0 : 320, which had 11 replicates. Containers were maintained under constant environmental conditions (28 ± 1°C, 14 : 10 L : D photoperiod). Pupae were removed from containers and stored in 20 ml water-filled vials until adult emergence. Containers were monitored until all individuals had emerged as adults or died as immatures. Emerged adults and cohorts were measured to evaluate competitive treatment effects on population growth correlates. We measured size (wing length in millimetres), development time to adulthood (days) and survivorship to adulthood, and used these measures to calculate λ' which estimates the finite rate of population increase;

$$\lambda' = \exp(r') = \exp\left(\frac{\ln\left[(1/N_0)\sum_x A_x f(w_x)\right]}{D + \left[\sum_x x A_x f(w_x)/\sum_x A_x f(w_x)\right]}\right),$$

where λ' is a composite index of population performance (Juliano 1998) and a transformation of r' which describes the *per capita* growth rate (Livdahl & Sugihara 1984); N_0 is the initial number of females in a cohort (assumed to be 50% of initial larvae); A_x is the number of females emerging to adulthood on day x; w_x is mean female size on day x; $f(w_x)$ is a function relating the number of eggs produced by a female to her size; and D is the time (in days) from emergence to adulthood to oviposition. For *A. albopictus* and *A. aegypti*, D is estimated to be 14 and 12 days, respectively (Livdahl & Willey 1991; Juliano 1998). We used the following fecundity–size relationships ($f(w_x)$) to calculate λ':

Aedes aegypti (Briegel 1990)

$$f(w_x) = 2.505\left(w_x^3\right) - 8.616,$$
$$r^2 = 0.875, \quad N = 206 \quad \text{and} \quad p < 0.001,$$

Aedes albopictus (Lounibos *et al.* 2002)

$$f(w_x) = 78.02(w_x) - 121.24,$$
$$r^2 = 0.713, \quad N = 91 \quad \text{and} \quad p < 0.001.$$

In both cases, w_x is wing length in millimetres, used as a measure of mean size of females emerging on day x and $f(w_x)$ is a function relating the number of eggs produced by a female to her size.

Measurements on individuals and cohorts were used to evaluate competitive treatment effects on *A. albopictus* and *A. aegypti* population growth. Only female mosquitoes transmit virus horizontally by bite, or vertically to offspring, so they were the focus of all analyses. Mean female size (wing length in millimetres from alula to wing tip) and time to adult female emergence (days) were determined for each treatment and replicate. Female survivorship per replicate was calculated as (number of adult females)/(total number of original larvae) of a given species. Estimated finite rate of increase (λ') was calculated for each replicate. Population growth correlates (time to emergence, size of females assayed for infection and survivorship) were analysed, separately for female *A. albopictus* and *A. aegypti*, by multivariate analyses of variance

Table 1. Multivariate ANOVA for main effects and multivariate pairwise contrasts of competitive treatment effects on female *A. albopictus* and *A. aegypti* population growth correlates: time to emergence, survivorship to emergence and adult size. Competition treatments consisted of numbers of *A. albopictus* : *A. aegypti* per container—160 : 0, 320 : 0, 160 : 160, 0 : 320 and 0 : 160—as treatments.

				standardized canonical coefficients (SCC)		
comparison	d.f.	Pillai's trace	*p*	time	survivorship	size
Aedes albopictus						
competitive treatment	6	0.86	<0.0001	0.84	−0.31	−1.13
160 : 0 versus 320 : 0	3	0.71	<0.0001	0.76	−0.43	−1.13
160 : 0 versus 160 : 160	3	0.74	<0.0001	0.90	−0.22	−1.12
320 : 0 versus 160 : 160	3	0.11	0.3826			
error d.f.	28					
Aedes aegypti						
competitive treatment	6	0.88	<0.0001	0.96	−0.27	−1.62
0 : 160 versus 0 : 320	3	0.85	<0.0001	0.99	−0.28	−1.59
0 : 160 versus 160 : 160	3	0.78	<0.0001	0.90	−0.23	−1.69
0 : 320 versus 160 : 160	3	0.17	<0.1844			
error d.f.	28					

(MANOVA) to quantify the effect of competition. MANOVA used sizes of females assayed for DENV infection and λ' was calculated based on sizes of females assayed for infection as well as all unfed females obtained over the duration of the experiment. Significant effects were further analysed by all possible pairwise multivariate contrasts using the sequential Bonferroni method (experimentwise $\alpha = 0.05$). Standardized canonical coefficients (SCCs) were used to describe the relative contribution of each population growth correlate to significant multivariate effects as well as their relationship to each other (e.g. positive or negative association; Scheiner 2001; SAS Institute 2002). Competitive treatment effects on *A. albopictus* and *A. aegypti* λ' were analysed by separate one-way ANOVA, and significant effects were further analysed by pairwise comparisons of main effect means (Ryan–Einot–Gabriel–Welsch test, SAS Institute 2002). The distribution of *A. albopictus* development times departed from normality, which was not improved by common transformations. However, MANOVA, using Pillai's trace, is relatively robust to departures from normality (Scheiner 2001). Also, the highly significant treatment effects and similar direction of competitive effects suggest that this departure from normality had little effect on interpretations of the results.

(b) *Infection study*

Adult females produced by each treatment were provided with blood meals, using a silicone membrane feeder system (Butler *et al.* 1984; Alto *et al.* 2003, 2005), containing measured aliquots of a Southeast Asian dengue virus-2 strain (DENV-2). The DENV (strain 16803) was originally isolated from a patient in Thailand in 1974. This virus isolate had been passed once in the mosquito *Toxorhynchites amboinensis*, thrice in Vero cells, twice in *A. albopictus* C6/36 cells and three additional passages in Vero cells. DENV-2 titres used in blood-feeding trials (6.2 log plaque-forming units (PFUs)/0.2 ml) were determined by plaque assays in six-well plates with a monolayer of Vero cells. Blood-fed females were held for a 12-day incubation period, and surviving females were individually stored at −80°C until assayed to determine the proportion infected, proportion with disseminated infection and body titre of females with disseminated infections. Plaque assays were used to determine mosquito infection and disseminated infections, whereas DENV-2 body titres were determined by quantitative RT-PCR. Virus titre refers to the amount of virus in solution. In this study, dissemination describes the proportion of infected mosquitoes with DENV-2 in secondary target organs (specifically legs). Non-disseminated infections are represented by mosquitoes with DENV-2 in their bodies, presumably limited to the midgut and an absence of virus in their legs (Turell *et al.* 1984; Alto *et al.* 2005).

Interspecific (*A. aegypti* versus *A. albopictus*) differences in susceptibility to DENV-2 infection were evaluated by MANOVA and SCC on the response variables proportion infected and proportion with disseminated infection (SAS Institute 2002). A one-way ANOVA tested for interspecific differences in body titre of *Aedes* females with disseminated DENV-2 infections (SAS Institute 2002). Next, individual one-way MANOVAs and SCCs, for each *Aedes* species were calculated to determine competitive treatment effects on proportion infected and proportion with disseminated infection. Significant effects were further analysed by all possible pairwise contrasts of bivariate means using the sequential Bonferroni method (experimentwise $\alpha = 0.05$). Competitive treatment effects on *A. albopictus* and *A. aegypti* body titre were analysed by separate one-way ANOVAs, and significant effects were further analysed by all possible pairwise comparisons of means (Ryan–Einot–Gabriel–Welsch test, SAS Institute 2002). Canonical correlation analyses were used for each species to determine the overall relationship between the recorded population growth correlates (size, survivorship, development time and λ') and vector competence for DENV-2 (infection and dissemination; Sherry & Henson 2005). We excluded viral titre from the canonical analyses because it was only determined for females with disseminated infections.

3. RESULTS

(a) *Competition study*

For both *A. albopictus* and *A. aegypti*, competitive treatments (numbers of *A. albopictus: A. aegypti* per container—160 : 0, 320 : 0, 160 : 160, 0 : 320 and 0 : 160) significantly affected population growth correlates (table 1) in the pattern 160 larvae < 320 larvae = 160 : 160 larvae (figure 1). Greater competition consistently resulted in significantly smaller adult female size, longer time to emergence and lower survivorship (*A. albopictus* least square (LS)

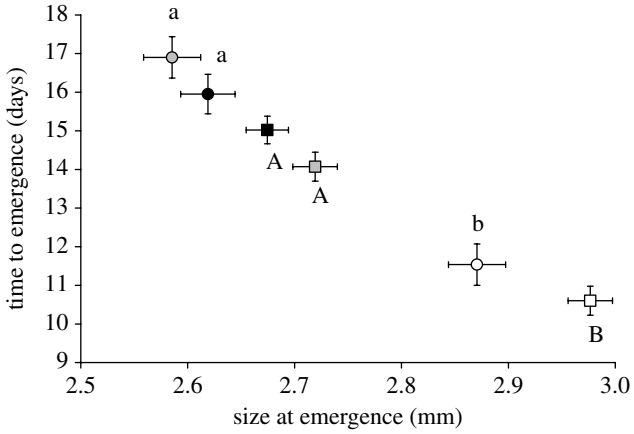

Figure 1. Bivariate plot of least square (LS) means (±s.e.) for *A. albopictus* and *A. aegypti* female size and time to emergence. Competition treatments consisted of numbers of *A. albopictus* : *A. aegypti* per container—160 : 0 (open circle), 320 : 0 (filled circle), 160 : 160 (grey circle and grey square), 0 : 320 (filled square) and 0 : 160 (open square). Different lower- and uppercase letters indicate significant differences between bivariate means for *A. albopictus* and *A. aegypti*, respectively. *Aedes albopictus* are represented by circles and *A. aegypti* by squares.

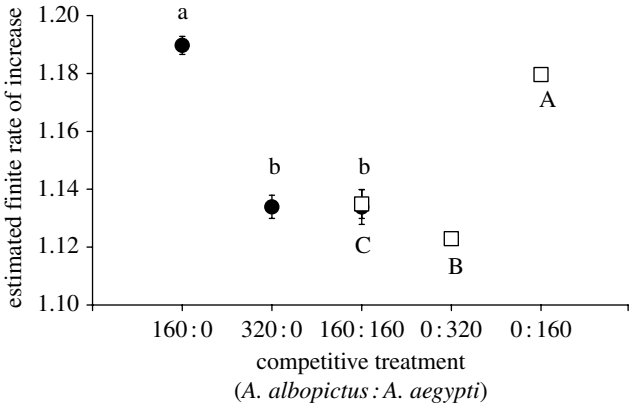

Figure 2. Least square (LS) means (±s.e.) for estimated finite rate of increase, λ', for *A. albopictus* (filled circles) and *A. aegypti* (open squares). Points without bars have standard errors too small to be visible. Different lower- and uppercase letters indicate significant differences between means for *A. albopictus* and *A. aegypti*, respectively.

mean±s.e. proportion surviving: 160 : 0, 0.42±0.03; 320 : 0, 0.27±0.03; 160 : 160, 0.32±0.03 and *A. aegypti* LS mean±s.e. proportion surviving: 0 : 160, 0.36±0.02; 0 : 320, 0.31±0.01; 160 : 160, 0.33±0.02) than all less intense competitive treatments (figure 1). For both species, SCCs showed that differences in adult size and time to emergence contributed the most to the significant effect of competition as well as to subsequent treatment differences (table 1). Both species had significantly decreased population performance, as measured by estimates of finite rate of population increase (*A. albopictus*, $F_{2,28}=50.02$, $p<0.0001$; *A. aegypti*, $F_{2,28}=82.60$, $p<0.0001$; figure 2), under conditions of intense larval competition.

(b) *Infection study*

Larval competition significantly increased the proportion of *A. albopictus* infected with DENV-2 and the proportion with disseminated infection (table 2; figure 3). The larval treatments had a greater effect on the proportion of infected mosquitoes than that with disseminated infection (SCC; table 2). *Aedes albopictus* reared at low larval density alone (160 per container) had significantly lower infection and dissemination rates compared with females of this species that had emerged from high densities (both intra- and interspecific; table 2; figure 3). Competitive treatments resulted in similar trends of infection and dissemination for *A. aegypti*, but the effects for this species were not significant (table 2; figure 3). ANOVA showed no significant competitive treatment effects on DENV-2 body titre for *A. albopictus* ($F_{2,17}=1.99$, $p=0.1672$) or *A. aegypti* ($F_{2,26}=2.38$, $p=0.1123$) with disseminated infections.

Canonical correlation analyses relating population growth correlates (size, survivorship, development time and λ') and vector competence for DENV-2 (infection and dissemination) showed a significant canonical relationship between growth correlates and *A. albopictus* vector competence parameters (Pillai's $\text{trace}_{8,44}=0.75$, $p=0.0046$; table 3) for the first pair of canonical variables (canonical correlation=0.78). SCCs were used to determine the relative contribution and relationship (e.g. positive or negative) among population growth and vector competence variables (Scheiner 2001). SCC showed that female *A. albopictus* adult size was the most important contributor to the canonical function for *A. albopictus* population growth correlates, followed by survivorship (table 3). Canonical vector competence parameters were equally determined by proportion infected and proportion with disseminated infections (table 3). Canonical structure coefficients showed that the larval development time, which increased under high levels of intra- and interspecific competition, was positively correlated with the vector competence canonical variate, whereas size and λ' were negatively correlated with the vector competence canonical variate (table 3). Canonical structure coefficients showed approximately similar contributions of proportion infected and proportion with disseminated infections (table 3). Thus, we found consistent associations of reduced population growth correlates (i.e. fitness measurements) with enhanced *A. albopictus* vector competence, suggesting a generalizable relationship between competitive stress and infection. No significant correlations were found for *A. aegypti* (Pillai's $\text{trace}_{12,72}=0.41$, $p>0.50$). Univariate product–moment correlations showed no significant correlations between single growth correlates and DENV-2 body titre for either mosquito species.

The vector competence of *A. aegypti* and *A. albopictus* for DENV-2 was significantly different in our studies. MANOVA demonstrated significant interspecific differences (i.e. *A. albopictus* versus *A. aegypti*) in proportion infected (SCC=−0.88) and proportion with disseminated infection (SCC=1.19; Pillai's $\text{trace}_{2,56}=0.58$, $p<0.0001$). Although both infection and dissemination contributed to the differences between the species in vector competence, dissemination was the more important contributor by approximately 25% (SCCs: $|-0.88|<|1.19|$). There was an inverse relationship between the proportion of mosquitoes infected and the proportion with a disseminated infection, such that *A. albopictus* was significantly more susceptible to infection, but had a significantly lower dissemination rate compared with *A. aegypti* (LS means± s.e. for *A. albopictus* and *A. aegypti*, respectively: proportion infected, 0.90±0.02 and 0.77±0.02 and proportion with

Table 2. Multivariate ANOVA for main effects and multivariate pairwise contrasts of competitive treatment effects on female *A. albopictus* and *A. aegypti* proportion infected and proportion with disseminated infection. SCCs indicate the relative contribution of each measurement to significant multivariate effects. Competition treatments consisted of numbers of *A. albopictus* : *A. aegypti* per container—160 : 0, 320 : 0, 160 : 160, 0 : 320 and 0 : 160—as treatments.

				standardized canonical coefficients (SCC)	
comparison	d.f.	Pillai's trace	*p*	infection	dissemination
Aedes albopictus					
competitive treatment	4	0.51	0.0057	1.04	0.61
160 : 0 versus 320 : 0	2	0.41	0.0022	1.11	0.49
160 : 0 versus 160 : 160	2	0.36	0.0060	0.89	0.77
320 : 0 versus 160 : 160	2	0.06	0.4897		
error d.f.	24				
Aedes aegypti					
competitive treatment	4	0.23	0.1394		
error d.f.	28				

disseminated infection, 0.37 ± 0.03 and 0.63 ± 0.03). Body titre of mosquitoes with disseminated infections was significantly higher in *A. albopictus* than in *A. aegypti* ($F_{1,47} = 85.26$, $p < 0.001$; LS means $\pm$ s.e. for *A. albopictus* and *A. aegypti*, respectively; 4.6 ± 0.06 log PFU/0.2 ml and 3.9 ± 0.04 log PFU/0.2 ml).

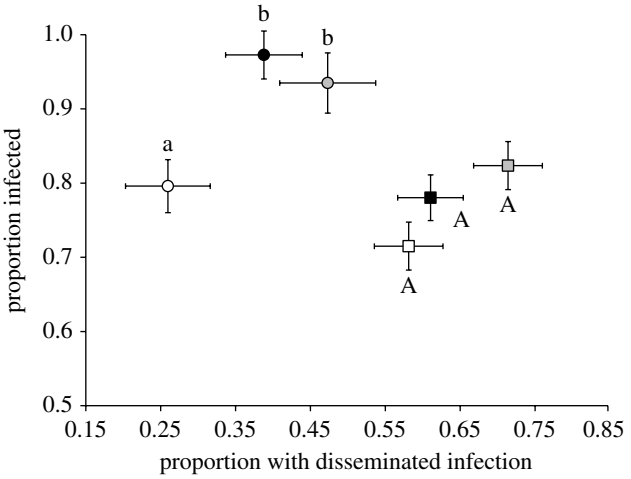

Figure 3. Bivariate plot of least square (LS) means ($\pm$s.e.) for proportion of *Aedes* females infected and proportion with disseminated infections after fed on a dengue-2 virus (Southeast Asian strain 16803) blood meal. Competition treatments consisted of numbers of *A. albopictus* : *A. aegypti* per container—160 : 0 (open circle), 320 : 0 (filled circle), 160 : 160 (grey circle and grey square), 0 : 320 (filled square) and 0 : 160 (open square)—as treatments. Different lower- and uppercase letters indicate significant differences between bivariate means for *A. albopictus* and *A. aegypti*, respectively. *Aedes albopictus* are represented by circles and *A. aegypti* by squares.

4. DISCUSSION

The goal of these experiments was to test the hypothesis that larval competition affects vector competence of *A. albopictus* and *A. aegypti* for DENV. We postulated that higher competition would be associated with increases in vector competence. Our results are consistent with our prediction, but these effects differed between *Aedes* species. All population growth measurements showed consistently poorer performance for mosquitoes reared at high larval density and were significantly correlated with infection parameters for *A. albopictus* but not *A. aegypti* (table 1; figures 1 and 2). These results are similar to those of our previous work using *Aedes* species in tests of competitive treatment effects on SINV infection (Alto *et al.* 2005). In both the SINV and the current DENV experiments, it was necessary to maximize adult mosquito production to assess vector competence without negating the effects of larval competition. To achieve this, a combination of natural (oak leaf infusion) and artificial (yeast and albumin) larval food resources was used. Previous laboratory and field research show contrasting outcomes of competition between these *Aedes* species dependent upon larval resource type, where *A. aegypti* is the superior competitor with protein-rich resources (e.g. liver powder, yeast) and *A. albopictus* is the superior competitor with plant detritus (e.g. leaves; Black *et al.* 1989; Barrera 1996; Juliano 1998; Braks *et al.* 2004; Juliano & Lounibos 2005). The present study's results are consistent with past work that suggests high-protein diets may benefit survivorship of *A. aegypti* in competitive conditions (Barrera 1996).

Higher levels of intra- and interspecific competition significantly enhanced the proportion of *A. albopictus* infected with DENV-2 and the proportion with disseminated infection. Larval treatments had a greater effect on the proportion of infected mosquitoes than that with disseminated infection, suggesting that initial infection in the adult mosquito midgut is more influenced by larval competition than escaping the midgut and infecting other organs (i.e. dissemination). Susceptibility to infection and dissemination matched closely the competitive treatment effects on *A. albopictus* population growth correlates, such that significantly reduced population growth correlates at high densities were associated with significantly enhanced DENV-2 infection relative to low densities (figures 1–3). Canonical correlations found consistent associations of reduced mosquito fitness measurements with enhanced vector competence, suggesting a generalizable relationship between competitive stress and infection. However, we found no significant competitive treatment effects on DENV-2 body titre for mosquitoes with disseminated infections, suggesting that overall DENV-2 replication in adults is unaffected by competitive interactions among larvae. Thus, competition-enhanced vector competence is probably not due to depression in innate immune response

Table 3. Standardized canonical coefficients (SCCs) and canonical structure coefficients for the relationship between population growth measurements (development time, survivorship, size and λ') and vector competence (infection, dissemination) for *A. albopictus*. A structure coefficient is a bivariate correlation between an observed variable (e.g. size) and a synthetic variable (e.g. vector competence parameters as a whole). Values greater than 0.45 are in italics and represent strong factor loading contributions.

canonical correlation analysis	growth correlate	vector competence
standardized canonical coefficients		
development time	0.29	.
survivorship	*0.52*	.
size	*−0.73*	.
λ'	−0.23	.
infection	.	*0.67*
dissemination	.	*0.62*
canonical structure coefficients		
development time	*0.87*	*0.68*
survivorship	−0.16	−0.13
size	*−0.94*	*−0.73*
λ'	*−0.65*	*−0.50*
infection	*0.62*	*0.80*
dissemination	*0.58*	*0.75*

that limits virus replication *per se* but is more likely associated with a reduction in 'barriers' (morphological or physiological) to virus infection and dissemination.

Infection studies using mosquito *A. triseriatus* and LACV showed higher infection, dissemination and oral transmission rates of LACV by nutritionally stressed small mosquitoes than by large well-fed mosquitoes after oral feedings on low doses of LACV infectious blood through an artificial membrane feeder (Grimstad & Walker 1991). Similar results were found in experiments using high doses of LACV infectious blood, except no differences were found for infection rate which were uniformly high (more than 98%; Grimstad & Haramis 1984; Grimstad & Walker 1991). The lack of differences in infection rate may be, in part, attributable to the overall high infection rates, thus making identification of potential nutrient-dependent differences in LACV infection less probable than for lower infectious blood meals resulting in moderate infection rates. Studies bypassing the normal route of infection (e.g. imbibing infectious blood and deposition in the midgut) via intrathoracic inoculation of LACV demonstrate similar effects with small nutrient-stressed mosquitoes having higher rates of vertical transmission (e.g. transovarial) and horizontal transmission (by bite) than large well-fed mosquitoes (Patrican & DeFoliart 1985). Although direct evidence is lacking, our results for DENV are consistent with the observation that differences in midgut morphology (basement membrane thickness), and by extension similar basal lamina in other organs, attributable to variations in larval nutrition were associated with altered vector competence for LACV (Grimstad & Walker 1991).

Laboratory colonies of these *Aedes* species have traditionally been accepted as representative of their natural populations. However, laboratory colonization may alter infection parameters due to founder effects, genetic drift and artificial selective forces imposed in laboratory colonies (e.g. Lorenz *et al.* 1984; Munstermann 1994). Laboratory colonization with associated selection and drift, even after only a few generations, may reduce heterozygosity and the number of alleles (Munstermann 1980, 1994). Further, geographical strains of both these *Aedes* species vary in their susceptibility to DENV infection (e.g. Gubler *et al.* 1979; Boromisa *et al.* 1987; Failloux *et al.* 2002). Despite these shortcomings, the current experiment on competition and infection used well-established laboratory colonies of *Aedes* mosquitoes. These mosquitoes were used for two reasons. Firstly, it was unclear whether larval competition would have strong, subtle or any effect on adult DENV infection parameters. Minimizing intra-population variation in response to viral infection, associated with greater genetic variability, should increase the likelihood of detecting competition-induced effects on adult infection parameters. Secondly, the use of identical laboratory mosquito strains and similar experimental design as a previous study with SINV allowed for robust comparisons of infection parameters for SINV and DENV. The current experiment showed similar directional effects of competition-induced changes in SINV and DENV vector competence for *A. albopictus*, suggesting a generalizable outcome linking the larval environment and adult *Aedes* vector competence (Alto *et al.* 2005). However, it is unclear whether genetically more heterogeneous mosquitoes (e.g. natural populations) would have similar infection responses. Future studies should consider experiments on larval competition and infection parameters using F_1 generation *Aedes*, whose parents were collected from natural field populations. The outcome of such additional experiments would answer whether conclusions based on *Aedes* laboratory colonies apply to field populations, and whether trends in infection parameters induced by competitive interactions are similar among more genetically heterogeneous populations. Additionally, mosquito infection parameters may be altered by different serotypes and strains of DENV (e.g. Gubler *et al.* 1979; Moncayo *et al.* 2004; Anderson & Rico-Hesse 2006). Thus, different types of DENVs may respond differently to competitive-induced alterations in virus–vector interactions.

Species-specific differences in vector competence (*A. aegypti* versus *A. albopictus*) may be caused by fundamental differences between these *Aedes* species in their physiological interactions with DENV-2. The actual proportion of probable DENV-2-transmitting mosquitoes is equivalent to the product of the proportion of females infected and disseminated (*A. albopictus*, 0.33 and *A. aegypti*, 0.49). However, this estimate also depends on the competitive environment (e.g. low versus high), especially for *A. albopictus*, so that more intense competitive conditions enhance the proportion of transmitting mosquitoes (figure 3). The mosquitoes used in the current research were derived from well-established laboratory colonies. Although laboratory colonization may alter *A. albopictus* and *A. aegypti* susceptibility for arboviruses (Lorenz *et al.* 1984; Vazeille *et al.* 2003), the current research results do agree with results for multiple mosquito strains ($F_{1–2}$ generation) and different DENV serotypes (DENV-1, mosquitoes $\geq F_5$; Chen *et al.* 1993). *Aedes aegypti* had significantly greater DENV-2 disseminated infection compared with *A. albopictus* using three Southeast Asian DENV-2 strains, including an almost identical strain used in the current experiment (Vazeille *et al.* 2003). The current study did not include a test of actual transmission of virus to

hosts by mosquito bite or a test of virus presence in the mosquito salivary glands. Thus, our estimates of potential to transmit based on disseminated infections may overestimate the proportion of actual DENV-2-transmitting mosquitoes. However, Vazeille *et al.* (2003) used a similar strain of DENV-2 virus as the current study and showed similar interspecific differences in virus infection measures for these *Aedes* species using head squash assays, an equivalent measure of virus detection in the salivary glands and a strong indicator of potential to transmit. Further, vector competence measurements for another Flavivirus, West Nile virus, showed a close correspondence between levels of disseminated viral infections, determined by plaque assays on mosquito legs, and rates of viral transmission by bite for several mosquito species, including *A. aegypti* and *A. albopictus* (Turell *et al.* 2001).

The current experiment allows a detailed comparison of DENV-2 vector competence between *A. aegypti* and *A. albopictus*, including information on species-specific infection, dissemination and body titre. Accurate description of relative viral susceptibility of *A. aegypti* and *A. albopictus* requires a comparison of multiple infection measurements, especially since some measures of viral competence are in complete opposition to each other (e.g. infection: *A. albopictus* > *A. aegypti* and dissemination: *A. albopictus* < *A. aegypti*). These results have important epidemiological consequences and are consistent with the observation that *A. aegypti* is a superior vector of DENV in nature, due to its higher rate of dissemination and, hence, transmission (Chen *et al.* 1993; Vazeille *et al.* 2003). The continued spread of Southeast Asian DENV genotypes, which are more virulent than American DENV genotypes, coupled with establishment of either of these invasive mosquito species in new regions increases the risk of severe forms of dengue (Rico-Hesse *et al.* 1997; Gubler 2002; Cologna *et al.* 2005).

Larval competition increases adult mosquito susceptibility to DENV-2 infection and increases the potential for disease transmission. Our results suggest that initial susceptibility to infection in the adult mosquito midgut is influenced more by larval competition than is viral dissemination to secondary tissues. These effects are strikingly similar to results for *A. albopictus* infected with the unrelated alphavirus SINV (Alto *et al.* 2005). Furthermore, as larval competition is very common among mosquitoes, these findings are likely to apply to other species of mosquitoes and arboviruses. Investigation of the specific mechanism(s) responsible for these results was beyond the scope of these studies. However, similar responses of *A. albopictus* to both SINV (Alto *et al.* 2005) and DENV suggest a common immunological (e.g. repressed innate immune function; Sanders *et al.* 2005) or a general mechanical (e.g. leaky midgut: Weaver 1986; Weaver *et al.* 1991; Chandler *et al.* 1998) mechanism(s) responsible for the effects of competition on viral infection. *Aedes aegypti* did not respond as strongly to the competition treatments as did *A. albopictus*, demonstrating interspecific differences in the responses to virus infection influenced by the larval environment.

These results demonstrate the importance of an unappreciated connection between mosquito larval ecology and the transmission of arboviruses. Models of arthropod-borne infectious diseases generally ignore the potential effects of the larval environment on vector competence, assuming that individual mosquitoes are equally likely to acquire and to transmit arboviruses. However, we have shown that a change in the intensity of larval competition, attributable to doubling the number of competing larvae (*A. albopictus* or *A. aegypti*), led to a 62% increase in the proportion of *A. albopictus*-transmitting DENV, as indicated by disseminated infection rates (low density = 0.26 and high density = 0.42). These results may be applicable to other arboviruses (e.g. SINV) and so may be used to parametrize models of disease transmission by mosquito vectors (Focks *et al.* 1995; Luz *et al.* 2003; Smith *et al.* 2004). Any vector control practice that reduced mosquito abundance, and perhaps competition-enhanced infection, may increase the numbers of larger, less competent mosquitoes. If competition-enhanced dissemination applies to ovaries (Patrican & DeFoliart 1985; Grimstad & Walker 1991), then competition, in addition to increasing the potential for transmission to hosts, may also enhance the ability of *A. albopictus* to maintain DENV in regions during inter-epidemic periods via high levels of vertical transmission.

Larval competition among mosquitoes may contribute to disease risk to humans in other ways, and it is important to consider all the effects of larval rearing environment on adult traits including fitness and subsequent vector potential. The number of infectious bites a person receives per day (the vectorial capacity) is determined by population level and behavioural attributes of mosquitoes, in addition to vector competence (Woodring *et al.* 1996). Smaller-sized adult females, associated with competition, may have reduced survivorship (e.g. Haramis 1985; Nasci 1986; Briegel *et al.* 2001) rendering them less effective vectors because they may undergo fewer biting cycles (i.e. blood feeding and egg production) during their lifetime than longer lived adults. Differences in adult survivorship are, in part, attributable to size related differences in nutrient reserves among newly emerged adults (Briegel 1990; Briegel *et al.* 2001). Small *A. aegypti* mosquitoes, derived from suboptimal larval diets in the laboratory or from field collections had reduced host-seeking behaviour (Nasci 1986; Klowden *et al.* 1988), biting persistence (Nasci 1991) and blood-feeding success (Nasci 1986) than larger mosquitoes. However, smaller adults imbibe less blood during feedings (Briegel 1990) and may have greater contact with hosts and blood feed more often than larger adults, potentially enhancing dengue transmission (Scott *et al.* 2000). Future studies should investigate the interplay of positive and negative effects of competitive-induced alterations in vector potential, including vector competence and vectorial capacity. Competition-enhanced vector competence is likely to contribute to the importance of DENV transmission by *A. albopictus*, especially in areas where *A. aegypti* is currently absent. Trends for *A. aegypti*, though not significant, suggest that probably some *A. aegypti* populations under competitive larval environmental conditions may also exhibit competition-altered vector competence for DENV. The results of this study suggest that larval conditions are certainly an important aspect of DENV transmission, and should be included in future evaluations of the epidemiology of other arboviruses. Competition is common in natural mosquito systems and should be considered with other ecological interactions among larval stages in estimates of mosquito vector potential.

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