# Size Alters Susceptibility of Vectors to Dengue Virus Infection and Dissemination

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## Abstract

The size of arthropod vectors may affect their ability to transmit pathogens. Here we test the hypothesis that body size alters the susceptibility of *Aedes aegypti* and *Aedes albopictus* mosquitoes to dengue virus (DENV) infection and subsequent dissemination throughout the body of the mosquito. After feeding on blood containing known quantities of virus, smaller-sized females were significantly more likely to become infected and to disseminate virus than larger individuals. The effects of size were stronger for *Ae. aegypti* and independent of rearing conditions. *Ae. albopictus* was more susceptible to DENV infection and had higher virus titer in the body than *Ae. aegypti*, yet infected *Ae. aegypti* disseminated DENV more readily than infected *Ae. albopictus*. These results are consistent with the concept that *Ae. aegypti* is a more competent vector of DENV and emphasize the importance of body size in determining adult infection parameters.

## INTRODUCTION

*Aedes aegypti* and *Aedes albopictus* are the primary vectors of dengue virus (DENV), an important human pathogen that causes ~50–100 million cases of dengue fever and hundreds of thousands of cases of dengue hemorrhagic fever annually. These *Aedes* species co-occur in Southeast Asia, Africa, and in the Americas, in areas where DENV is endemic and a serious health threat. Emerging dengue virus subtypes, especially more virulent forms in the Americas originally from the Paleotropics, coupled with uncontrolled urbanization and continued spread of *Ae. albopictus*, may endanger greater numbers of people for dengue-related illnesses in the future.[1] In the southern United States, the spread of invasive *Ae. albopictus* was associated with declines in range and abundance of resident *Ae. aegypti*.[2] The immature stages of these species occupy similar aquatic habitats such as water-holding tires and vases, and the asymmetric outcome of interspecific larval competition, which favors *Ae. albopictus*, is a likely contributor to the observed declines of *Ae. aegypti* in the southern United States.[3] Plastic responses in life history traits (e.g., size), induced by larval competition between and among *Ae. albopictus* and *Ae. aegypti*, may influence the vector competence of each species.

Ontogenetic niche shifts, which often entail a dramatic change in habitat or diet, occur among many taxa that undergo complete metamorphosis.[4] Abiotic factors and interactions with other species at earlier stages of development can influence later stages and shape plastic responses for individual traits such as body size,[5,6] feeding ability,[5] fitness,[6] and population dynamics.

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[7] In many instances, these carryover effects between stages may be classified as indirect effects (e.g., trait- or density-mediated indirect effects), where interactions between two species (e.g., interspecific competition) alter a subsequent interaction with a third species.[8] However, the epidemiologic consequences of ecological interactions that transcend life history stages of holometabolous insect vectors of pathogens have not been well studied. Species interactions (e.g., competition, predation) and abiotic factors (e.g., temperature) affecting the larval stage may play an important role in determining individual traits, such as size, and may thereby affect vector potential of adult mosquitoes for pathogens, such as arboviruses.[9]

The size of a female mosquito, determined by its genetic background and larval environment, has long been suspected to be an important contributing factor to vectorial capacity,[10] although there is a debate over the magnitude and even sign of the effect. Positive relationships have been observed between size and vector longevity, which enhances vector potential,[11–13] whereas negative relationships have also been observed between size and blood feeding frequency such that smaller mosquitoes may take blood meals more often than larger individuals.[14] In addition to effects on the vector–host interaction, size may influence vectorial capacity by altering vector–pathogen interactions (vector competence, i.e., the intrinsic permissiveness of a vector to become infected and subsequently to transmit a pathogen). Transmission requires acquisition of the virus from a blood meal, establishment of an initial viral infection in the midgut, viral dissemination to other organs, and sub-sequent transmission to a host by bite.[15] The time from initial acquisition of infection in the vector until transmission is referred to as the extrinsic incubation period. Successful transmission requires that the virus over-come numerous barriers to infection and dissemination within the mosquito,[15] many of which are under genetic control.[16–19]

Environmental factors, although less studied than genetic factors, are also likely to play an important role in determining vector–virus interactions by influencing the strength of barriers to infection and dissemination. An inverse relation-ship between temperature experienced by the adult vector and viral dissemination to secondary tissues has been observed.[20–22] Nutrient deprivation or crowded conditions at the larval stage may also influence adult virus infection and subsequent transmission. Nutrient-deprived *Ae. triseriatus* larvae produced small adults that more readily became infected and transmitted La Crosse virus (LACV) than did larger adults derived from well-fed larvae.[23–25] Enhanced transmission efficiency of these small *Ae. triseriatus* females was associated with higher virus titers and dissemination rates compared with larger females. Size-dependent differences in midgut thickness were consistent with observed differences in vector competence among mosquitoes.[23] Additionally, field-collected *Ae. triseriatus* pupae, exposed to natural ecologic conditions as larvae, had disseminated infection and transmission rates that were inversely correlated with body size, after oral infection of adults with LACV.[26] Similar effects of size have been shown for other arboviruses (e.g., Ross River virus,[27] Sindbis virus[9]) on vector competence of their mosquito hosts. However, some studies have shown opposite (dengue-2 virus),[28] or no effects (Murray Valley virus, western equine encephalitis and St. Louis encephalitis viruses).[29,30] Thus, effects of body size on infection and transmission of mosquito-borne viruses may be dependent on the particular mosquito–virus system. Here we test the hypothesis that variation in adult size, determined in part by larval conditions, subsequently alters the responses of *Ae. aegypti* and *Ae. albopictus* to DENV infection and dissemination.

## MATERIALS AND METHODS

### Larval treatments

Mosquitoes were laboratory strains: *Ae. albopictus* Lake Charles and *Ae. aegypti* Rockefeller. We conducted a laboratory experiment with numbers of *Ae. albopictus*: *Ae. aegypti* larvae per container of 320:0, 160:160, and 0:320, respectively, as treatments. We used 5-L containers

with 4000 mL tap water, 500 mL oak leaf infusion water, and 0.2 g larval food (1:1 albumin:yeast) to rear larvae.[9] Larval food was incubated for 5 days at 28°C, after which newly hatched (< 24 hours old) mosquitoes were added to containers. Three days later, 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. Containers were maintained under constant environ-mental conditions (28 ± 1°C, 14:10 L:D photoperiod). Density of mosquitoes used in this experiment was held constant and within the range observed in tires occupied by *Ae. albopictus*, *Ae. aegypti*, or both species in field conditions.[9] A competition experiment studying the effects of different larval density treatments (e.g., 160 versus 320) on dengue virus infection of adult mosquitoes is reported in a companion study.[31] All treatments had 11 replicates, except for the 160:160 treatment, which had 10 replicates. Containers were monitored every 48 hours when pupae were removed and placed in sealed vials until emergence to adulthood. The experiment continued until all individuals had emerged as adults or im-matures died. Because each container was a replicate, individuals emerged from each container were kept together.

## Infection experiment

**Oral infection of mosquitoes—**Adult females were offered a southeast Asian dengue-2 virus (DENV-2, strain 16803). DENV-2 virus was propagated in confluent 75-$cm^2$ tissue culture flasks, rocked for 1 hour at 37°C to allow for attachment and entry, after which media (Leibovitz L-15 media, 10% fetal bovine serum [FBS], 50 μg/mL gentamicin) were added to bring the total volume to 10 mL/flask, and incubated at 35°C. Media in 75-$cm^2$ tissue culture flasks were recovered, with replacement of fresh media, on Days 4 and 8 and harvested to prepare infectious blood meals on Day 11 after infection. Freshly recovered media-virus from Day 11 was used as the source of DENV-2 for infectious blood meals offered to mosquitoes, because of the superiority of freshly harvested virus over frozen stock.[32] Adult mosquitoes from the larval rearing treatments (intra-and interspecific conditions) were placed in cylindrical card-board containers (14 cm high × 11 cm diameter) according to larval treatment and species. Mosquitoes were provided with an oviposition cup, 20% sucrose, water, and a 14:10 L:D photoperiod. Sucrose and water were renewed every 48 hours. Mosquitoes were deprived of sucrose, but not water, 24 hours before blood feeding. Infectious blood meals, warmed at 37° C for 20 minutes, were offered to females for 30 minutes using a silicone membrane feeder system.[33] Blood meals consisted of a suspension of citrated bovine blood and freshly harvested media, from tissue cultures infected with DENV-2, in a 4 to 1 ratio. Samples of infectious blood were stored at −80°C and later tested to determine DENV titers offered to mosquitoes during feeding trials.

DENV-2 titers used in bloodfeeding trials (mean ± SE = 6.47 ± < 0.01 $log_{10}$ plaque forming units [PFU]/0.2 mL, range = 6.2–6.8 $log_{10}$ PFU/0.2 mL) were determined by plaque assays in duplicate 6-well plates with a monolayer of Vero cells (see methods below). Six separate blood-feeding trials, administered over 18 days, were offered to female mosquitoes 5–9 days after emergence to adulthood. A smaller number of mosquitoes (5% of total) were 12 or 14 days old at blood feeding. Housing numerous adult mosquitoes emerged from each larval replicate required the use of multiple cages for each treatment replicate. Each larval treatment (intra-and interspecific) was present for both *Ae. aegypti* and *Ae. albopictus* in all feeding trials. Immediately after feeding trials, mosquitoes were cold anesthetized, and unengorged females were separated from engorged females, which were returned to their original cages for a 12-day incubation period and subsequently stored at −80°C. Individual mosquitoes were dissected to remove legs (assayed to determine disseminated infection) and wings to measure female size. Mean female size (wing length in millimeters from alula to wing tip) was measured for each treatment and replicate. Plaque assays were used to determine individual mosquito infections and disseminated infections, as well as the proportions per replicate. Quantitative

reverse transcriptase-polymerase chain reaction (qRT-PCR) was used to determine the body titer of females with disseminated infections (see methods below), where titer refers to amount of virus. In this study, we define dissemination as the proportion of infected mosquitoes with DENV-2 in legs, whereas 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.[34]

**Plaque assays—**For each mosquito, wings and legs were dissected from bodies followed by separate virus assays of bodies and legs. Wing lengths were measured as indicators of mosquito size. The detailed procedures used for the plaque assays are described elsewhere. [31] Bodies and legs were homogenized separately in 2-mL flat-bottom vials containing 1 mL media and two zinc-plated steel BBs followed by homogenization and centrifugation ($3,148 \times g$ for 4 minutes at 4°C). Mosquitoes were tested for the presence of virus infection by plaque assays using undiluted body and leg stock solutions. Wells were scored as positive or negative depending on the presence or absence of plaques, respectively. Only females determined to have disseminated DENV-2 infections were subsequently assayed for body titer by RNA extractions subject to qRT-PCR. Plaque assays to determine blood meal titer in infectious feedings were performed similar to those assays used in determining mosquito infection and dissemination, except that a series of 10-fold serial dilutions were performed in duplicate 6-well plates to identify endpoints. Plaques were counted and expressed in PFUs per 0.2 mL of test inoculum.

**Determination of viral body titer—**DENV-2 body titer was determined for mosquitoes with disseminated infections using qRT-PCR standardized with a plaque assay. RNA was extracted from mosquitoes using one of two methods: QIAamp viral RNA Mini Kits (Qiagen Inc., Valencia, CA) or Mag-NApure Total Nucleic Acid kits (Roche Diagnostics, Chicago, IL). Preliminary examination determined these two methods to be equivalent for use in qRT-PCR to determine viral titer, and separate standard curves based on plaque as-says were generated for each RNA extraction method (unpublished data). Quantitative RT-PCR was conducted using SuperScript III Platinum one step qRT-PCR (Invitrogen Co., Carlsbad, CA) and fluorogenic probe hydrolysis (TaqMan) technology. DENV-2 virus–specific primers targeted the capsid gene (forward 237–251 bp, reverse 305–284 bp).[35] Primer sequences were: forward (5′-CATGGCCCTKGTGGCG-3′) and reverse (5′-CCCCATCTYTTCAGTATCCCTG-3′).[35] The DENV-2–specific dual-labeled fluorogenic oligonucleotide probe included a 5′-reporter dye and a 3′-quencher dye (250 nm DLB 5′ 6-FAM/3′ BHQ-2 [5′-TCCTTCGTTTCCTAACAATCC-3′]).[35] Reactions were performed in glass capillary tubes or in 96-well reaction plates, each containing: 0.4 µL SuperScript III RT/Platinum *Taq* mix, 10 µL 2× reaction mix (a buffer system, $MgSO_4$, dNTPs, and stabilizers), 1 µL forward primer (10 µmol/L), 1 µL reverse primer (10 µmol/L), 0.5 µL fluorogenic probe (10 µmol/L), 4.2 µL diethyl procarbonate (DEPC)-treated $H_2O$, and 2 µL test sample (positive or negative for DENV-2 RNA). Glass capillary tubes, containing reaction reagents, were placed in a thermocycler, LightCycler 1.2 instrument, equipped with LightCycler software version 3.5 (Roche Molecular Biochemicals, Indianapolis, IN). Ninety-six-well reaction plates were placed in a thermocycler, LC480 (Roche Molecular Biochemicals). For both instruments, the thermal cycle was as follows: 30 minutes at 48°C and 2 minutes at 95°C, followed by 45 cycles of PCR, 15 seconds at 95°C, and 1 minute at 60°C. A negative control (water as template) and positive control standard (DENV-2 stock RNA, $10^{-5}$ dilution) were included in each reaction run. PFUs were calculated by a standard curve method that compared cDNA synthesis (i.e., positive control) to *in vitro* PFU for the same full range of positive DENV-2 RNA stock virus titrated in parallel by qRT-PCR and plaque assay (Qiagen extraction: $N = 3$, slope = −3.007, intercept = 34.54, $r^2 = 0.9604$; MagNA Pure extraction: $N = 21$, slope = −3.46 intercept = 34.47, $r^2 = 0.9962$).[36]

### Statistical analyses

We compared the size of females between species and infection status among those individual females that blood fed and completed the extrinsic incubation period using a two-way analysis of variance (ANOVA). Main effects were species (*Ae. aegypti* versus *Ae. albopictus*) and DENV-2 infection status that consisted of uninfected (no virus detected), positive infection limited to the midgut (non-disseminated infection), and positive infection disseminated to other tissues such as the legs (disseminated infection). Thus, we tested for differences in the wing lengths of mosquitoes in different states of infection as well as between species, both of which are categorical variables. We also tested for a species × infection status interaction. Significant effects were further analyzed by pairwise comparisons of main effect means (Ryan-Einot-Gabriel-Welsch test).[37] There was significant departure from normality (Kolmogorov Smirnov test, $D = 0.0835$, $P = 0.0397$), so data were transformed reciprocally to satisfy the assumption of normality. Results for follow-up tests did not differ among untransformed versus transformed data, so untransformed means ± SE are reported for ease of interpretation.

For each *Aedes* species and larval treatment, separate logistic regressions were used to determine the relationship between individual mosquito size and the state of DENV-2 infection while controlling for the potential random, idiosyncratic effects within each replicate (PROC GLIMMIX in SAS).[37] Thus, comparisons are within species and within larval treatment. Probability of state of DENV-2 infection (infected = 1, uninfected = 0) and state of DENV-2 dissemination (disseminated = 1, non-disseminated = 0) were regressed against size, a continuous variable. To determine whether there were differences in DENV-2 infection and dissemination status between intra- and interspecific treatments, we used a two-sample *t* test (two-tailed) to compare estimates derived from each of the separate logistic regressions.

Separate linear regressions were analyzed for both *Ae. ae-gypti* and *Ae. albopictus*, with female size (per replicate) as a continuous independent variable, to study the relationship between female size and body titer of females with disseminated infection using PROC GLM in SAS.[37] Thus, these analyses pool all larval treatments of the two *Aedes* species. The aim of these analyses is to determine whether a measure of mosquito body size (wing length) is a predictor of subsequent vector–virus interactions (viral replication as measured by body titer). To address larval treatment effects (i.e., intra-versus interspecific effects), as well as species effects (*Ae. albopictus* versus *Ae. aegypti*), we used a two-way ANOVA and tested for differences in body titer.[37]

Interspecific (*Ae. aegypti* versus *Ae. albopictus*) differences in DENV-2 susceptibility to infection were evaluated by multivariate ANOVA (MANOVA) and standardized canonical coefficients (SCCs) on the response variables infection and dissemination rates (expressed as percentage of mosquitoes from each replicate). These analyses form the basis for comparing DENV-2 infection and dissemination rates between these two *Aedes* species. MANOVA is superior to univariate approaches for these comparisons because it yields information on both infection and dissemination rates simultaneously. Furthermore, SCCs determine the relative contribution of each of the response variables to significant multivariate effects and their relationship to each other (e.g., positive or negative).[38] Raw data adequately met assumptions of univariate normality and homogeneous variances for analyses.

## RESULTS

A two-way ANOVA showed significant size differences between species (*Ae. aegypti* versus *Ae. albopictus*; $F_{1,114} = 14.84$, $P = 0.0002$; Figure 1) and dengue infection status categories ($F_{2,114} = 3.17$, $P = 0.0457$; Figure 1). There was no significant species × infection status interaction ($F_{2,114} = 0.28$, $P = 0.7563$; Figure 1). Thus, differences between infection status were consistent in the two species. The species effect showed that *Ae. aegypti* mean wing length

was significantly larger than that of *Ae. albopictus* (least squares means ± SE, 2.69 ± 0.01 and 2.62 ± 0.01, respectively). Tests of the contrasts for the significant DENV infection status effect showed a consistent pattern, for both *Aedes* species, in mean wing lengths and DENV-2 infection status (uninfected > infected [non-disseminated] > disseminated infection). For both species, uninfected mosquitoes were significantly larger than mosquitoes with disseminated DENV-2 infections ($P = 0.0350$; Figure 1).

Logistic regressions using 547 *Ae. albopictus* and 742 *Ae. aegypti* mosquitoes showed significant size effects on DENV-2 infection and dissemination for *Ae. aegypti* (Table 1). Smaller-sized *Ae. aegypti* had a consistent and significantly greater probability of a DENV-2 infection or disseminated infection, the latter an indicator of transmission, than larger individuals (Table 1). Significant effects of size on DENV-2 infection and dissemination were not observed for *Ae. albopictus* (Table 1). These results differed from the previous ANOVA analyses that detected significant size effects common to both *Aedes* species. Two-sample *t* tests were used to compare intra- and interspecific treatments for each species. There were no significant differences for any of the comparisons (all $t_s < 0.615$, $P > 0.5388$), indicating similar effects of intra- and interspecific treatments on DENV-2 infection and dissemination for both species.

There were significant effects on DENV-2 body titer of *Ae. aegypti* (Figure 2) when size is considered a continuous independent variable and titer is considered a dependent variable. In particular, slopes of DENV-2 body titer of *Ae. aegypti* with disseminated infections versus size showed a significant positive relationship (Figure 2). No significant size effects on body titer were found for *Ae. albopictus* (Figure 2). A two-way ANOVA showed significant differences between *Ae. aegypti* and *Ae. albopictus* in DENV-2 body titer ($F_{1,37} = 5.75$, $P = 0.0216$), with *Ae. albopictus* (least squares mean ± SE, 3.88 ± 0.06 $\log_{10}$ PFU/0.2 mL) having greater body titer than *Ae. aegypti* (least squares mean ± SE, 3.67 ± 0.06 $\log_{10}$ PFU/0.2 mL). Larval treatment (intra- versus interspecific) and the species × larval treatment interaction were not significant (both $F_{1,37} \leq 2.42$, $P = 0.1283$).

Interspecific (*Ae. aegypti* versus *Ae. albopictus*) differences in DENV-2 susceptibility to infection were compared, which showed that infection and dissemination rates were significantly different between the two *Aedes* species (Pillai's trace$_{2,39} = 0.82$, $P < 0.0001$). Infection rate (SCC = 1.88) contributed more to the overall interspecific differences than dissemination rate (SCC = −0.71). The opposite signs of the SCCs showed a negative relationship between infection and dissemination, because *Ae. albopictus* had a greater percent of infected females but a lower percent of disseminated infections compared with *Ae. aegypti* (Table 2).

## DISCUSSION

The results showed that the susceptibility to infection and dissemination of DENV among adult *Aedes* is altered by body size and species. Smaller individuals of both species were more likely to have disseminated DENV-2 infections than larger individuals. Significant negative slopes of size plotted against probability of DENV-2 infection and dissemination suggested effects were stronger for *Ae. aegypti* than *Ae. albopictus*. Significant relationships between body size and virus susceptibility found common to both mosquito species in the ANOVA, but limited to *Ae. aegypti* in the logistic regressions are, in part, attributable to a more statistically conservative approach obtained when analyzing each mosquito species and larval treatment separately in the logistic regression analyses. The absence of significant logistic regressions between size and infection status in *Ae. albopictus* may also be attributable, in part, to the overall high infection rates in this species (> 90%), thus making detection of potential size-dependent differences in infection less likely than would be possible for moderate infection

rates. Moreover, there was far less variation in the sizes of *Ae. albopictus* relative to *Ae. aegypti* (Figure 1), thus decreasing ability to detect size-related differences in infection and dissemination. These results showed size-dependent vector competence for DENV and its primary mosquito vectors. Whether the larvae experienced intra- or interspecific competition did not alter these size effects. We also recorded species-specific differences in infection and dissemination rates as well as body titer for DENV-2, which may determine, in part, the relative vectorial ability of these two species beyond other recognized factors (e.g., host choice for blood feeding).

Ontogenetic niche shifts are ubiquitous among holometabolous insects. Here we show how alterations in size, in many instances mediated by ecological conditions in the aquatic larval stage, can alter vector–virus interactions in the adult female mosquito with potential consequences for human health. We found size effects common to both *Aedes* species, with larger individuals more resistant to infection, suggesting that these phenomena might be generalizable to other populations and species. Although these effects of size on infection seem to be stronger for *Ae. aegypti*, there were no significant species × size interactions, indicating that the trends caused by size were the same in each species for both infection and dissemination. This is consistent with the hypothesis that the mechanisms responsible for size-dependent susceptibility may be similar for *Ae. aegypti* and *Ae. albopictus*. However, we cannot rule out the possibility that different mechanisms were responsible for similar outcomes in the two species.

In contrast to the negative relationships between infection and dissemination status and body size, there was a positive relationship between size and body titer for *Ae. aegypti*. This latter result may be explained simply as a body mass phenomenon, because more tissue is available for virus propagation in large mosquitoes than small mosquitoes, as observed in a different mosquito–virus system.[9] Other research on unre-lated arboviruses confirmed similar relationships between size and arbovirus infection and dissemination rates in mosquitoes. [9,23,24,26]

Beyond the effects of size, the interspecific comparison (*Ae. aegypti* versus *Ae. albopictus*) showed different responses to dengue virus infection in these *Aedes* species. *Ae. albopictus* was more susceptible to viral infection but less likely to disseminate DENV compared with *Ae. aegypti*, whereas *Ae. aegypti* was less susceptible to infection but more likely to permit dissemination than *Ae. albopictus*. These differences in infection status showed the importance of measuring multiple infection parameters to characterize vector competence, especially when species comparisons are being made. Genetic studies on *Ae. aegypti* have mapped several quantitative trait loci controlling DENV-2 midgut infection[17] and dissemination.[19] Similar studies have not been done on *Ae. albopictus* but may help resolve species differences in infection outcomes.

*Aedes aegypti* is presumed to be the most important vector of dengue viruses worldwide. The current results seem to support this contention from the physiological standpoint that barriers operating to limit viral dissemination, and thus transmission, were less efficient in *Ae. aegypti* than in *Ae. Albopictus*. Considering only viral passage in the vector, the competence in terms of dissemination rates of *Ae. aegypti* for dengue virus (horizontal transmission) is superior to that of *Ae. albopictus*. However, the actual percent of probable dengue virus transmitting mosquitoes is equivalent to the product of the proportion of female mosquitoes infected and disseminated expressed as a percent (*Ae. aegypti*, 41%; *Ae. albopictus*, 35%). Thus, although infection and dissemination rates of DENV-2 were markedly different between these two species, the number of potential transmitting mosquitoes was similar with *Ae. aegypti* only slightly greater than *Ae. albopictus*. Therefore, it is likely that the relative vector potential of these mosquito species for dengue viruses may be determined largely by factors

other than vector competence such as host choice in obtaining blood meals. However, these species-specific effects of vectoring ability may also depend on the size of mosquitoes so that smaller mosquitoes could enhance the percent of transmitting mosquitoes, as indicated by disseminated infections (Figure 1). There have been outbreaks of dengue fever in Hawaii and Macao attributed to *Ae. albopictus*, suggesting that, under the right circumstances, *Ae. albopictus* can be a satisfactory vector.[39,40] Therefore, additional studies are needed to evaluate the relative importance of factors that determine the vectoring ability of these mosquitoes species and ultimately evaluate the risk of dengue transmission in areas where one or the other or both species exist.

In addition to differences in infection and dissemination rates, *Ae. albopictus* had significantly greater viral body titer than *Ae. aegypti* in disseminated infections yet, given infection, it was less likely to be disseminated. A previous study showed that DENV-2 dissemination rates in *Ae. aegypti* were independent of midgut virus titer.[41] Interspecific differences in body titer of *Aedes* females with disseminated infections suggest that factors limiting viral replication in *Ae. aegypti*, but not viral dissemination (see above), were more efficient compared with *Ae. albopictus*. A more efficient midgut escape barrier in *Ae. albopictus* than *Ae. aegypti* may select for greater viral replication, and associated body titer, because failure to escape the midgut results in failure to infect the next host. Alternatively, the *Ae. albopictus* midgut may merely be a better physiologic environment for DENV-2 replication than the *Ae. aegypti* midgut, irrespective of barriers. Few studies have quantified DENV body titer in these *Aedes* species, so it is not clear whether other mosquito and DENV strains show similar *Aedes*-specific effects of viral load.

Results of this study, as well as a companion study,[31] may have important implications for vector control strategies and evaluation of disease risk for dengue. Previous studies have shown that larval control strategies (e.g., larvicides) may release surviving larvae from competition, resulting in the production of larger adult mosquitoes.[42,43] An analogous situation occurs when mosquito immatures escape predators and emerge as larger adults after predator-mediated release from competition.[44–47] This study suggests an additional potential benefit of control strategies, mainly that the production of larger, less competent adults, in terms of dengue virus infection and dissemination, potentially mitigating DENV transmission. Also, information on temporal and spatial differences in sizes has the potential to be informative for disease risk and aid in control efforts. However, release from competition may also result in greater numbers of mosquitoes (i.e., enhanced survivorship to adulthood) potentially offsetting any benefits of altered infection and dissemination of DENV. Larger adults, although less competent in this study, may have greater longevity under field conditions,[11–13] but some studies have shown no such effects.[48] Thus, effects of body size on longevity may depend largely on the particular ecological conditions. Vector competence is only one of several factors that determine vectorial capacity that also includes population and behavioral attributes of mosquitoes.[15]

There is high variability in mosquito size within many mosquito species,[49] and in some instances, adult size may be related to adult longevity, an important contributing factor to vectorial capacity.[10] Smaller sized adults, associated with competition or warmer larval environments, may have reduced survivorship making them less effective vectors.[11–13] However, reduced size among adults may allow for greater contact with hosts and more frequent blood feeding than larger adults, potentially enhancing transmission.[14] Also, size effects on susceptibility to infection and vectorial capacity may differ among mosquito populations, which have different genetic backgrounds.[50] This study serves as a starting point for future research directed at evaluating the interplay of positive and negative effects of altered life history traits (e.g., size, development time, survivorship) and its role in determining vector potential, including vector competence and vectorial capacity. The complex nature of these

questions may generate competing hypotheses and requires invoking more complex and rigorous analytical tools (e.g., path analysis) to evaluate control strategies and disease risk.

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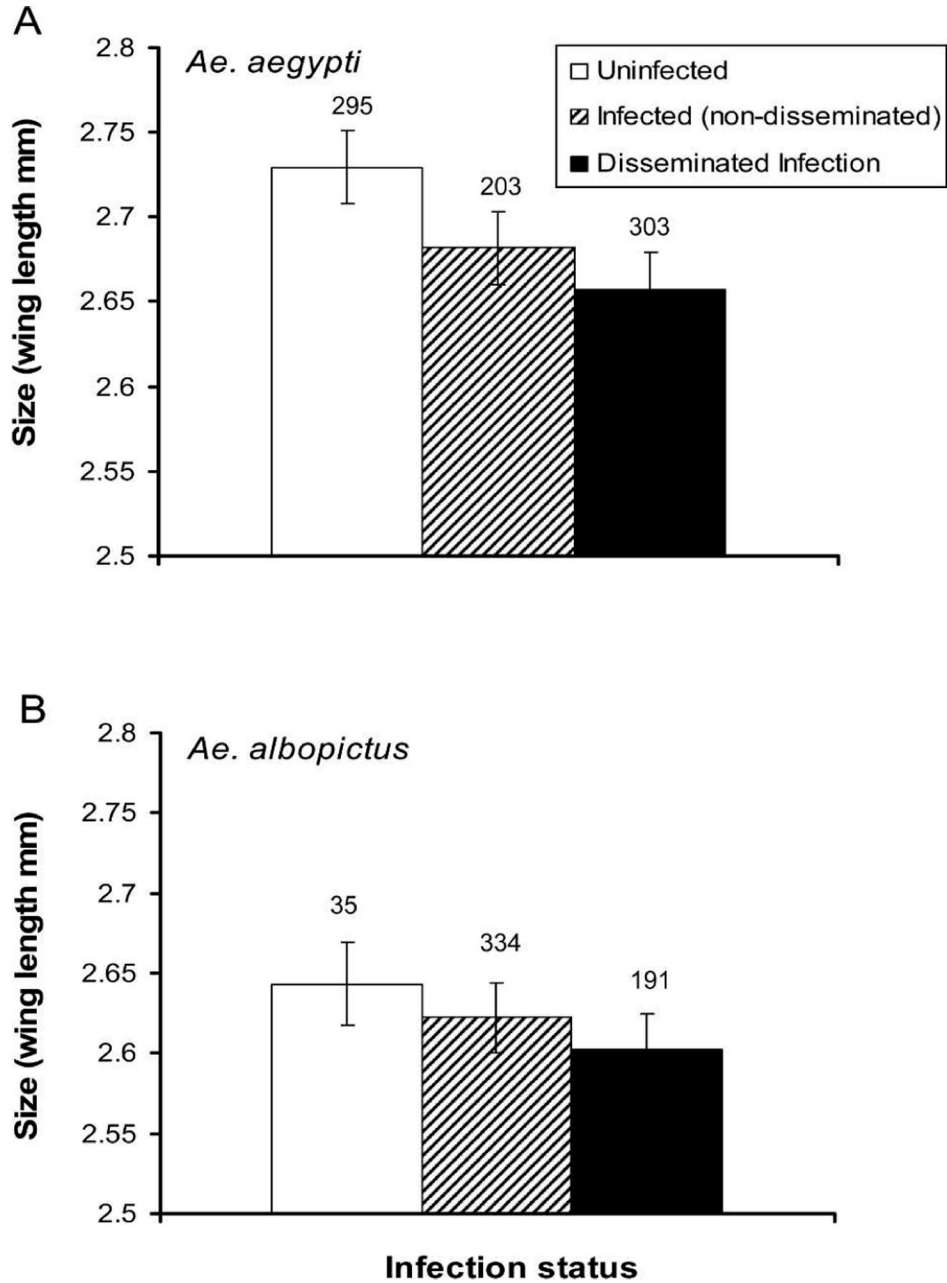
**FIGURE 1.**
Least squares means (SE) for sizes of adult *Aedes* mosquitoes in different states of DENV-2 infection (uninfected [no virus detected], positively infected [non-disseminated infection], and positively infected [disseminated infection]). Some replicates for *Ae. albopictus* treatments had all mosquitoes infected with dengue virus (100% infection in 6 of 21 replicates). These results did not yield information on sizes of uninfected mosquitoes. The design was un-balanced so least squares means are reported. Pooled over mosquito species (**A** and **B**), an analysis of variance showed that uninfected mosquitoes were significantly larger than mosquitoes with disseminated dengue virus infections. Also, *Ae. aegypti* were significantly larger than *Ae. albopictus* (see Results section). Sample sizes are indicated above each bar.
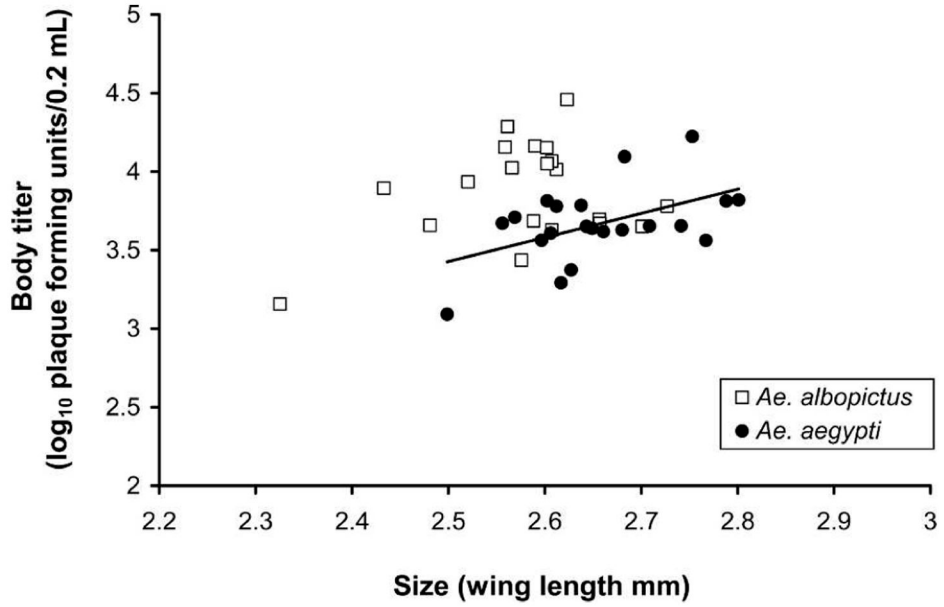
**FIGURE 2.**
Mean (per replicate) size and body titer for *Ae. aegypti* and *Ae. albopictus* mosquitoes with disseminated dengue virus infections. Line drawn through means shows the best-fit linear regression for *Ae. aegypti* (intercept [SE] =–0.4177 [1.5941]; slope [SE] = 1.5379 [0.5997]; $r^2 = 0.2571$, $F_{1,19} = 6.58$, $P = 0.0190$). No significant size by body titer relationship was observed for *Ae. albopictus* (intercept [SE] = 1.3232 [2.0153]; slope [SE] 0.9902 [0.7807], $r^2 = 0.0820$, $F_{1,18} = 1.61$, $P = 0.2209$).

**TABLE 1**

Logistic regressions testing the relationship of adult size on dengue virus infection and dissemination in *Ae. albopictus* and *Ae. aegypti*

| Species | Larval treatment | df | Logit (SE) | t-value | P |
|---|---|---|---|---|---|
| *Ae. albopictus* infection | Intraspecific | | | | |
| Intercept | | 10 | 4.2584 (4.0076) | 1.06 | 0.3130 |
| Size | | 363 | −0.5254 (1.5114) | −0.35 | 0.7283 |
| *Ae. albopictus* infection | Interspecific | | | | |
| Intercept | | 9 | 2.1498 (4.4739) | 0.48 | 0.6423 |
| Size | | 161 | 0.1117 (1.7253) | 0.06 | 0.9485 |
| *Ae. albopictus* dissemination | Intraspecific | | | | |
| Intercept | | 10 | 1.2975 (1.9500) | 0.67 | 0.5209 |
| Size | | 363 | −0.8079 (0.7408) | −1.09 | 0.2762 |
| *Ae. albopictus* dissemination | Interspecific | | | | |
| Intercept | | 9 | 3.7506 (2.5260) | 1.48 | 0.1718 |
| Size | | 161 | −1.5940 (0.9763) | −1.63 | 0.1045 |
| *Ae. aegypti* infection | Intraspecific | | | | |
| Intercept | | 10 | 6.5405 (1.6202) | 4.04 | 0.0024 |
| Size | | 445 | **−2.2054** (0.6047) | −3.65 | **0.0003** |
| *Ae. aegypti* infection | Interspecific | | | | |
| Intercept | | 9 | 6.5283 (2.0906) | 3.12 | 0.0123 |
| Size | | 274 | **−2.0775** (0.7615) | −2.73 | **0.0068** |
| *Ae. aegypti* dissemination | Intraspecific | | | | |
| Intercept | | 10 | 3.7340 (1.5405) | 2.42 | 0.0358 |
| Size | | 443 | **−1.6156** (0.5795) | −2.79 | **0.0055** |
| *Ae. aegypti* dissemination | Interspecific | | | | |
| Intercept | | 9 | 4.1147 (1.8557) | 2.22 | 0.0538 |
| Size | | 275 | **−1.5598** (0.6819) | −2.29 | **0.0229** |

Significant slopes and associated *P* values are shown in bold.

**TABLE 2**

Least squares means (SE) for rates of infection and dissemination for *Ae. aegypti* and *Ae. albopictus* after fed a DENV-2 virus blood meal

| Species | Measure of infection | Mean percent (SE) |
| --- | --- | --- |
| *Ae. albopictus* | Infection | 94 (2) |
| | Dissemination | 38 (3) |
| *Ae. aegypti* | Infection | 68 (2) |
| | Dissemination | 61 (3) |