ECOLOGY AND POPULATION DIFFERENTIATION OF THE ASIAN TIGER MOSQUITO, *AEDES ALBOPICTUS*

A Dissertation submitted to the Faculty of the Graduate School of Arts and Sciences of Georgetown University in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Biology

By

Deborah Ladner O’Donnell

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ECOLOGY AND POPULATION DIFFERENTIATION OF THE ASIAN TIGER MOSQUITO, *Aedes albopictus*

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ABSTRACT

This research investigates factors affecting variation in life-history traits across the life cycle of a medically important mosquito, *Aedes albopictus*. First, to evaluate ecological differences between *Ae. albopictus* and *Ae. japonicus*, I compared their larval foraging behavior. Based on results from a previous competition experiment, I predicted that *Ae. albopictus* would be a more active forager than *Ae. japonicus*. However, *Ae. japonicus* exhibited greater activity than *Ae. albopictus*, suggesting that more active foraging does not connote superior competitive ability.

In addition, I performed common-garden and line-cross experiments in order to examine the genetic differentiation of fitness for *Ae. albopictus* at three spatial scales. I found that North American populations had lower fitness than populations from outside North America. Population crosses resulted in significant heterosis and outbreeding depression of hybrids, but the results did not depend on the geographic distance separating populations. My results imply a role for local genetic drift affecting the life-history differentiation of *Ae. albopictus* populations.

Local genetic drift is known to influence genetic differentiation among
populations for a variety of vector species. Next, I created inbred *Ae. albopictus* lines to test the boundary conditions under which local genetic drift might affect pathogen susceptibility. I predicted that inbreeding would significantly decrease life history traits and increase susceptibility to infection by the model pathogen, *Plasmodium gallinaceum*. Inbred mosquitoes had significantly reduced larval survivorship and female adult longevity, but I found no effect of inbreeding on susceptibility to infection.

Finally, I compared egg volume of populations from across a latitudinal gradient in the native (Japan) and invasive (US) range of *Ae. albopictus*. Egg size clines are frequently observed in insect populations, so I predicted that egg size would increase with population latitude for both native and invasive populations. I measured egg size for 11 populations from Japan and the US over the same approximate latitudinal range. However, I did not find a cline of increasing egg size correlated with increasing latitude for *Ae. albopictus* in either country. Overall, these results provide valuable insight regarding ecological and evolutionary processes during the invasion and range expansion of *Ae. albopictus* in North America.
Acknowledgements

I would like to gratefully acknowledge the assistance of my advisor, Dr. Peter Armbruster, and my thesis committee members, Dr. Martha Weiss, Dr. Tovi Lehmann and Dr. Gina Wimp, who were all integral to the completion of this research. In addition, I would like to thank Dr. Jennifer Hume and Ph.D. candidate Jennifer Urbanski for their role in facilitating the progress of my experiments. This work was supported by The Cosmos Club Foundation, the National Science Foundation Doctoral Dissertation Improvement Grant (Award ID 0808297) and Georgetown University.
Dedication

To my mom, dad and sis
who stuck with me like glue,
and my husband Chris-
it is all thanks to you!
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Chapter I

Comparison of Larval Foraging Behavior of Aedes albopictus and Aedes japonicus

(Diptera: Culicidae)

*Aedes albopictus* and *Ae. japonicus* are invasive, container-inhabiting mosquitoes that have recently become established in North America. *Aedes albopictus* was introduced into North America from Japan in 1985 and spread rapidly across ca. 15 degrees of latitude in the eastern United States from southern Florida to New Jersey, Ohio, and Illinois (Hawley et al. 1987, Moore 1999). Although considerable information is available regarding the invasion dynamics of *Ae. albopictus* in the United States (Lounibos 2002, Juliano and Lounibos 2005), far less is known about *Ae. japonicus* (but see Fonseca et al. 2001, Widdel et al. 2007). *Aedes japonicus* was first identified in the US in 1998 (Peyton et al. 1999), and is believed to have been introduced multiple times from Japan (Fonseca et al. 2001). Although *Ae. japonicus* is a cold-adapted species (Tanaka et al. 1979) with a more northerly native range than *Ae. albopictus*, there is significant habitat overlap between these species in the United States. *Aedes japonicus* has been reported on the east coast of the United States from Maine (Foss and Dearborn 2002) to Georgia (Reeves and Korecki 2004) as well as in Washington state (Roppo et al. 2004).

*Aedes albopictus* and *Ae. japonicus* are capable of transmitting various arthropod-borne diseases and are thus of considerable public health concern. *Aedes*
*Ae. albopictus* is a vector of dengue and dengue hemorrhagic fever in Asia (Gratz 2004), and in the United States it is a potential vector of several other arthropod-borne viruses including eastern equine encephalitis (Lounibos 2002, Gratz 2004). Although not considered an important disease vector in Japan, *Ae. japonicus* adults have been shown to be capable of transmitting Japanese encephalitis (Takashima and Rosen 1989), eastern equine encephalitis (Sardelis et al. 2002a), La Crosse encephalitis (Sardelis et al. 2002b) and St. Louis encephalitis (Sardelis et al. 2003). Furthermore, *Ae. albopictus* and *Ae. japonicus* are both able to efficiently transmit West Nile virus in the laboratory (Turell et al. 2001a, b), and naturally infected individuals of both species have been discovered ((CDC) 2000a, b, c, d, Bernard et al. 2001, White et al. 2001, Turell et al. 2001a, b, Holick et al. 2002). Consequently, the relative abundance of *Ae. albopictus* and *Ae. japonicus* throughout North America may have important human health implications.

Larvae of both *Ae. albopictus* and *Ae. japonicus* are found in water-filled man-made container habitats (e.g. used automobile tires), where, like other container-inhabiting mosquitoes, they feed on both microorganisms and dead particulate matter (Merritt et al. 1992). *Aedes* larvae feed in multiple modes, including browsing and filtering (Merritt et al. 1992). Resources are an important and often limiting component in container communities (Fish and Carpenter 1982, Paradise 2004), suggesting that larval foraging behavior can have a significant impact on survivorship and reproductive success.
Foraging behavior has also been linked to competitive interactions between species of container-breeding mosquitoes. For example, increased foraging activity by *Ae. albopictus* as compared to *Ae. aegypti* (Yee et al. 2004a) may be one of the mechanisms underpinning the competitive advantage of the former species under a wide range of conditions (Barrera 1996, Juliano 1998, Daugherty et al. 2000, Braks et al. 2004).

Recent studies have reported that *Ae. albopictus* is a superior larval competitor to *Ae. japonicus* under near-natural field conditions (Armistead 2007). Based on these results and previous studies linking larval competitive ability to foraging activity, I predicted that *Ae. albopictus* would be a more active forager than *Ae. japonicus*. I tested this hypothesis by quantifying the behavior of individual *Ae. albopictus* and *Ae. japonicus* larvae in six different feeding environments.

**Materials and Methods**

**Collections.** Mosquito eggs for the foraging behavior trials were collected from a county tire disposal site in Virginia (Prince William County Landfill, Manassas, Virginia, 38° 43’ 59 N, 77° 28’ 26 W) in July and August of 2006. Polystyrene oviposition traps (measuring 12 cm X 5 cm X 2 cm) as described by Scott and Crans (2003) were placed in 15 water-filled tires in the late afternoon and retrieved the next morning. Field collected eggs were kept moist in the laboratory where they were maintained at 21 ± 1°C on a 18:6 (L:D) photoperiod. Hatch was stimulated between three and five days post-oviposition by submerging eggs in water (Novak and Shroyer...
1978) mixed with ca. 3 ml larval food slurry. Larval food consisted of 80 g dried dog food (Nutro Brand Large Breed Adult, Nutro Products Inc., City of Industry, CA) and 40 g whole brine shrimp (Sally’s Frozen Brine Shrimp, San Francisco Bay Brand, Newark, CA) in 1 L distilled water homogenized in a kitchen blender.

**Foraging behavior.** Newly hatched larvae were placed into individual wells of 24-well cell culture plates as described in Armbruster and Conn (2006). Larvae were maintained at 21 ± 1°C on a 18:6 (L:D) photoperiod. Every Monday, Wednesday, and Friday larvae were transferred to fresh water in clean 24-well cell culture plates and fed 15 µl of larval food slurry. Pupae were retained in individual adult cages and species designations were confirmed based on adult morphology. A total of 180 individuals (90 *Ae. albopictus*, 90 *Ae. japonicus*) were used for behavior trials. Foraging behavior trials were based on previously established methods of measuring feeding behavior in *Aedes* larvae (Juliano and Reminger 1992, Grill and Juliano 1996, Juliano and Gravel 2002, Kesavaraju and Juliano 2004, Yee et al. 2004a, b). Six food environments with varying nutrient levels were set in 50 ml petri dishes (the plastic and leaf substrates are described in the next paragraph): 1) “plastic only” (plastic strip and 50 ml distilled water, a control treatment); 2a) “plastic + liquid” (plastic strip, 1.5 ml of liquid stock food and 50 ml of distilled water); 2b) “liquid only” (1.5 ml liquid stock food and 50 ml distilled water); 3) “leaf only” (leaf strip and 50 ml distilled water) 4a) “leaf + liquid” (leaf strip, 1.5 ml of liquid stock food and 50 ml of distilled water); and 4b) “leaf + tire water” (leaf strip and 50 ml of filtered tire water). I predicted different behavior modes
for each type of environment. Non-feeding behavior was expected for “plastic only” and filtering behavior was expected for “plastic + liquid” and “liquid only”. Browsing behavior was expected for “leaf only” while filtering and browsing behaviors were expected for “leaf + liquid” and “leaf + tire water.”

Senescent white oak leaves (*Quercus alba*) were collected from Glover-Archbold Park in Washington, DC. Leaves were cut into strips (1 cm x 5 cm) and soaked in distilled water at room temperature for two days to allow for growth of microorganisms before being used in a behavior trial. Clean plastic sheets were cut into strips (1 cm x 5 cm) and used as a control for the presence of leaf strips. The “leaf + tire water” food environment includes microorganisms that occur in tire water (as opposed to distilled water). Tire water was collected from four tires that contained *Aedes* larvae at the Manassas, Virginia field site. Water was removed from the tires and filtered through a fine-mesh net in order to eliminate field larvae and particulate matter and then homogenized and stored at room temperature.

Foraging behavior trials were conducted by recording the behavior and position of the individual larva by instantaneous scan every 30 seconds for 30 minutes. Prior to each behavior trial, fourth instar larvae were starved for 24 hours. Each larva was then allowed to acclimate in the 50 ml petri dish for 5 minutes before the 30 minute observation period began and all observations were made by the same researcher (DO). Behavior trials were performed between 2:00 and 6:00 pm each day in order to control for circadian variation in activity levels with the order of species and food treatment.
haphazardly assigned. Activity was defined as “browsing” if a larva moved along the surface of the leaf or plastic propelled by the feeding movements of its mouth parts, “resting” if a larva was motionless, “filtering” if a larva drifted through the water propelled by feeding mouth parts and “thrashing” if a larva rapidly flexed its body laterally through the water (Juliano and Reminger 1992, Grill and Juliano 1996, Juliano and Gravel 2002, Kesavaraju and Juliano 2004, Yee et al. 2004a, b). Positions were defined as “surface” if a larva’s siphon was in contact with the water surface, “wall” if the larva was within 1 mm of the wall and “leaf” if the larva was within 1 mm of the leaf or plastic strip (Juliano and Reminger 1992, Grill and Juliano 1996, Juliano and Gravel 2002, Kesavaraju and Juliano 2004, Yee et al. 2004a, b).

**Analysis.** Data from the behavior trials were transformed to proportions of observations in each activity and position category (Juliano and Reminger 1992, Grill and Juliano 1996, Juliano and Gravel 2002, Kesavaraju and Juliano 2004, Yee et al. 2004a, b). Arcsine-square-root transformation was then applied to meet the assumptions of normality and to homogenize variances. Principal components analysis was performed in S-Plus 6.0 (Mathsoft 1999) in order to derive independent composite indicators of larval behavior. Multivariate analysis of variance (MANOVA, PROC GLM, SAS Institute 2004) was then performed on retained PC scores. Standardized canonical coefficients were calculated and multivariate pairwise contrasts performed in order to interpret MANOVA results (Scheiner 2001). Following MANOVA, univariate ANOVAs were performed on retained PCs with *a posteriori* comparison between
species within each food environment in order to identify environment-specific differences in foraging behavior. A sequential Bonferroni (Rice 1989) was applied to control for experiment-wise error ($\alpha = 0.05$).

**Results**

The first two principal components collectively explained 77.9% of the variation in larval behavior (Table 1). Principal component 1 (PC1) explained 54.5% of the variance in behaviors and quantified time browsing on the leaf (high positive scores) versus time spent near the surface (low negative scores; Table 1). The second principal component (PC2) explained 23.4% of the variance in behaviors and quantified time filtering (high positive scores) versus time resting (low negative scores; Table 1). PC1 and PC2 were retained for further analysis because their eigenvalues are greater than 1.0 (Mathsoft 1999). Loadings greater than 0.4 and below -0.4 were used for interpretation of the principal components.

The results of a MANOVA on PC1 and PC2 scores indicated that behavior differs significantly among environments and between species (Table 2). There also was a significant species by environment interaction, indicating that species responded differently to changes in food environment. Standardized canonical coefficients indicated that differences between species were strongly associated with PC1 and weakly associated with PC2, differences among food environments were strongly associated with PC1 and moderately associated with PC2, and differences in the response of species to differing food environments (S x E interaction) were strongly
associated with PC1 but not with PC2.

Pairwise *a posteriori* contrasts revealed that in the plastic only environment, *Ae. japonicus* spent significantly more time browsing along the plastic strip compared to *Ae. albopictus* (Fig. 1A), but time spent filtering was not significantly different (Fig. 1B). Foraging behavior was not significantly different for *Ae. albopictus* and *Ae. japonicus* in the plastic + liquid food environment or liquid food environment (Fig. 1A, B). In the leaf environment, filtering did not differ between species (Fig. 1B), but *Ae. japonicus* spent significantly more time browsing along the leaf when compared to *Ae. albopictus* (Fig. 1A). *Aedes japonicus* also spent significantly more time browsing in the leaf + liquid environment (Fig. 1A) but filtering behavior was similar for both species (Fig. 1B). In the leaf + tire water environment, *Ae. japonicus* spent significantly more time browsing (Fig. 1A) and filtering (Fig. 1B) as compared to *Ae. albopictus*.

**Discussion**

Studies of larval competition between *Ae. albopictus* and *Ae. japonicus* provided with leaves in containers outdoors indicated that *Ae. albopictus* realized a higher finite rate of increase (\( \lambda' \)) than *Ae. japonicus* due to a higher survivorship and shorter development time of *Ae. albopictus* relative to *Ae. japonicus* (Armistead 2007). These results thus demonstrate that *Ae. albopictus* is a superior larval competitor to *Ae. japonicus*, similar to the results of larval competition experiments between *Ae. albopictus* and *Ae. aegypti* (Barrera 1996, Juliano 1998, Daugherty et al. 2000, Braks et al. 2004), and between *Ae. albopictus* and *Cx. pipiens* (Costanzo et al. 2005).
Furthermore, previous studies have suggested that in some cases larval foraging behavior contributes to the competitive interactions between container-breeding mosquito species. For example, a foraging behavior study performed by Yee et al. (2004a) indicated higher levels of foraging activity, especially browsing on leaf surfaces, of *Ae. albopictus* relative to *Ae. aegypti*. *Aedes albopictus* is a superior larval competitor to *Ae. aegypti* under a wide range of conditions (Barrera 1996, Juliano 1998, Daugherty et al. 2000, Braks et al. 2004); the results of Yee et al. (2004a) support the hypothesis that these differences in competitive ability may be related to foraging behavior.

Based on the results of Armistead (2007) and the considerations noted above, I predicted that *Ae. albopictus* would be a more active forager than *Ae. japonicus*. However, contrary to my expectations, I found that *Ae. japonicus* was a more active forager than *Ae. albopictus* in four of six experimental environments. In the control treatment (plastic only), *Ae. japonicus* exhibited more browsing and filtering behavior while *Ae. albopictus* spent more time resting (Fig. 1A, B). This control treatment indicates that *Ae. japonicus* is a more active forager than *Ae. albopictus* even without direct cues from food resources. Overall levels of browsing tended to increase for both *Ae. albopictus* and *Ae. japonicus* in environments that include a leaf relative to the plastic only control (Fig. 1A). When leaf resources are available, phagostimulants (such as nucleic acid) stimulate larvae to feed along leaf surfaces (Merritt et al. 1992). *Aedes japonicus* was a more active browser than *Ae. albopictus* in the leaf and leaf +
liquid food and leaf + tire water food environments (Fig. 1A), suggesting that *Ae. japonicus* may be more receptive to phagostimulants than *Ae. albopictus*. The increased foraging activity of *Ae. japonicus* relative to *Ae. albopictus* in response to the presence of a solid foraging substrate is similar to the results of Nilsson (1986), who found that the presence of a leaf altered the feeding strategy for *Ae. communis* (DeGeer).

*Aedes japonicus* was not a more active feeder in the presence of only liquid larval food slurry; there were no significant differences in foraging behavior between species in the liquid only and plastic strip + liquid environments (Fig. 1A, B). However, *Ae. japonicus* was a more active filter feeder in the leaf + tire water environment (Fig. 1B). Because this treatment was composed of water from *Aedes* larval habitats, the results suggest that *Ae. japonicus* is an active filter feeder under field conditions. Thus, under a wide range of environments, *Ae. japonicus* exhibited higher foraging activity levels than *Ae. albopictus*, and differences between species tended to be most pronounced in the presence of solid foraging substrate (i.e., plastic or leaf strip).

Several factors may explain the observation that *Ae. albopictus* is less active in larval foraging but a superior larval competitor relative to *Ae. japonicus*. First, increased foraging in low nutrient environments may decrease fitness due to inappropriate energy expenditure. Optimal foraging models (Stephens and Krebs 1989) assume that fitness is maximized by optimizing either the net rate (energy acquired per
unit time) or efficiency (energy acquired per unit of energy expenditure) of foraging. My results show that *Ae. japonicus* was a more active forager in the low-nutrient plastic only environment, while *Ae. albopictus* conserved energy by resting (Fig. 1A, B). These results imply that *Ae. albopictus* exhibited a more appropriate foraging strategy because resources were absent in this environment. Similar effects also may occur under low nutrient conditions, such as those employed by Armistead (2007) or in nature.

It is also possible that factors other than foraging behavior affect competitive interactions between *Ae. albopictus* and *Ae. japonicus*. For instance, foraging behavior comparisons revealed a similar amount of leaf browsing for *Ae. albopictus* and *Culex pipiens* (Yee et al. 2004b). However, in competition experiments, *Cx. pipiens* larva had lower survivorship, longer development times, and reduced per capita rate of increase (r’) compared to *Ae. albopictus* (Costanzo et al. 2005). Priority effects demonstrating inhibition of younger instars by older larvae have been demonstrated for a variety of mosquito species, and in some cases these effects appear to be mediated by water soluble chemicals (Broadie and Bradshaw 1991, Sunahara and Mogi 2002, Bédhomme et al. 2005). Sunahara and Mogi (2002) demonstrated asymmetric chemical priority effects between *Ae. albopictus* and its interspecific competitor, *Tripteroides bambusa*. There is also evidence for asymmetrical priority effects between fast and slow growing larvae that colonize a habitat simultaneously (Livdahl 1984, Sunahara and Mogi 1997), although the extent to which these effects may be mediated by chemical factors as opposed to feeding behavior is unknown. Thus, although clear examples of
allelopathy (common in plants) have not been demonstrated in insects, it is possible
that differences in larval growth rate and sensitivity to nitrogenous waste products or
other water-borne chemicals could contribute to competitive interactions between
erspecies, including Ae. albopictus and Ae. japonicus or Cx. pipiens.

My results also have implications for the relative predator susceptibility of Ae.
albopictus and Ae. japonicus in container habitats. Grill and Juliano (1996) showed that
the invasive species Ae. aegypti was more active and thus more vulnerable to predation
by Toxorhynchites rutilus than the native Aedes triseriatus. Similarly, Kesavaraju and
Juliano (2004) showed that Ae. triseriatus shifted to low-risk behavior in the presence
of water-borne chemical cues from predatory Tx. rutilus while the invasive Ae.
albopictus maintained a high level of foraging activity, causing Ae. albopictus larvae to
be more vulnerable to predation. Furthermore, larval predation has been shown to
ameliorate the competitive effects of Ae. albopictus on Ae. triseriatus (Griswold and
Lounibos 2005). Because Ae. japonicus is an active larval forager that has been found
to co-occur with T. rutilus (Scott 2003, Joy 2004), I predict it will also be highly
susceptible to predation by T. rutilus and that predation by T. rutilus may enhance the
fitness of Ae. albopictus relative to Ae. japonicus in container habitats where all three
species co-occur. Future comparisons of competition, susceptibility to predation, and
resource use for Ae. albopictus and Ae. japonicus should help elucidate how ecological
factors influence the interactions and relative abundance of these species throughout
North America.
Table 1. Principal component analysis of foraging behavior, showing the variance and factor loadings for each component. Boldface text indicates principal components retained for further analysis.

<table>
<thead>
<tr>
<th>Variable</th>
<th>PC1</th>
<th>PC2</th>
<th>PC3</th>
<th>PC4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Variance:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eigenvalue</td>
<td>3.816</td>
<td>1.635</td>
<td>0.837</td>
<td>0.479</td>
</tr>
<tr>
<td>Proportion of variance</td>
<td>0.545</td>
<td>0.234</td>
<td>0.120</td>
<td>0.068</td>
</tr>
<tr>
<td>Cumulative proportion of variance</td>
<td>0.545</td>
<td>0.779</td>
<td>0.898</td>
<td>0.967</td>
</tr>
<tr>
<td><strong>Loadings:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resting</td>
<td>-0.377</td>
<td>-0.497</td>
<td>-0.136</td>
<td>0</td>
</tr>
<tr>
<td>Browsing</td>
<td>0.486</td>
<td>0</td>
<td>0.105</td>
<td>-0.164</td>
</tr>
<tr>
<td>Thrashing</td>
<td>-0.231</td>
<td>0.257</td>
<td>0.888</td>
<td>-0.218</td>
</tr>
<tr>
<td>Filtering</td>
<td>0</td>
<td>0.721</td>
<td>-0.200</td>
<td>0.454</td>
</tr>
<tr>
<td>Surface</td>
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<td>0.140</td>
<td>0.532</td>
</tr>
<tr>
<td>Wall</td>
<td>-0.342</td>
<td>0.390</td>
<td>-0.329</td>
<td>-0.658</td>
</tr>
<tr>
<td>Leaf</td>
<td>0.493</td>
<td>0</td>
<td>0.120</td>
<td>0</td>
</tr>
<tr>
<td>Interpretation</td>
<td>Browsing, Leaf (+) vs. Filtering (+) vs. Thrashing (+) Wall (-)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Surface (-) Resting(-)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


Table 2. Results of two-way (species, food environment) MANOVA on PC1 and PC2 behavior scores.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>error df</th>
<th>Pillai’s Trace</th>
<th>Approx. F</th>
<th>P value</th>
<th>PC1</th>
<th>PC2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Environment (E)</td>
<td>10</td>
<td>336</td>
<td>1.210</td>
<td>51.47</td>
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<td>0.431</td>
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<td>40.58</td>
<td>&lt; 0.001</td>
<td>1.655</td>
<td>0.957</td>
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<tr>
<td>S x E</td>
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<td>336</td>
<td>0.245</td>
<td>4.69</td>
<td>&lt; 0.001</td>
<td>1.874</td>
<td>-0.08</td>
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Figure 1. Larval foraging behavior of 90 *Ae. albopictus* and 90 *Ae. japonicus* as measured by mean scores (+1 SE) for (A) PC1 and (B) PC2 in plastic only (Pl), plastic + liquid (Pl+ Liq), liquid only (Liq), leaf only (Lf), leaf + liquid (Lf+Liq) and leaf + tire water (Lf+TW) food environments. Open bars (□) represent *Ae. japonicus* and closed bars (■) represent *Ae. albopictus*. Significant differences between species within treatments after sequential Bonferroni adjustment (experiment-wise $\alpha = 0.05$) are indicated by *.
In this chapter, I assessed individual larval foraging behavior of *Ae. albopictus* in a diverse set of environments. In conjunction with other studies that have quantified larval competition, these results provide insight into mechanisms mediating species interactions. I investigated larval foraging behavior because it has a significant impact on mosquito survivorship and reproductive success. Life-history traits that underpin population growth rates are also important determinates of abundance and can thus strongly affect both invasion success and rates of disease transmission by vector species. In the next chapter, I examine genetic differentiation of fitness related life-history traits among North American and non-North American populations of *Ae. albopictus*. 
Chapter II
Evolutionary differentiation of fitness traits across multiple geographic scales in *Aedes albopictus* (Diptera: Culicidae)

*Aedes albopictus* (Skuse) is an invasive, container-inhabiting mosquito that was introduced into North America from Japan in 1985 and subsequently spread rapidly across the eastern United States from Houston, TX south to Florida and north to New Jersey, Ohio, and Illinois (Hawley et al. 1987, Moore 1999). This medically important mosquito is capable of transmitting a wide variety of arthropod-borne pathogens including dengue, eastern equine encephalitis, West Nile, and chikungunya viruses (Gratz 2004, Angelini et al. 2007). Life-history traits that underpin population growth rates are important determinants of abundance and can thus strongly affect both invasion success (Lounibos 2002) and rates of disease transmission by vector species (Black and Moore 2005). It is therefore of fundamental importance to vector biology as well as the field of life-history evolution to understand how ecological and genetic factors influence the diversification of life-history traits underpinning population growth rate parameters. However, surprisingly few studies have considered genetically-based population differentiation of life-history traits in vector species (but see Lehmann et al. 2003, Armbruster and Conn 2006, Leisnham et al. 2008).

In contrast, there have been a large number of previous studies utilizing neutral biochemical markers to study population differentiation of vector species. In *Ae.*
*albopictus*, such molecular studies have consistently found evidence for high levels of local and regional population structure in the native and invasive ranges (Black et al. 1988a, b, Kambhampati and Rai 1991, Birungi and Munstermann 2002, Lourenço-de-Oliveira et al. 2003, Usmani-Brown et al. 2009). For example, Black et al. (1988a) conducted an analysis of allozyme variation and found that populations from New Orleans, Houston and near Indianapolis all contained unique (= “private”) alleles. An excess of homozygous genotypes within populations was found, suggesting that local inbreeding may have contributed to population differentiation. Black et al. (1988a) suggested that high local population structure in *Ae. albopictus* was likely the consequence of genetic drift during local population establishment of discrete habitat patches by a small number of individuals. Local mosquito control efforts may have also caused reductions in population size and increased local genetic drift (Black et. al 1988a). In a subsequent study, Black et al. (1988b) found similar patterns of population structure of *Ae. albopictus* in its native range in Malaysia and Borneo.

Subsequent molecular studies have further supported the conclusions of Black et al. (1988a, b). Analysis of mitochondrial DNA sequence variation by Birungi and Munstermann (2002) indicated that US *Ae. albopictus* populations exhibited significant genetic differentiation among populations on both a local (within Florida) and regional (between Florida and New Orleans, Georgia, Tennessee and Illinois) spatial scale. Birungi and Munstermann (2002) also found that Brazilian populations contained private (= unique) haplotypes, indicating no genetic exchange between the US and
Brazil, a conclusion that was further corroborated by the more recent study of Usmani-Brown et al. (2009). In addition, Birungi and Munstermann (2002) found that global *Ae. albopictus* populations have high NADH dehydrogenase 5 subunit (ND5) variation among populations (*H*$_{ST}$ = 0.835). Finally, an isoenzyme analysis by Lourenço-de-Oliveira et al. (2003) indicated that both North American and Brazilian *Ae. albopictus* populations are highly differentiated, with respective F$_{ST}$ values of 0.289 and 0.136. Furthermore, *Ae. albopictus* populations from Florida exhibit a strong local pattern of isolation by distance (slope of Fst/(1-Fst) vs. ln distance in km = 0.63, *P* = 0.018; Lourenço-de-Oliveira et al. 2003). Thus, studies using different types of molecular markers and sampling unique populations across a broad geographic and temporal scale consistently indicate high levels of local and regional genetic structure among populations of *Ae. albopictus*.

However, neutral biochemical markers can exhibit different evolutionary dynamics relative to genes underpinning polygenic life-history traits (Spitze 1993, Lynch 1996, Armbruster et al. 1998, Reed and Frankham 2001). I therefore initiated a study to investigate processes of evolutionary differentiation of fitness related life-history traits among North American and non-North American populations of *Ae. albopictus*. Based on the results of molecular studies noted above, I hypothesized that genetic drift would be an important factor influencing the evolutionary differentiation of populations.
First, I examined the differentiation of performance at three spatial scales: 1) local (among populations within Florida), 2) regional (between populations from Florida and New Jersey), and 3) long distance (between populations from Florida and Hawaii, Malaysia and Japan). I performed a common garden experiment and measured a composite index of fitness in semi-natural microcosms for three populations from each of Florida, New Jersey and outside of North America (ONA- Hawaii, Malaysia, Japan). I predicted that if genetic drift was an important factor mediating population differentiation, then fitness would differ between populations at a local, regional, and long distance scale.

I also addressed the question of whether populations differ in the underlying genetic basis of fitness related traits on a local, regional and/or long distance spatial scale. To do so, I measured a composite index of fitness for F₁ and reciprocal F₁ hybrid lines created by crossing populations separated at each spatial scale. If populations differ in the frequency of (partially) recessive deleterious alleles due to random genetic drift, then F₁ and reciprocal F₁ hybrid lines would be expected to exhibit increased performance relative to the average of the parental populations (i.e. heterosis). I predicted that if Ae. albopictus populations experience local genetic drift due to founder effects during local colonization or as a consequence mosquito control efforts then hybrid populations would exhibit significant heterosis.
Materials and Methods

All populations were collected between early and late summer of 2006. A minimum of 200 larvae were collected from at least 20 containers at each of three locations in New Jersey and Florida (Table 1). Larvae were also collected from Shimonoseki, Japan. In addition, *Ae. albopictus* eggs collected in field oviposition traps from Honolulu, Hawaii were kindly provided by Mr. Pingjun Yang (Department of Health, Hilo, Hawaii) and from Kuala Lumpur, Malaysia by Dr. Indra Vythilingam (Institute for Medical Research, Jalan Pahang, Kuala Lumpur). My approach to population sampling was designed to examine differences 1) between the southern and northern extremes of the range of *Ae. albopictus* in North America, and 2) between recently colonized North American populations and more long-term established populations from outside North America (ONA). Both Kuala Lumpur and Shimonoseki, Japan are within the native range of *Ae. albopictus*, and colonization of Hawaii is thought to have occurred sometime during the 1890s (Joyce 1961). Phylogeographic analysis based on mtDNA haplotypes (Mousson et al. 2005) suggests that the Hawaii invasion occurred from an Indian Ocean source.

All populations were reared under standardized laboratory conditions for between three (North America and Japan) and six (Hawaii, Malaysia) generations as described in Armbruster and Hutchinson (2002). Larvae were maintained at a density of 30 larvae per 100 ml petri dish at 21 ±1°C on a 18:6 (L:D) photoperiod. Every Monday, Wednesday, and Friday (M-W-F) larvae were transferred to fresh water and
fed 1.5 ml of a larval food slurry consisting of 80 grams dried dog food (Nutro Brand Large Breed Adult, Nutro Products Inc., City of Industry, CA) and 40 grams whole brine shrimp (Sally’s Frozen Brine Shrimp, San Francisco Bay Brand, Newark, CA) in 1 litre distilled water homogenized in a kitchen blender. Every M-W-F, pupae were collected and transferred into population-specific cages. Adult cages were constructed from inverted 9.5-litre containers with 8- by-14 cm mesh windows and mesh tops. Adults were allowed to feed *ad libitum* on organic raisins and cages were lined with filter paper and watered daily to maintain approximately 80 percent humidity. For all populations, females were allowed to feed to repletion from a human host. Each cage was subsequently provided with a 200 ml black jar half-filled with distilled water and lined with a moist paper towel to stimulate oviposition. Each M-W-F eggs were collected from oviposition jars, gently dried, and maintained at approximately 80 percent humidity in Tupperware containers with a saturated aqueous potassium sulfate solution at 21 ±1°C on a 18:6 (L:D) photoperiod. Egg hatch was stimulated by submerging eggs in water mixed with ca. 3 ml larval food (Novak and Shroyer 1978).

I generated F₁ and reciprocal F₁ hybrid lines by establishing two hybrid mating cages per cross while maintaining the parental populations. Crosses were performed among populations at three levels of spatial separation: (1) local (57- 105 km), (2) regional (1,409- 1,665 km) and (3) long distance (7,737- 16,590 km). Local crosses were conducted between populations from Florida and included FL1xFL2, FL1xFL3, and FL2xFL3 (Table 1). Regional crosses were conducted between populations from
Florida and New Jersey and included NJ1xFL3, NJ2xFL1 and NJ3xFL2 (Table 1). Long distance crosses were conducted between populations from Florida, Hawaii, Malaysia and Japan and included HAWxFL3, MALxFL1, JPNxFL2 (Table 1). I crossed one of each of the Florida populations to one of each of the New Jersey and ONA populations in order to examine whether there were consistent regional effects independent of individual populations. This approach is analogous to the design of my common garden experiment, where individual populations represent random effects within each region. For each cross, pupae were collected from each parental stock population every M-W-F, sexed, and split into three groups before transfer to separate adult mating cages. One of the three groups was used to maintain the parental population and the other two groups were used to generate F\textsubscript{1} and reciprocal F\textsubscript{1} hybrid lines. Adult mating cages were comprised of at least 50 adult males and 50 adult females for each of the 27 populations (9 parental and 18 hybrid). Population crosses were performed after parental populations were reared for two (North America and Japan) or five (Hawaii, Malaysia) generations in the laboratory. Consequently, F\textsubscript{1} hybrid cross populations were simultaneously reared with F\textsubscript{3} and F\textsubscript{6} parental populations in the fitness experiment described below.

I reared experimental cohorts in microcosms designed to simulate natural field conditions. The aquatic larvae of *Ae. albopictus* are found in a variety of natural and artificial container habitats (e.g. tree holes, used automobile tires), where, like other container-inhabiting mosquitoes, they feed on both microorganisms and dead particulate
matter (Merritt et al. 1992). Because *Ae. albopictus* larvae are often found in discarded tires, microcosms were created from small rubber tires (13-cm diameter; Mammoth Pet Tire Biter, Mammoth Lakes CA) that were cut in half. Miniature-tire microcosms contained 75 ml of distilled water and 0.5 g of dried leaves maintained at 21 ±1°C. Senescent white oak leaves (*Quercus alba*) were collected from Glover-Archbold Park in Washington, DC. Leaves were oven-dried for three days at 37°C and then soaked in distilled water at room temperature for six days to allow for growth of microorganisms.

To conduct fitness assays, eggs from all 27 populations were simultaneously hatched and reared under standardized conditions. First-instars on the day of hatch were placed into five replicate cohorts of 40 individuals for each population with each cohort reared in a separate miniature-tire microcosm. Pupae were collected, sexed and weighed to the nearest 0.01 mg every M-W-F. Pupal mass determined using this protocol has a high repeatability (> 99 percent) and is highly correlated with fecundity in *Ae. albopictus* (Armbruster and Hutchinson 2002). Development time was measured as number of days from hatch to pupation and larval survivorship was calculated as the total number of individuals that pupated divided by the original cohort number (40).

Mass-specific fecundity relationships were determined independently for each of the 27 populations. First, I determined the relationship between pupal mass and wing length by rearing larvae from each population at a density of 20-80 larvae per 100 ml petri dish as described above. Upon pupation, female pupae were weighed to the nearest 0.01 mg and transferred to individual glass vials. Upon eclosion, wing length
was measured to the nearest 0.1 mm as the distance from the axial incision to the marginal vein excluding fringe setae using a slide graticle at a 20X magnification (Armbruster and Hutchinson 2002). In a second experiment, I determined the relationship between wing length and fecundity for each population. Larvae from each population were reared as described above. Female pupae were transferred to population-specific mating cages and after eclosion adults were blood fed to repletion on a human host. At five days post-blood meal, females were removed for dissection and the number of mature (stage V) follicles (eggs) was counted as described in Armbruster and Hutchinson (2002). The two regression equations were then combined as described by Lounibos et al. (2002) to obtain a single equation relating pupal mass to fecundity for each of the 27 populations (Appendix A). I also used the wing length and egg number data to calculate size-specific fecundity as the number of eggs divided by wing length.

I calculated $r'$, a composite indicator of performance, as described in Livdahl and Sugihara (1984):

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

where $N_0$ is the number of females (assumed to be 50 percent of the initial cohort), $A_x$ is the number of females eclosing on day $x$, $w_x$ is the female size measure, $f(w_x)$ is the function relating female size (pupal mass) to fecundity, and $D$ is the number of days.
required for a newly eclosed female to mate, take a bloodmeal and oviposit. I used a \( D \) of 14 days, which is typical for \( Ae. \) albopictus (Livdahl and Willey 1991, Juliano 1998, Costanzo et al. 2005).

**Statistical Analysis.** The pupal mass, development time, mass-specific fecundity, and \( r' \) data were approximately normally distributed; however, variances were not homogeneous. Natural log (ln) transformation was therefore applied in order to homogenize variances, but in all cases the conclusions were identical to those based on untransformed data. I therefore have presented statistics and figures based on untransformed data to facilitate biological interpretation. Larval survivorship data were arcsine square-root transformed to homogenize variances and meet the assumptions of normality. Statistical analyses for larval survivorship are based on transformed data but I present untransformed data for biological interpretation.

To compare \( r' \), survivorship, and mass-specific fecundity among Florida, New Jersey and ONA populations, I performed a nested mixed-model analysis of variance (ANOVA) on cohort means with populations (random effect) nested in regions (fixed effect, PROC MIXED, SAS Institute 2004). I used population nested in region as the error term for region in these analyses (Partridge and Fowler 1992, Zani et al. 2005). If the effect of region was significant, I performed \textit{a posteriori} comparison of regional means to test for specific pair-wise differences with a sequential Bonferroni (Rice 1989) to control for experiment-wise error (\( \alpha = 0.05 \)).
I used a nested multivariate analysis of variance (MANOVA, PROC GLM, SAS Institute 2004) on cohort means of sex-specific development time and pupal mass to test for differences among regions and among populations nested within regions. As above, if differences among regions were significant I then performed an *a posteriori* comparison of regional means with a sequential Bonferroni (Rice 1989) to control for experiment-wise error (*α* = 0.05). To determine the relative contribution of each variable to the MANOVA results, I calculated standardized canonical coefficients as described by Scheiner (2001).

If the genetic differentiation of parental populations is due to genes with additive effects, then the fitness of *F*₁ and reciprocal *F*₁ hybrids is expected to equal the average of the parental values (i.e., the midparent value; Armbruster et al. 1997, Lynch and Walsh 1998). Alternatively, if genetic drift and/or local inbreeding leading to fixation of deleterious recessives has contributed to genetic divergence among parental populations, then *F*₁ hybrid lines are expected to exhibit significantly greater fitness (*r¹*) than the mid-parent expectation (i.e., heterosis; Lynch and Walsh 1998). Because of similar performance, I pooled *F*₁ and reciprocal *F*₁ hybrid lines before testing for the fit of the 3 generation means (2 parents, pooled *F*₁ hybrid and reciprocal *F*₁ hybrids) to a purely additive model of population differentiation using the joint-scaling tests of Mather and Jinks (1982) as in Armbruster et al. (1997). For this goodness-of-fit test, rejection of the simple additive model indicates that alleles with dominance effects
and/or epistasis have contributed to the divergence of parental populations (Lynch and Walsh 1998).

**Results**

**Fitness comparisons.** ANOVA of $r'$ indicated significant differences among regions ($F = 8.73; \text{df} = 2, 6; P = 0.017$) but there were no significant differences between populations within regions ($F = 1.19; \text{df} = 6, 36; P = 0.33$) (Fig. 1). Pairwise contrasts revealed no significant differences in fitness ($r'$) between Florida and New Jersey populations. However, both Florida and New Jersey populations differed significantly from ONA populations (Fig. 1). There were no significant differences in larval survivorship among regions ($F = 1.64; \text{df} = 2, 42; P = 0.15$) or between populations within a region ($F = 1.64; \text{df} = 6, 36; P = 0.16$) (Fig. 2A). Size-specific fecundity did not differ between regions ($F = 0.38; \text{df} = 2, 6; P = 0.69$), but populations varied significantly within regions ($F = 112.31; \text{df} = 6, 251; P < 0.001$) (Fig. 2B).

The results of MANOVA on sex-specific pupal mass and development time showed no effect of region for males but a significant effect of region for females (Table 2; Fig. 3). *A posteriori* comparison of regional female means indicated that all three regions were significantly different from each other (Fig. 3B). Standardized canonical coefficients indicated that differences between regions were strongly associated with female development time and weakly associated with pupal mass. MANOVA also indicated a significant effect of population nested within region on pupal mass and development time for males but not females (Table 2; Fig. 3). This
indicates that male traits varied significantly between populations within a region. Standardized canonical coefficients indicated that the differences were strongly associated with development time and weakly associated with pupal mass.

**Hybrid populations.** A purely additive model of population differentiation was rejected for five of the nine population crosses (Fig. 4). Three population crosses, two local and one regional, revealed significant fitness heterosis (Fig. 4A, B, F). This indicates that hybrid populations had enhanced fitness relative to the midparent expectation due to effects of dominance and/or epistasis at the local and regional level. For one regional and one long distance cross, hybrids had significantly reduced fitness as compared to the midparent expectation (Fig. 4E, G). This indicates that population crosses at a larger geographic level (i.e. regional and long distance) resulted in outbreeding depression.

**Discussion**

Populations of *Ae. albopictus* from distinct geographic regions (NJ, FL, ONA) exhibited significantly different mean phenotypes for several life-history traits. Because these phenotypes were measured under a single set of standardized conditions (i.e., a “common garden” design), these differences reflect underlying genetic differences among populations (Falconer and Mackay 1996). My results indicate significant regional differences for overall fitness ($r'$; Fig. 1), as well as female development time and pupal mass (Fig. 3B). There was no effect of region on larval survivorship and size-specific fecundity (Fig. 2), but larval survivorship did exhibit a
non-significant pattern similar to $r'$ (Fig. 1), suggesting that variation in larval survivorship contributed to regional differences in $r'$. There were also no significant differences among regions for male development time and male pupal mass. Neither of these traits are components of $r'$ and their regional trends were not consistent with the $r'$ results (Fig. 3A).

*Ae. albopictus* populations from both North American regions (NJ, FL) had significantly lower fitness than did two populations from the native range (MAL, JPN) and one long established population from Hawaii (Fig. 1). However, overall fitness ($r'$) did not differ between regions within North America (i.e., NJ vs. FL). This result contrasts with previous studies showing increased fitness for invasive verses native populations in plants (Siemann and Rogers 2001, Blair and Wolfe 2004, Brown and Eckert 2005, Lavergne and Molofsky 2007, Cano et al. 2008). These results in plants are usually interpreted in the context of the “enemy release” hypothesis (Colautti et al. 2004, Liu and Stiling 2006) or evolution of increased competitive ability in invasive species (Blossey and Notzold 1995). Increased fitness has also been reported for introduced versus native populations of the Argentine ant (Tsutsui et al. 2000, Tsutsui and Case 2001).

My results showing decreased fitness of the recently invasive North American populations relative to the more long-term established ONA populations are consistent with the hypothesis of increased genetic load due to genetic drift caused by restricted population size, an inference which is further supported by the results of my line-cross
analyses discussed below (Fig. 4). However, it is important to note that Kambhampati et al. (1991) found similar levels of overall allelic variation at allozyme loci in the US and Japan. Because *Ae. albopictus* is thought to have invaded the US through a shipment of used tires from temperate Japan (Hawley et al. 1987), the results of Kambhampati et al. (1991) suggest that the colonization of North America did not involve a population bottleneck. These considerations, in conjunction with the results of Black et al. (1988a, b), suggest that if genetic drift due to restricted population size has led to reduced fitness in North American *Ae. albopictus* relative to ONA populations, this effect has taken place at the individual population level (i.e., during colonization of individual populations or due to the effects of local mosquito control) rather than at the regional level (i.e., during the colonization of North America). Regardless of the specific underlying mechanism, the decreased fitness of North American populations relative to ONA populations as determined under my experimental conditions has not impeded *Ae. albopictus* from spreading broadly across the eastern US and excluding *Ae. aegypti* from throughout much of its range in the southeastern US (O'Meara et al. 1995, Juliano and Lounibos 2005).

An alternative interpretation of the decreased fitness of North American relative to ONA populations is that these differences are due to the specific environmental conditions utilized in my experiment. I attempted to mimic natural conditions by rearing experimental cohorts in miniature-tire microcosms provisioned with natural resources (i.e., leaf litter). However, under the conditions utilized in my study both the
mean (± SE) female pupal mass (1.56 mg ± 0.03) and male pupal mass (1.12 ± 0.02) of North American populations was lower than the mean (± SE) female (1.92 ± 0.33) and male (1.47 ± 0.23) mass of pupae collected from 10 haphazardly selected tires across a two month period during 2004 at a Manassas, VA tire dump. Because both $r'$ and wing length (closely correlated to pupal mass in *Ae. albopictus*; Armbruster and Hutchinson 2002) are highly sensitive to conditions during larval development (Lord 1998), these results suggest that the conditions in my experiment may have been relatively stressful compared to natural container habitats due to low nutrient levels, high larval density, or other factors. Further research of inter-population differences in population performance under a range of environmental conditions merits further investigation to better understand if environmental gradients differentially affect particular populations.

The regional differences between FL and NJ for female pupal mass and development time (Fig. 3) are consistent with previous studies of *Ae. albopictus* that also have indicated regional differentiation within North America for various life-history traits (Kambhampati and Rai 1991, Lounibos et al. 2003, Armbruster and Conn 2006). Taken together, these studies and my current results clearly indicate that despite the relatively recent invasion of *Ae. albopictus* into North America and a lack of evidence for regional differentiation within the US at the level of overall fitness ($r'$; Fig. 1), regional populations exhibit genetically-based differentiation for a variety individual life-history traits. Consistent differences among replicate regional populations in female pupal mass and development time implies that these differences are due to
deterministic (i.e., natural selection) rather than stochastic (i.e., random genetic drift) processes.

In contrast to the results for females (Fig. 3B), male development time and pupal mass did not differ among regions but did vary significantly among populations within regions (Fig. 3A). Size-specific fecundity (Fig. 2B) also varied significantly among populations but not regions. These results are similar to those of Leisnham et al. (2008) who found population-level but not regional differences in female adult survival and reproductive allocation among southern (Tampa, FL and Fort Meyers, FL) and northern (Bloomington, IL and Manassas, VA) populations of North American Ae. albopictus. Leisnham et al. (2009) also found that Ae. albopictus vary between populations but not regions with regard to interspecific competitive ability. The existence of population-level but not regional differences could be due to either local adaptation or a stochastic process (i.e., genetic drift) driving population differentiation. At least in the case of male pupal mass and development time, these traits may be under weaker selection than traits such as female pupal mass and development time that show significant regional differentiation but not significant population-level variation. The replicate populations within the two North American regions (FL, NJ) have presumably diverged over less than 20 years, while the ONA populations have been isolated from one another for a century (HAW) or millennia (MAL, JPN). Relative to North American populations, the ONA populations exhibit slightly greater among-population variation for overall fitness
(r') (Fig. 1), larval survivorship (Fig. 2A) and size-specific fecundity (Fig. 2B), but somewhat surprisingly, not for male pupal mass and development time (Fig. 3A).

Despite the fact that I did not find significant differences between populations within North America at the level of overall fitness (r'; Fig. 1), I did find evidence for differences in the underlying genetic basis of fitness revealed by significant heterosis for two local (within Florida; 4A, B) and one regional (NJ-FL, 4F) cross. Heterosis in F\textsubscript{1} hybrids is a common observation in the animal breeding and evolutionary genetics literature (Barlow 1981, Whitlock et al. 2000). Although the possibility of overdominance cannot be excluded, this phenomenon is most commonly attributed to the existence of different (partially) recessive deleterious alleles in the parental populations that are masked by dominance effects in the hybrid generation (Lynch and Walsh 1998). Therefore, the results in Fig. 4, in conjunction with results of allozyme analysis conducted by Black et al. (1988a, b), suggest that local genetic drift either during colonization of local container habitats or due to mosquito control efforts may permit the accumulation of (partially) recessive deleterious alleles within at least some local populations.

At a more distant spatial scale, population crosses lead to decreased fitness in hybrids relative to the midparent value (i.e. outbreeding depression) in one regional (Fig. 4E) and one long-distance (Fig. 4G) cross. This result is also consistent with a number of studies showing a transition from heterosis to outbreeding depression in F\textsubscript{1} hybrids as the spatial scale separating parental populations increases (see review by
Edmands 2002). Although less well characterized than heterosis, outbreeding depression in the F₁ hybrid generation can be caused by underdominance, epistasis, and/or chromosomal incompatibilities (Turelli and Orr 2000). Previous investigators have documented a three-fold variation in the haploid nuclear DNA content among populations of *Ae. albopictus* (Kumar and Rai 1990), suggesting that chromosomal incompatibilities could contribute to the outbreeding depression I observed in the F₁ hybrids of two crosses. However, it is also important to note that for two long distance crosses in which the parental populations differed for overall fitness (*r’*), the fitness in the F₁ hybrids almost exactly matched the midparent (additive) expectation. Thus, differences in the underlying genetic architecture appear to be highly population-specific and do not depend on distance in a consistent, predictable manner. These conclusions further support the inference that stochastic processes such as colonization history and genetic drift are important forces mediating the genetic differentiation of life-history traits underpinning fitness among populations of *Ae. albopictus* across a range of spatial scales.

In summary, understanding how ecological and genetic factors interact to influence life-history traits underpinning population growth rates of vector species is important because these traits can have a major impact on invasion success (Lounibos 2002) and disease transmission (Black and Moore 2005). The current study indicates regional differentiation of overall fitness (*r’*, Fig. 1) and female pupal mass and development time (Fig. 3B). The decreased fitness of North American populations
relative to populations from outside North America (Fig. 1) and the results of line-cross experiments (Fig. 4) imply a role of local random genetic drift affecting the differentiation of fitness ($r'$) among populations of *Ae. albopictus*. These conclusions corroborate earlier studies of population structure in *Ae. albopictus* using neutral biochemical markers (Black et al. 1988a, b, Kambhampati and Rai 1991, Birungi and Munstermann 2002, Lourenço-de-Oliveira et al. 2003, Usmani-Brown et al. 2009). Additional studies tracking allele frequency changes across temporal samples from multiple populations and exploring the life-history consequences of inbreeding will further elucidate the role of local genetic drift in the evolution of these populations.
Table 1. Geographic origin of nine populations of *Aedes albopictus* collected for fitness experiments.

<table>
<thead>
<tr>
<th>City</th>
<th>State</th>
<th>Country</th>
<th>Latitude and Longitude</th>
<th>Region</th>
<th>Code</th>
<th>Gen&lt;sup&gt;a&lt;/sup&gt;</th>
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<td>Florida</td>
<td>USA</td>
<td>27° 35’ N, 80° 22’ W</td>
<td>FL</td>
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<td>FL</td>
<td>FL2</td>
<td>3</td>
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<tr>
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<td>USA</td>
<td>26° 45’ N, 80° 41’ W</td>
<td>FL</td>
<td>FL3</td>
<td>3</td>
</tr>
<tr>
<td>Burlington</td>
<td>New Jersey</td>
<td>USA</td>
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<td>NJ1</td>
<td>3</td>
</tr>
<tr>
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<td>USA</td>
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<td>NJ</td>
<td>NJ2</td>
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</tr>
<tr>
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<td>USA</td>
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<td>NJ</td>
<td>NJ3</td>
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<tr>
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<td>USA</td>
<td>21° 18’ N, 157° 49’ W</td>
<td>ONA&lt;sup&gt;b&lt;/sup&gt;</td>
<td>HAW</td>
<td>6</td>
</tr>
<tr>
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<td>---</td>
<td>Malaysia</td>
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<tr>
<td>Shimonoseki</td>
<td>---</td>
<td>Japan</td>
<td>33° 57’ N, 130° 56’ W</td>
<td>ONA</td>
<td>JPN</td>
<td>3</td>
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</tbody>
</table>

<sup>a</sup> = number of generations in the lab  

<sup>b</sup> = outside of North America
Table 2. MANOVA results for male and female development time (DT) and pupal mass (PM).

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>error df</th>
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<th>Approx. F</th>
<th>P value</th>
<th>DT</th>
<th>PM</th>
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Fig. 1. Mean fitness ($r' \pm 1$ SE) for North American populations from Florida (FL 1, 2, 3) and New Jersey (NJ 1, 2, 3) and populations outside North America (ONA) from Hawaii (HAW), Malaysia (MAL) and Japan (JPN). Legend in the upper left indicates the results of an ANOVA testing for effect of region and population nested within region. Significant differences ($P < 0.05$) are indicated by *, ns = $P > 0.05$. The results of a posteriori pair-wise contrasts of region are indicated by letters (A, B), where regions sharing the same letter are not significantly different from one another after sequential Bonferroni adjustment (experiment-wise $\alpha = 0.05$).
Fig. 2. Mean (± 1 SE) larval survivorship (A) and size-specific fecundity (B) for populations labeled as in Fig. 1. The legend in the upper right indicates the results of ANOVA testing for effects of region and population nested within region. Significant differences ($P < 0.001$) are indicated by ***, ns = $P > 0.05$. 
Fig. 3. Mean (± 1 SE) development time and mean (± 1 SE) pupal mass for males (A) and females (B) in North American populations from Florida (○) and New Jersey (△) and outside of North America (□). The legend in the upper right indicates results of MANOVA testing for effects of region and populations nested within region. Significant differences (\( P < 0.05 \)) are indicated by *, ns = \( P > 0.05 \).
Fig. 4. Mean (± 1 SE) fitness ($r'$) of parental and pooled F1 and reciprocal F1 hybrid populations. The results of a chi-squared goodness-of-fit test (df = 1) comparing expected population means to an additive model of population differentiation are indicated under each individual graph label, ** indicates, $P < 0.01$, *** indicates $P < 0.001$, ns = $P > 0.05$. 
In this chapter, I have investigated the differentiation of a composite fitness index between *Ae. albopictus* populations at multiple spatial scales. In order to test the hypothesis that local genetic drift contributed to population differentiation, I also measured a composite index of fitness for hybrid lines created by crossing geographically disparate populations at multiple spatial scales. In addition to contributing to local population structure, genetic drift leading to fixation of (partially) deleterious alleles may also affect the ability of vectors to transmit pathogens. Reduced genetic variability is known to decrease fitness and increase disease susceptibility in a number of vertebrate taxa. It has been know for some time that many vector species exhibit significant intraspecific variation in vector competence (i.e., the ability to transmit disease). However, almost nothing is known about the linkages between variation in immune function, life- history traits, and the underlying genetic causes of this variation in natural populations of medically important mosquitoes. In the next chapter, I test the novel hypothesis that reduced genetic variation due to inbreeding leads to increased pathogen susceptibility using *Ae. albopictus* as a model system.
Chapter III

Inbreeding depression affects life-history traits but not susceptibility to infection by *Plasmodium gallinaceum* in the Asian tiger mosquito, *Aedes albopictus*

The re-emergence of mosquito-borne diseases including malaria, yellow fever, and dengue fever represents one of the most significant public health challenges of the 21st century (Breman 2001, Guzman and Kouri 2002, WHO 2002, Barrett and Higgs 2007). The re-emergence of these diseases is due to complex interactions amongst sociological, ecological and evolutionary factors. For example, invasions by novel vector species may shift the ecological dynamics of a community to increase the prevalence of vector-borne disease (Juliano and Lounibos 2005). Additionally, the evolution of pesticide resistance in mosquito vectors (Hemingway and Ranson 2000) and drug resistance in pathogens (Wellems and Plowe 2001) have clearly been of central importance in many cases. While a great deal of progress has been made in understanding the physiological and molecular bases of vector-pathogen interactions (Dimopoulos et al. 2001, Lowenberger 2001, Barillas-Mury et al. 2005, Christensen et al. 2005, Michel and Kafatos 2005), determining the evolutionary forces that influence the ability of vector species to transmit pathogens has received less attention. This is surprising given the clear role of evolutionary processes in causing current patterns of re-emerging infectious disease noted above.
Vector competence is defined as the ability of a vector to become infected by and subsequently transmit a pathogen (Higgs and Beaty 2005). It is well established in mosquitoes that vector competence is at least partially genetically determined, and can vary due to differences in a mosquito’s susceptibility, infection, dissemination and transmission efficiency (Beerntsen et al. 2000, Higgs and Beaty 2005). Inherent physiological characteristics, such as the midgut and transmission barriers (i.e., sites that can impede pathogen progress), may reduce a mosquito’s susceptibility to viruses, *Plasmodium* and other pathogens (Grimstad and Walker 1991, Beerntsen et al. 2000, Vinetz et al. 2000, Bennett et al. 2002, Black et al. 2002, Higgs and Beaty 2005). Additionally, mosquito immune responses, including the up-regulation of antimicrobial peptides (Beerntsen et al. 2000) and the melanization response (Paskewitz and Riehle 1994, Nayar and Knight 1999, Shiao et al. 2001, Hillyer et al. 2003, Barillas-Mury et al. 2005, Christensen et al. 2005), have been shown to inhibit bacterial, filarial worm and *Plasmodium* proliferation. More recently, it has been demonstrated in *Aedes aegypti* that the RNA interference machinery can inhibit viral replication (Sanchez-Vargas et al. 2009).

A series of elegant studies have demonstrated that ecological factors such as larval competition (Alto et al. 2005, Bevins 2008, Alto et al. 2008a), pre-existing pathogen infection (Paulson et al. 1992, Comiskey et al. 1999) and nutritional stress (Comiskey et al. 1999, Vaidyanathan et al. 2008) affect the vector competence of mosquitoes. Nutrient manipulation studies have also found that pathogen susceptibility
or transmission may be positively (Lyimo and Koella 1992, Sumanochitrapon et al. 1998, Okech et al. 2007) or negatively (Grimstad and Haramis 1984, Grimstad and Walker 1991, Paulson and Hawley 1991, Alto et al. 2008b) correlated with adult size, depending on the population and species examined.

However, it is also known that geographic populations of mosquito vectors maintained under standardized (i.e., “common garden”) laboratory conditions often exhibit substantial variation in susceptibility to pathogen infection and/or vector competence. For example, populations of *Aedes albopictus* (Gubler and Rosen 1976, Boromisa et al. 1987) and *Ae. aegypti* (Gubler et al. 1979, Sumanochitrapon et al. 1998, Failloux et al. 1999, Bennett et al. 2002, Black et al. 2002, Failloux et al. 2002) vary in susceptibility and vector competence of dengue viruses. Populations of *Ae. aegypti* have also been shown to vary in vector competence of yellow fever virus (Black et al. 2002), as have populations of *Ae. polynesiensis* for filarial pathogens (Failloux et al. 1995) and *Culex* species for West Nile virus (Vaidyanathan and Scott 2007, Reisen et al. 2008). These studies thus demonstrate genetically-based differences among populations in susceptibility to pathogen infection and/or the ability to transmit pathogens, indicating that in addition to ecological factors discussed above, evolutionary forces must also contribute at least in part to this among-population variation. However, the evolutionary causes of this variation have received very limited theoretical and empirical attention (but see Lambrechts et al. 2009).
Random genetic drift is known to influence genetic differentiation among populations for a variety of vector species (Black and Tabachnick 2005). Based on studies of neutral molecular markers, random genetic drift has been implicated as an important factor mediating population differentiation for the container-inhabiting mosquitoes *Culex pipiens* (Chevillon et al. 1995), *Ae. aegypti* (Bosio et al. 2005, Scarpassa et al. 2008), and *Ae. albopictus* (Black et al. 1988a, b, Kambhampati et al. 1990, Kambhampati et al. 1991, O'Donnell and Armbruster 2009). For example, $F_{st}$ values are as high as 0.12 for *Ae. polynesiensis* (Failloux et al. 1997), 0.249 for *Ae. albopictus* (de Oliveira et al. 2003), and 0.39 for *Ae. aegypti* (Bosio et al. 2005). In *Ae. albopictus*, line-cross experiments have corroborated the results of molecular studies by showing $F_{1}$ heterosis for fitness ($r'$) between Florida populations separated by as little as 98 km (O'Donnell and Armbruster 2009). These results thus imply that even in nearby populations, local random genetic drift and restricted gene flow have led to the fixation of alternative (partially) deleterious alleles affecting fitness. Black et al. (1988a; b) suggested that for *Ae. albopictus*, high local population structure was likely the consequence of genetic drift during local population establishment of discrete habitat patches by a small number of individuals or local mosquito control efforts that caused a reduction in population size.

In this study, I tested the hypothesis that inbreeding would lead to an increased susceptibility to pathogen infection in the Asian tiger mosquito, *Ae. albopictus*. My rationale was that consanguineous mating would exaggerate the effects of local drift
causing the fixation of (partially) deleterious recessive alleles, and thereby provide a test of the most extreme conditions under which local genetic drift might affect susceptibility to pathogen infection in a natural population. Populations with reduced genetic diversity due to inbreeding or population bottlenecks have reduced fitness (Charlesworth and Charlesworth 1987, Hedrick and Kalinowski 2000) and are more susceptible to pathogen infection across many taxa (O'Brien and Evermann 1988, Stevens et al. 1997, Coltman et al. 1999, Spielman et al. 2004, Pearman and Garner 2005, Acevedo-Whitehouse et al. 2006, Calleri et al. 2006, Luong et al. 2007, Ilmonen et al. 2008), including a diverse group of insects (Stevens et al. 1997, Spielman et al. 2004, Calleri et al. 2006, Luong et al. 2007; but see Gerloff et al. 2003, Rantala and Roff 2006). However, no prior studies have investigated how reduced genetic variation influences the infection susceptibility of vectors.

*Aedes albopictus* is an invasive, container-inhabiting mosquito that was introduced into North America from Japan in 1985 and spread rapidly across the eastern United States from Texas to southern Florida, New Jersey, Ohio, and Illinois (Hawley et al. 1987, Moore 1999). *Aedes albopictus* is an aggressive biter of humans (Richards et al. 2006) capable of transmitting a wide variety of arthropod-borne diseases including dengue, eastern equine encephalitis (Gratz 2004), West Nile (Turell et al. 2001a, b, Holick et al. 2002) and chikungunya viruses (Angelini et al. 2007, Pages et al. 2009). In order to test the effect of inbreeding on life-history and immune function traits in *Ae. albopictus*, I measured larval survivorship, adult longevity, female wing length (body
size) and susceptibly to infection by *Plasmodium gallinaceum* in replicate control (expected \( f = 0.07 \)) and inbred (expected \( f = 0.375 \)) lines. Although *Ae. albopictus* are not commonly infected with *Plasmodium* species in nature (but see Ejiri et al. 2008), infection of aedine mosquitoes by *P. gallinaceum* has been used extensively as a model system to elucidate factors related to the genetic basis of mosquito immunity and vector competence (e.g. Thathy et al. 1994, Hillyer et al. 2003, Morlais et al. 2003, Alavi et al. 2004, Boete et al. 2004). I predicted that inbred lines of *Ae. albopictus* would have reduced larval survivorship, reduced adult longevity, reduced female wing length (body size) and elevated infection levels relative to non-inbred control lines.

**Materials and Methods**

*Laboratory husbandry.* In order to establish a laboratory colony (“New Jersey”), approximately 1,000 *Ae. albopictus* larvae were collected from at least 20 discarded tires located at a tire-recapping facility in Salem, New Jersey (39° 35’ N, 75° 29’ W). *Aedes albopictus* have been established in Salem, NJ since 1995 (CDC 2005) and this population has a relatively high fitness compared to other North American populations (O'Donnell and Armbruster 2009). Mosquitoes were reared under standardized laboratory conditions at 21°C and 80% RH for six generations with at least 50 males and 50 females in each generation as described in Armbruster and Conn (2006).

In order to generate replicate inbred lines with an expected inbreeding coefficient of \( f = 0.375 \), I performed two generations of full-sib mating as described in
Fig. 1. New Jersey F7 larvae were reared to pupation, sorted by sex, and placed in separate adult cages. Adult female mosquitoes were bloodfed to repletion on a human host, and then 200 replicate single-pair mating cages were established with one haphazardly selected male and one haphazardly selected female in each cage. Single-pair cages consisted of 32-ounce plastic containers (United States Plastic Corporation, Lima, Ohio) lined on the bottom with moist filter paper and secured with mesh tops. Adults were allowed to feed *ad libitum* on organic raisins and each cage was provided with a 60 mL amber jar half-filled with distilled water and lined with a moist non-bleached paper towel to stimulate oviposition. Eggs were collected every three days, dried two days after collection, and maintained at approximately 80% humidity in Tupperware containers with a saturated aqueous potassium sulfate solution at 21 ±1° C on a 18:6 (L:D) photoperiod. Females in single pair cages were offered 1-2 subsequent bloodmeals (approximately 7 days between feedings) in order to stimulate oviposition of additional egg batches. The full-sibling eggs produced by single-pair mating were subsequently hatched and the single pair mating process described above was repeated for another two generations. The offspring of the third generation of single pair mating (expected $f = 0.375$) were used for pathogen infection and life-history assays (Fig. 1).

Four control (i.e. outbred) lines were created from the same stock population used to generate the inbred lines (Fig. 1). After being reared under standard conditions for eight generations in the lab, F9 control eggs were split haphazardly into two control populations with at least 50 females and 50 males. The next generation, both larval F10
control populations were split haphazardly into larval dishes designated for small or standard adult cages. This was done in order to control for potential effects of cage size of female longevity and pathogen susceptibility. As adults, female control mosquitoes were reared in either a standard 2.5 gallon population cage with at least 50 females or a 32-ounce single-pair cage with approximately 20 females. The control adult females in these four cages (control 1: standard, small and control 2: standard, small) were measured for test assays of adult longevity and infection susceptibility along with adult females from the inbred lines (expected $f = 0.375$). All control cages were maintained in an identical manner to inbred line cages. Control mosquitoes were maintained for nine generations in the laboratory with a 50:50 sex ratio and at least 100 individuals per generation. Effective population size ($N_e$) of the control lines was calculated as in Armbruster et al. (1997) assuming that all females mate and are monogamous (Clements 1992), and that the average male mating success of 0.66. If mating is random, then $1 - e^{-2/3}$ of the males will mate, and the effective population size ($N_e$) of my control population may be estimated as $N_e \geq 65$. I calculated the expected inbreeding coefficient for the control (i.e. outbred) population relative to when the laboratory population was first established as in Armbruster et al. (2000) as $f = 1 - (1 - 1/2N_e)^9$ or $f = 0.07$ (Wright 1969).

Eggs from control and inbred lines were stimulated to hatch by submersion in distilled water mixed with ca. 3 ml larval food (Novak and Shroyer 1978). Larvae were reared in 100 mL petri dishes at a density of 2-30 larvae per dish; conditions which
provide a near-optimal environment for larval growth. Larvae from each line were reared in separate dishes. Every Monday, Wednesday and Friday, larvae were transferred to fresh water and fed 1.5 mL of a larval food slurry consisting of 120 grams dried dog food (Nutro Brand Large Breed Adult, Nutro Products Inc., City of Industry, CA) and 40 grams whole brine shrimp (Sally’s Frozen Brine Shrimp, San Francisco Bay Brand, Newark, CA) in 1 litre distilled water homogenized in a kitchen blender.

Larval survivorship. Larval survivorship was measured for each line as the proportion of first instar larvae on the day of hatch that survived to pupation (total hatch = 2,332 larvae; percent survivorship measured for 22 inbred lines and four control lines).

Adult longevity. All pupae were sorted by sex, and adult longevity was measured for all males (total n = 858, 22 inbred lines and four control lines) by individually placing male pupae into 5.5 mL glass vials. All vials contained 1ml of distilled water and were secured with a mesh top, provisioned with organic raisins, and examined daily in order measure adult longevity, which was calculated as difference between date of eclosion and death.

I also measured the adult longevity of females that were not used in the pathogen infection assay. In order to standardize adult female age and pathogen infection exposure (see below), all adult females between three to nine days old were used for the pathogen infection assay. The remaining females were used to measure adult female longevity, and these tended to be females that completed their larval
development more slowly. The mean pre-adult development period of females used for the pathogen infection assay was 14.70 (SD = 1.93) days, the mean pre-adult development period of females used for the female adult longevity assay was 21.76 (SD = 3.22) days. The pre-adult development period of control females used for the adult longevity assay (mean = 21.32, SD = 3.13) was closely matched to the pre-adult development period of inbred females used for the adult longevity assay (mean = 22.07, SD = 3.27). Adult females were maintained in 32-ounce cages as opposed to vials to measure longevity. Each day, eclosed females in each line (total n = 163, 17 inbred lines and three control lines) were collected and placed in a new 32 ounce cages maintained as described above. To measure individual longevity, the total number of adults per cage was noted and mortality was recorded daily. Female adult longevity was calculated for each individual mosquito as the number of days between adult eclosion and death.

**Body size.** I estimated adult body size by measuring the wing length of all females subjected to the *P. gallinaceum* infection assay. During the midgut dissections (see below), wing length was quantified by removing one haphazardly selected wing and measuring the length from the axial incision to the R4+5 vein edge to the nearest 0.1 mm on a slide graticule (Armbruster and Hutchinson 2002).

**Pathogen Infection.** In order to assay susceptibility to *P. gallinaceum* infection, three to nine day old adult females from each inbred or control line were allowed to feed for 30 minutes on a *P. gallinaceum* infected chicken (approximately 12-15% total
parasitaemia at time of feeding). Females from each line were contained in separate 10 dram vials with the number of individuals per vial ranging from 1 to 49 (mean = 17.59, SD = 16.12). During blood feeding, four vials were placed on the dorsal lateral area of the chicken while another four vials were placed on the ventral lateral area. A total of 23 lines (19 inbred, 4 control) were exposed to the same infected chicken over a 90 minute time period. Of the 23 lines exposed, 20 lines (16 inbred, 4 control) obtained a blood meal and survived the subsequent development period. Blood-fed females were maintained at 21°C with 80% relative humidity on a long day photoperiod (18L:6D) to allow for malarial oocyst development during the seven day development period. Instead of a typical development period of six days for females reared at 26°C to 30°C, I used a seven day development period for females maintained at 21°C based on preliminary pilot studies. Seven days post-bloodmeal, all mosquitoes were dissected, midguts were examined at 20X magnification and the number of oocysts was counted as a measure of infection susceptibility. The presence and prevalence of oocysts is linked to intensity of infection, therefore I interpret higher oocyst counts to indicate a higher susceptibility to infection (i.e., Lyimo and Koella 1992, Shahabuddin et al. 1995, Shahabuddin et al. 1998, Sattabongkot et al. 2003, Okech et al. 2004, LaPointe et al. 2005, Okech et al. 2007). Wing length was measured for each individual as described above.

Statistical Analysis. Larval survivorship data were square-root arcsine transformed to approximate a normal distribution and stabilize variances. I tested for
differences between larval survivorship of control and inbred lines using an approximate \( t \)-test as described by Lynch and Walsh (1998, p. 260-261). Male and female adult longevity data were subject to a \( \ln(\text{longevity} + 1) \) transformation. I did not perform a full factorial analysis on the adult longevity data because males were maintained in individual glass vials and females were maintained in either 32 ounce (small) or 2.5 gallon (standard) adult cages. Instead, I used separate approximate \( t \)-tests as described above to test for differences in the adult longevity of inbred vs. control males and inbred vs. control females. I also tested for differences in adult longevity of females maintained in standard vs. small adult cages by using a Welch’s modified two-sample \( t \)-test to account for unequal variance (S-Plus 6.2; Mathsoft 1999).

For the *P. gallinaceum* infection experiments, I used linear regression to test for an effect of vial density during blood feeding on the percentage of females that successfully fed, survivorship during the development period, and the mean number of oocysts per line (S-Plus 6.2; Mathsoft, 1999). I used Chi-square to test for an effect of vial location during blood feeding on the percentage of females that successfully fed, survivorship during the development period, and the mean number of oocysts per line (S-Plus 6.2; Mathsoft, 1999). In both sets of analyses all percentage data were square-root-arsine transformed. Previous investigators have reported a significant correlation between host body size and infection, transmission (Grimstad and Haramis 1984, Grimstad and Walker 1991, Paulson and Hawley 1991, Lyimo and Koella 1992, Sumanochitrapon et al. 1998, Okech et al. 2007, Alto et al. 2008b) or immune function.
(Suwanchaichinda and Paskewitz 1998). I therefore conducted an analysis of
covariance (ANCOVA) on oocyst count with wing length (body size) as a covariate and
treatment (inbred, control) as a fixed effect (S-Plus 6.2; Mathsoft 1999). Finally, to test
for differences in wing length (body size) between control and inbred adult females that
were used for the infection assays I used an approximate $t$-test as described above.

**Results**

Larval survivorship was significantly lower for inbred compared to control lines
($t = 17.4$, df = 21, $P < 0.001$; Fig. 2). Adult longevity of males did not differ between
control and inbred lines ($t = 0.33$, df = 21, $P = 0.74$; Fig. 3A), but inbred females had
significantly shorter adult longevity than control females ($t = 2.14$, df = 16, $P = 0.48$;
Fig. 3B). Adult longevity of control females maintained in large vs. small adult cages
did not differ ($t = 0.26$, df = 27, $P = 0.80$).

The density of adult females in 10 dram vials while blood feeding on the
infected chicken did not affect the percentage of females per vial that fed ($F_{1,20} = 3.68$,
$P = 0.07$; $r^2 = 0.38$), survivorship during the development period ($F_{1,18} = 1.92$, $P = 0.18$,
$r^2 = 0.09$), nor the mean number of oocysts per female per vial ($F_{1,18} = 0.10$, $P = 0.76$, $r^2$
= 0.01). Similarly, vial location (dorsal vs. ventral side of the infected chicken) did not
affect the percentage of females per vial that fed ($\chi^2 = 17.97$, df = 15, $P = 0.26$),
survivorship during the development period ($\chi^2 = 15.15$, df = 13, $P = 0.30$), nor the
mean number of oocysts per female per vial ($\chi^2 = 15.29$, df = 15, $P = 0.43$). Oocyst
counts did not differ between control females maintained in 32 ounce vs. 2.5 gallon
adult cages \((t = -1.04, \text{ df } = 1.36, \text{ } P = 0.45)\). For females that blood fed on the infected chicken, survivorship during the development period was 74.4% for inbred females and 47.2% for control females, but percent survivorship did not differ significantly between inbred and control lines \((t = 0.62, \text{ df } = 3.7, \text{ } P = 0.57)\). The mean number of oocysts per line ranged from 0 – 31 \((\text{mean } = 17.45, \text{ SD } = 16.85 \text{ for inbred lines and mean } = 17.71, \text{ SD } = 16.08 \text{ for control lines})\). Typical oocyst counts for \textit{Ae. aegypti} range from 7.33 (Alavi et al. 2004) to 44.8 (Shahabuddin et al. 1996); thus my oocyst counts were on the low end of the range. ANCOVA of oocyst counts (Fig. 4) indicated no significant effects of wing length \((F_{1,108} = 1.57, \text{ } P = 0.021)\), inbreeding \((F_{1,108} = 0.002, \text{ } P = 0.96)\) or wing length-by-inbreeding interaction \((F_{1,108} = 0.002, \text{ } P = 0.96)\). Finally, wing length (body size) did not differ between inbred and control females \((t = 0.258, \text{ df } = 27, \text{ } P = 0.80)\).

**Discussion**

interactions mediating vector competence (Beerntsen et al. 2000, Morlais et al. 2003, Barillas-Mury et al. 2005, Blandin et al. 2009), relatively little effort has been devoted to understanding the ultimate evolutionary causes of population variation in disease transmission (but see Lambrechts et al. 2009).

My study tested the novel hypothesis that reduced genetic variation could affect susceptibility to pathogen infection. This hypothesis was based on the observation that local genetic drift can be an important force affecting population differentiation in a broad range of vector species (Black and Tabachnick 2005), including *Ae. albopictus* (Black et al. 1988a, b, Kambhampati et al. 1990, Kambhampati et al. 1991, O'Donnell and Armbruster 2009). Because local genetic drift is expected to accelerate the fixation of (partially) recessive deleterious alleles (Lynch et al. 1995, Whitlock 2003), I performed two generations of full-sib mating in the laboratory to generate replicate inbred lines and test the most extreme conditions under which local genetic drift might affect susceptibility to pathogen infection in a natural population. Before a pathogen can be successfully transmitted by a mosquito vector, it must successfully establish an infection, and then pass through a series of infection “barriers” (i.e., sites that may impede pathogen progress). I only examined the effect of inbreeding on the first stage of this process, pathogen infection. I also quantified the effect of inbreeding on a variety of performance traits affecting fitness, including larval survivorship, adult longevity, and female wing length, which is highly correlated with body size and fecundity in *Ae. albopictus* (Armbruster and Hutchinson 2002). Based on a large

In agreement with my predictions, I did find that inbred mosquitoes had significantly reduced larval survivorship as compared to non-inbred mosquitoes, indicating that inbred mosquitoes would be less likely to survive to adulthood (Fig. 2). Longevity of adult females, but not males, was also significantly reduced for inbred as compared to control lines (Fig. 3). This particularly strong effect of inbreeding on female adult longevity may in part be due to the fact that in order to standardize the age of females used for the infection assay (see materials and methods section), I measured the adult longevity of females that completed their pre-adult development relatively late. However, it is important to note that both the control and inbred adult females used for the longevity assay were closely matched for pre-adult development period (see above).

It is also possible that females harbor an increased genetic load for mutations expressed later in life due to reduced efficacy of selection in removing deleterious mutations expressed after first reproduction (Hamilton 1966, Edney and Gill 1968, Partridge and
Barton 1993, Charlesworth 1994) since data on the parous rate of \textit{Ae. albopictus} suggests that the average female in nature completes only a single gonotrophic cycle (Hawley 1988).

Contrary to my prediction, I found no significant effect of inbreeding on susceptibility to infection by \textit{P. gallinaceum} (Fig. 4). My results differ from previous studies in insects that have found a significant effect of inbreeding on immune function and/or pathogen susceptibility (Stevens et al. 1997, Spielman et al. 2004, Calleri et al. 2006, Luong et al. 2007). In a study of inbreeding depression in \textit{Drosophila melanogaster}, Spielman et al. (2004) did detect significant inbreeding depression for susceptibility to pathogen infection, but noted that increased susceptibility of inbred lines was likely due to the loss of specific resistance alleles rather than more general physiological effects of inbreeding because the among-line correlations between pathogen susceptibility and overall fitness were low. This suggests that homozygosity \textit{per se} and immune function may not always be correlated. Furthermore, in the studies by Stevens et al. (1997), Calleri et al. (2006) and Luong et al. (2007), the effects of inbreeding on disease susceptibility may have been mediated by behavior, a factor that was unlikely to be important in my studies. Finally, my results are in accordance with a limited number of other insect studies (Gerloff et al. 2003, Rantala and Roff 2006) that also found no effects of inbreeding on immune function.

The effects of inbreeding on infection susceptibility may vary with different immune function challenges. For example, Calleri et al. (2006) found an effect of
inbreeding only for a relatively high concentration of fungal pathogens, but not for filament injection (immune function assay) or low-dose fungal pathogen and bacterial pathogen challenge. Because the effects of inbreeding may vary with different immune function challenges, *Plasmodium* may only be representative of certain pathogen challenges that elicit a similar immune response. As a model pathogen, *P. gallinaceum* infections are useful because they elicit a melanization and encapsulation response (Hillyer et al. 2003) similar to filarial infection (Shiao et al. 2001) and certain species of bacteria (Hillyer et al. 2003). Although my model system is expected to elicit an encapsulation response, it is not likely to induce RNAi, which has been found to inhibit dengue virus replication (Sanchez-Vargas et al. 2009). Furthermore, because *Ae. albopictus* is not commonly infected by avian *Plasmodium* species (Ejiri et al. 2008), *P. gallinaceum* may present a novel immune challenge that does not elicit a comprehensive immune response.

Both negative (Grimstad and Haramis 1984, Grimstad and Walker 1991, Paulson and Hawley 1991, Alto et al. 2008b) and positive (Lyimo and Koella 1992, Sumanochitrapon et al. 1998, Okech et al. 2007) relationships between body size and infection or transmission rate have been found in vector mosquito species. In addition, previous work has shown a correlation between smaller body size and reduced immune function (Suwanchaichinda and Paskewitz 1998). However, I did not find conclusive evidence that oocyst count was affected by wing length, a body size measure.
For my study, I intentionally chose a relatively long-established *Ae. albopictus* population with high fitness compared to other US populations (O'Donnell and Armbruster 2009). The decreased larval survivorship and female adult longevity of inbred as compared to control lines implies that my stock population was not highly inbred to begin with. The inbreeding depression I detected for life-history traits related to fitness in *Ae. albopictus* is also consistent with inbreeding depression for fitness traits documented by Armbruster et al. (2000) for the tree-hole breeding mosquito, *Aedes geniculatus*. It is important to note that my current results likely provide a conservative estimate of the negative impact of fitness consequences of inbreeding in natural environment, since the negative effects of inbreeding tend to be more pronounced under stressful conditions likely to be found in nature relative to more benign laboratory environments (Armbruster and Reed 2005, Swindell and Bouzat 2006). However, this does not imply that fixation of deleterious recessives would affect pathogen susceptibility in natural populations because my level of inbreeding ($f = 0.375$) was higher than would be expected in nature.

These considerations have important implications for ongoing efforts to use transgenic mosquito strains refractory to pathogen transmission in order to control vector-borne diseases. To create stable germ-line transformation, successfully transformed mosquitoes must be isolated and full-sib mated (Coates et al. 1998, Jansinskiene et al. 1998). My results suggest that the inbreeding that must occur during this process is likely to reduce the performance of transgenic lines. This is in
accordance with previous studies that have demonstrated that transgenic *D. melanogaster* (Woodruff 1992) and *Ae. aegypti* (Irvin et al. 2004) have significantly shorter adult longevity as compared to non-transgenic strains, as well as previous studies showing transgenic mosquitoes to be out-competed by wild type mosquito strains (Catteruccia et al. 2003, Li et al. 2008, but see Moreira et al. 2004). Because the effects of inbreeding are expected to vary substantially between lines (Fowler and Whitlock 1999), maximizing the number of independent transgenic lines may be an effective way to manage the effects of inbreeding in order to create competitive inbred strains.

In summary, the results of the current study do not support my hypothesis that inbreeding affects susceptibility to infection by a model pathogen. Nevertheless, given the strong evidence for drift in vector populations (Black and Tabachnick 2005), including *Ae. albopictus* (Black et al. 1988a, b, Kambhampati et al. 1990, Kambhampati et al. 1991, O'Donnell and Armbruster 2009), and the prevalent effects of reduced genetic variation on pathogen susceptibility in a wide range of organisms (O'Brien and Evermann 1988, Stevens et al. 1997, Coltman et al. 1999, Spielman et al. 2004, Pearman and Garner 2005, Acevedo-Whitehouse et al. 2006, Calleri et al. 2006, Luong et al. 2007, Ilmonen et al. 2008), similar studies in other host-pathogen systems are warranted. The more general topic of investigation of the evolutionary forces that may impact variation among populations in ability to transmit disease is also an area that merits further attention. For example, a recent study by Lambrechts et al. (2009)
suggests that local adaptation of viral lineages to spatially structured vector host
populations may contribute to variation in vector competence among natural
populations of the yellow fever mosquito, Aedes aegypti. Ultimately, further
elucidating the genetic underpinnings of variation in life-history and immune function
traits for medically important mosquitoes should provide important insight relevant to
developing urgently needed novel forms of vector control.
Figure 1. Experimental protocol for creating inbred and control lines in order to measure life-history and immune function traits. (* Indicates that adults were split into small and standard-sized cages to create 4 total control lines.)
Figure 2. Mean larval survivorship (+1 SE) for control ($f = 0.07$, ■) and inbred ($f = 0.375$, □) lines. The result of a $t$-test for inbreeding treatment is indicated in the upper right corner. $t_{21} = 17.4, P < 0.001$
Figure 3. Mean adult longevity (+1 SE) for A) male and B) female mosquitoes in control ($f = 0.07$, ■) and inbred ($f = 0.375$, □) lines. The results of $t$-tests for inbreeding treatment are indicated in the upper right corner. Note that males and females were not reared in a full-factorial design (see text for details).
Figure 4. Effect of individual wing length and inbreeding (control $f = 0.07$, inbred $f = 0.375$) on oocyst count. The legend in the upper right indicates results of ANCOVA testing for effects of wing length, inbreeding, and wing length-by-inbreeding interaction (Wl x Inb). ns indicates $P > 0.05$. 

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Bridge

In previous chapters, my research examined genetic differentiation of fitness related life-history traits among North American and non-North American populations of *Ae. albopictus*. Egg size is a life-history trait positively related to offspring fitness, and egg size clines over a latitudinal range have been observed among many taxa, including insects. The relationship between egg size variation and environmental variables, such as temperatures, may yield insight into the mechanistic basis by which egg size selection occurs. In the next chapter, I have compared egg volume across a broad geographic range for both the native and invasive ranges of *Ae. albopictus*. 
Chapter IV

Egg size variation of *Aedes albopictus* across native and invasive geographic ranges

Latitudinal clines are widespread for many life-history traits in insects. Body size clines are frequently observed in ectotherms (e.g. Arnett and Gotelli 1999, Gilchrist et al. 2004; for review, see Partridge and French 1996). Desiccation tolerance in *Drosophila subobscura* (Gilchrist et al. 2008), desiccation and starvation tolerance in *Drosophila ananassae, Drosophila melanogaster,* and *Zaprionus indianus* (Karan et al. 1998) and diapause incidence in *D. melanogaster* (Schmidt et al. 2005) all exhibit latitudinal clines. Reproductive traits also display latitudinal clines; clines for fecundity (egg number) and reproductive timing have been observed in *D. melanogaster* (Mitrovski and Hoffmann 2001). In addition, clines of increasing egg size with increasing latitude have been observed for spruce budworm larvae (*Choristoneura fumiferana*; Harvey 1983), fruit flies (*D. melanogaster*; Azevedo et al. 1996) and mosquitoes (*Wyeomyia smithii*; Armbruster et al. 2001).

Understanding the forces that influence the evolution of clines is imperative to understanding invasive species biology and also represents an important tool in discerning the effects of global climate change. The success of an invasive species is enhanced if it exhibits a rapid evolutionary response to environmental gradients, such as temperature clines (Lee 2002). The rapid evolution of clinal variation in invasive species may occur even if the genetics basis is distinct from that of its native-range cline.
(Huey et al. 2000). Studying clinal adaptation of invasive species also enhances our understanding of how species may respond to climate change. For example, the invasion and range expansion of *D. melanogaster* throughout eastern Australia provides an opportunity to examine evolutionary responses to a variety of climatic conditions, which may offer insight into adaptation to climate change (Hoffmann and Weeks 2007). In addition, shifts in an existing cline for genetic polymorphisms in *D. melanogaster* indicate that this species is already responding to global climate change, and that changes in clinal genetics may serve as indicators of this response (Umina et al. 2005). Furthermore, changes in the genetically-based photoperiodic response of *W. smithii* mosquito populations are consistent with adaptation to more southerly conditions that have occurred due to global climate change (Bradshaw and Holzapfel 2001).

Although slope or intercept may vary, markedly similar clines may evolve in native and invasive ranges (e.g. Gilchrist et al. 2004). The evolution of similar body size clines across different continents has been well documented for *D. subobscura* (Huey et al. 2000, Calboli et al. 2003, Gilchrist et al. 2004) and *D. melanogaster* (David and Capy 1988, Imasheva et al. 1994, Gilchrist and Partridge 1999, Zwaan et al. 2000). Comparable clines for desiccation tolerance have also been observed for *D. subobscura* in Europe and North America (Gilchrist et al. 2008). Finally, similar egg size clines for *D. melanogaster* have been observed in Australia and South America (Azevedo et al. 1996).
There are a number of reasons to believe that temperature might be the selective pressure causing latitudinal clines in body size. First, temperature is known to decline with increasing latitude across continents in the northern hemisphere (Gilchrist et al. 2004). Second, the evolution of clines in the same direction on separate continents implies that clines are maintained by a deterministic (e.g., natural selection) rather than stochastic (e.g., genetic drift) process (Partridge and French 1996). Finally, laboratory selection under cooler conditions has been shown to lead to the evolution of larger body size in D. melanogaster (Partridge et al. 1994). Thus, a combination of patterns in nature and the results of laboratory evolution experiments suggest that temperature may be the driving force in generating clines for body size (Partridge and French 1996, Gilchrist et al. 2004). Similar results suggest that thermal selection may also be causing clines in other life-history traits, such as egg size (Azevedo et al. 1996, Armbruster et al. 2001), longevity, fecundity, and fertility (Partridge et al. 1995).

Although there is compelling evidence for thermal selection as the causal selective mechanism driving clinal evolution for a variety of life-history traits, the arguments noted above are complicated by the fact that many biotic and/or abiotic factors may co-vary with temperature under either laboratory or field conditions. One example is the length of growing season, which is known to vary with latitude (Tauber et al. 1986). Clinal variation in humidity and precipitation has a more significant effect than temperature on body size clines in the seed-feeding beetle Stator limbatus (Stillwell et al. 2007). The results of a common garden experiment by Arnett and
Gotelli (1999) indicate that selection related to food availability likely influences clinal variation in growth, body size, and survivorship for the ant lion *Myrmeleon immaculatus*. Conspecific larval density is known to vary across a latitudinal gradient for the mosquito *W. smithii* (Bradshaw and Holzapfel 1983, 1986). In addition, Santos et al. (2005) found that apparent thermal selection on body size in *D. subobscura* may instead be an effect of larval density, where more crowded conditions in populations maintained under relatively warm conditions select for rapid development and reduced adult size. These studies suggest that local adaptation to larval density, rather than temperature, may be the proximate selective mechanism driving clinal variation in nature.

Further complicating efforts to identify direct targets of selection that cause clinal variation, it is important to note that phenotypic traits may evolve in response to direct selection on genetically correlated traits (Lande 1982). For example, in *D. melanogaster*, body size is genetically correlated with fecundity (Roff 1981). In addition, egg size is genetically correlated to body size in the butterfly *Parnara guttata guttata* (Seko et al. 2006) and the beetle *S. limbatus* (Czesak and Fox 2003). As a result, apparent evolution in insect egg size may be due to direct selection acting on body size. Thus, it can be difficult to discern if selection is acting directly on an individual trait based on patterns of geographic variation.

Egg size is an important trait positively related to offspring fitness in a wide variety of organisms (Smith and Fretwell 1974, McGinley et al. 1987, Fox and Czesak
Crossing experiments have found positive maternal effects of egg size on embryo viability and development, hatchling weight, feeding rate and larval and pre-adult development time (Azevedo et al. 1997). As noted above, *D. melanogaster* and *W. smithii* from populations at higher latitudes with colder habitats tend to produce larger eggs under common garden conditions (Azevedo et al. 1996, Armbruster et al. 2001), indicating a genetic basis to egg size variation in these taxa. In addition to insects, egg size clines for natural populations have been found for other taxa such as marine invertebrates (Clarke 1992), pacific salmon (Morita et al. 2009), and wood frogs (Berven 1982).

Variation for egg size in natural populations due to environmental effects has also been observed across many taxa (see references in Azevedo et al. 1996). This pattern of phenotypic plasticity indicates that in some taxa egg size variation across a latitudinal cline may not necessarily be due to genetic differentiation. Laboratory manipulations of temperature for field-caught *Drosophila simulans, Drosophila phalerata* and *D. subobscura* resulted in low egg volumes for high temperature treatments (Avelar 1993), supporting observations that environmental variation in temperature affects egg size in natural populations. It is interesting that the phenotypically plastic response of egg size to temperature appears to be the same as the evolutionary response to temperature (see references in Azevedo et al. 1996).

*Aedes albopictus* is a container-inhabiting mosquito that was introduced into the United States in 1985 from Japan via the used tire trade (Hawley et al. 1987, Craven et
al. 1988) and subsequently spread from Texas to southern Florida, New Jersey, Ohio and Illinois (Hawley et al. 1987, Moore 1999). *Aedes albopictus* is a medically important mosquito able to transmit dengue, eastern equine encephalitis, West Nile and chikungunya viruses (Gratz 2004, Angelini et al. 2007). Thus, the ability of *Ae. albopictus* to expand and rapidly adapt to new habitats has important implications for the potential of this invasive disease vector to spread pathogens (Moore 1999). As a result, the evolution of life-history traits for *Ae. albopictus* is of public health concern (Lounibos 2002).

In addition to being a potential public health concern, the establishment and range expansion of invasive species presents an intriguing natural experiment in evolution (Sakai et al. 2001). Several studies have documented rapid clinal evolution in invasive species. For example, a wing length cline evolved within 20 years of the introduction and range expansion of *D. subobscura* to North America (Huey et al. 2000). A latitudinal cline for size and fecundity evolved for populations across the invasive range of the plant *Hypericum perforatum* that was similar to the latitudinal clines for size and fecundity exhibited in ancestral populations (Maron et al. 2004). In order to investigate the potential evolution of a latitudinal cline in an invasive species, I designed an experiment to test the null hypothesis that there is no cline for egg size in *Ae. albopictus*. Because *Ae. albopictus* is native to Japan, there has been more evolutionary time for an egg size cline to evolve in its native as compared to invasive United States range. Thus, an egg size cline for *Ae. albopictus* may exist in Japan, but
be absent in the more recently established US populations. Alternatively, egg size clines may exist for *Ae. albopictus* populations from both continents. In order to test these hypotheses, I measured egg volume for populations across a similar latitudinal gradient in Japan and the United States (US). I predicted that I would find a cline of increasing egg size correlated with increasing latitude for *Ae. albopictus* populations. I also predicted that, due to the time that has elapsed since invasion, egg size for populations from both Japan and the US would exhibit similar clines.

**Materials and Methods**

**Collection.** Mosquito populations were collected from a range of latitudes across Japan and the United States (Table 1). A minimum of 200 larvae were collected from at least 10 containers for 11 populations in the US ranging from Newark, New Jersey (40°44’N) to West Palm Beach, Florida (26°40’N). Eleven populations were collected from Japan, ranging from Sakata, Yamagata (38°55’N) to Okinawa, Okinawa (26°30’N). Sendai, Tokyo, Hiroshima, Shimonoseki, and Okinawa populations were collected as above. All other populations from Japan were collected from the field as larvae and pupae, and laboratory populations were established from *F*₁ eggs kindly provided by Dr. Motoyoshi Mogi.

All populations were reared under standardized laboratory conditions for three to 11 generations (Table 1) as described in Armbruster and Conn (2006). The mosquitoes that oviposited eggs used in this experiment were reared at a density of 30 larvae per 100 ml petri dish at 21 ±1°C on a long day (18L:6D) photoperiod. There
were seven dishes of larvae (210 individuals) per population. Every Monday, Wednesday, and Friday (M-W-F) larvae were transferred to fresh water and fed approximately 1.5 ml of larval food slurry. Pupae were collected every M-W-F and transferred into population-specific cages constructed from inverted 2.84 litre containers with 8-by-14 cm mesh windows and mesh tops. Adults were maintained and oviposition was stimulated as in Armbruster and Conn (2006).

**Measurements.** In order to quantify the accuracy of egg volume measurements, I determined the repeatability. One investigator, who was unaware of egg source or identity, measured the length and width of 12 eggs at 5 X with an ocular micrometer. Each egg was measured six times in a haphazard order. Length and width were then used to calculate egg volume \( V \) as that of prolate spheroid as in Armbruster et al. (2001):

\[ V = \frac{\pi L W^2}{6} \]

where \( L \) is egg length and \( W \) is egg width. Repeatability of egg measurements was computed as described by Lessells and Boag (1987).

Eggs used to study geographic variation of egg volume were collected from population-specific cages every M-W-F and dried two days after collection. All eggs were placed in Tupperware containers with potassium sulfate to maintain constant humidity and stored under a short day (8L:16D) photoperiod. Egg volume was measured as described above nine days post-oviposition. All measurements were performed blind with respect to population origin by the same investigator. Thirty to 45
eggs were measured per population from two oviposition dates (with the exception of eggs from Vero Beach, US and Utsunomiya, Japan for which eggs were only available from a single oviposition date). Eggs were selected haphazardly from across each day’s collection in order to obtain a representative sample of eggs for each population.

To quantify climatic variation across the latitudinal gradient of Japan and the United States, I used temperature data to calculate a temperature index as in Gilchrist et al. (2004). I obtained US climate data from the National Oceanic & Atmospheric Administration (NOAA) from 1971-2000 (NOAA 2004) and transformed all data to degrees Celsius from degrees Fahrenheit. I obtained Japanese climate data (in °C) from the Japanese Meteorological Agency (JMA) and used the subset of years 1971-2000 in order to match my US temperature data (JMA 2002). For each population considered, I selected the closest meteorological station available (Table 1). I calculated mean minimum ($T_{\text{min}}$) and maximum ($T_{\text{max}}$) temperature for winter, spring, summer and fall at each location, and transformed the data into a temperature index ($T_I$) by computing principal component scores (see statistical analyses).

**Statistical Analysis.** Egg volumes were approximately normally distributed with homogenous variance. I conducted an analysis of variance (ANOVA) on individual egg volume measurements to compare the effect of country and population nested within country. Although analysis of individuals within a population increases power, this approach assumes that individuals from within a population are fully independent (i.e. unrelated). Therefore, for analysis of clinal variation, I chose to be
conservative and use population means (as in Gilchrist and Partridge 1999, Calboli et al. 2003, Gilchrist et al. 2004). To compare mean egg volume between countries and determine if egg size is correlated with latitude, I conducted an analysis of covariance (ANCOVA) with country (Japan or US) as a fixed effect and latitude as a covariate. I also examined the interaction of country and latitude. In order to compare mean egg volume between countries and determine if egg size is correlated with climate, I calculated the first principal component, $T_I$, based on $T_{\text{min}}$ and $T_{\text{max}}$ data. I also examined the interaction of country and climate. In order to determine how my temperature index ($T_I$) was correlated with latitude for *Ae. albopictus* populations from Japan and the US, I conducted an ANCOVA with country (Japan or US) as a fixed effect and latitude as a covariate. Then, I conducted an analysis of covariance (ANCOVA) with country (Japan or US) as a fixed effect and climate ($T_I$) as a covariate. I also examined the interaction of country and climate. All analyses were performed in S-Plus 6.0 (Mathsoft 1999) with the exception of the ANOVA which was performed in SAS (SAS Institute 2004).

**Results**

Egg measurements had a high repeatability ($r = 91.28\%$). Egg size comparisons revealed similar egg volume for both countries. Populations from Japan had a mean ($\pm$ SE) egg volume of $6.94 \text{mm}^3 \times 10^{-3} \pm 0.05 \times 10^{-3}$, and populations from the US had a mean ($\pm$ SE) egg volume of $6.87 \text{mm}^3 \times 10^{-3} \pm 0.03 \times 10^{-3}$. ANOVA indicated no significant effect of country on egg volume ($F_{1, 20} = 0.18, P = 0.68$) (Table 2), but
populations nested within country differed significantly in egg volume ($F_{1,668} = 10.28, P < 0.001$) (Table 2). ANCOVA revealed that egg volume was not significantly affected by country of origin (Japan or US) ($F_{1,18} = 0.17, P = 0.68$) or latitude ($F_{1,18} = 1.45, P = 0.24$) (Fig. 1). Also, there was no interaction between country and latitude ($F_{1,18} = 0.18, P = 0.68$) (Fig. 1). Principal components analyses revealed that the first principal component ($T_1$, see materials and methods) explained 81.3% of the variance in seasonal $T_{\text{min}}$ and $T_{\text{max}}$ data and consisted of positive loadings for all temperatures. Thus, large $T_1$ scores indicate higher temperature locations. Because the second principal component explained relatively little of the variance (16%), it was excluded from analyses. Temperature index ($T_1$) and latitude ANCOVA revealed that $T_1$ was significantly affected by country of origin (Japan or US) ($F_{1,18} = 31.01, P < 0.001$) and latitude ($F_{1,18} = 683.14, P < 0.001$) (Fig. 2), but there was no interaction between country and latitude ($F_{1,18} = 2.36, P = 0.14$) (Fig. 2). Similar to the ANCOVA with latitude as a covariate described above, ANCOVA revealed that egg volume was not significantly affected by country of origin (Japan or US) ($F_{1,18} = 0.17, P = 0.68$) or climate ($T_1$) ($F_{1,18} = 1.44, P = 0.25$). There was also no interaction between country and climate ($T_1$) ($F_{1,18} = 0.01, P = 0.91$).

**Discussion**

I did not find a cline of increasing egg size correlated with increasing latitude for *Ae. albopictus* in either native (Japan) or invasive (US) populations (Fig. 1). This is surprising given that latitudinal clines for egg size have been found in spruce budworm
larvae (C. fumiferana; Harvey 1983), fruit flies (D. melanogaster; Azevedo et al. 1996) and mosquitoes (W. smithii; Armbruster et al. 2001). However, other studies have failed to find the expected clines for insects (Blanckenhorn and Fairbairn 1995, Robinson et al. 2000, Snook 2001, Hallas et al. 2002, David et al. 2006). Although an egg size cline was documented for water striders (Blanckenhorn and Fairbairn 1995), egg volume was found to increase with decreasing latitude, presumably due to the longer growing seasons of more southerly populations. In addition, the presence of a cline may depend on the trait measured; Hallas et al. (2002) found clines for chill coma recovery and body size but not desiccation or starvation resistance for the Australian fruit fly Drosophila serrata. Other studies have found no clines in life-history traits for insects. For example, sperm length is a highly variable trait in D. subobscura, but variation in sperm length was not found to be a function of latitude (Snook 2001). One previous study that investigated egg volume failed to find a significant cline; no latitudinal cline in egg size was observed for Arctic charr (Power et al. 2005), although it had been found in another salmon species (Morita et al. 2009). Also, it is important to note that publication bias is likely to result in fewer studies published with negative results.

The presence of latitudinal clines may also vary by continent. For instance, Robinson et al. (2000) found no clines for starvation resistance or fat content for D. melanogaster in South America although clines were discovered in India (Karan et al. 1998). David et al. (2006) found no body size cline for the drosophilid Z. indianus in
South America, despite the fact that a cline was observed in its native African range. To explain these results, David et al. (2006) suggested that the absence of a cline in South America was due to the relatively recent invasion of *Z. indianus*. However, in the current study it is clear that the results are not simply a consequence of the recent invasion of the US by *Ae. albopictus* because there was no correlation between egg size and latitude in Japan, its native range.

I examined the correlation of egg volume with climate (\(T_1\)) in addition to latitude because latitude may not account for local microclimatic variation. In addition, the use of a temperature index allowed me to assess the relationship between temperature and latitude independently for collection locations from the US and Japan. I found that there was no correlation between egg size and climate (\(T_1\)) in either country, which suggests that climate does not influence evolution of egg size for *Ae. albopictus*. Furthermore, if egg size was evolving in response to climate, I would expect differentiation between populations from the US and Japan because climate (\(T_1\)) is significantly different between countries (Fig. 2). The lack of differentiation in egg size despite a significant effect of country on climate further corroborates my finding that climate is not a factor affecting the evolution of egg size in *Ae. albopictus*. The selective mechanism that drives increased egg size with increased latitude for some species is not known, although temperature has been implicated (Azevedo et al. 1996, Armbruster et al. 2001). My results imply that the evolution of egg size among *Ae. albopictus* populations is not the straightforward result of thermal selection. Other
potentially confounding ecological factors may play a role in affecting the evolution of egg size among *Ae. albopictus* populations, thereby obscuring any clinal variation.

The contrast between my current results and the latitudinal variation in egg size found in other Diptera may be due to ecological differences between these species. For example, selection related to larval density may be causing latitudinal clines in egg size of *W. smithii* (Bradshaw and Holzapfel 1983, 1986) and larval density may also influence body size clines in *Drosophila* (Partridge and French 1996). Santos et al. (2005) found that larval density, not climate, played a significant role in affecting the evolution of body size in replicate laboratory populations of *D. subobscura*. Santos et al. (2005) hypothesized that adaptation to high larval densities in southern populations resulted in selection for rapid development time and reduced body size. Body size is known to be genetically correlated to egg size in the butterfly *Parnara guttata guttata* (Seko et al. 2006) and the beetle *S. limbatus* (Czesak and Fox 2003). If egg size is genetically correlated with body size in *D. melanogaster*, then clinal selection on body size could lead to clinal evolution in egg size in this species. Although larval density clines have not been examined in *Ae. albopictus*, this issue clearly warrants attention given my current results because the absence of a cline in larval density would further support the larval crowding hypothesis.

Another possibility that may explain the lack of an egg size cline in both the ancestral and invasive range of *Ae. albopictus* is that effects of selection due to temperature are confounded by selection for desiccation resistance, resulting in no
apparent cline. Dried containers are known to cause egg mortality for *Ae. albopictus* (Sota and Mogi 1992), suggesting that desiccation resistance may be a significant selective force affecting eggs of *Ae. albopictus*. Furthermore, selection for desiccation-resistant eggs is known to influence egg size. Sota (1993) found that selection for desiccation resistance resulted in larger *Ae. albopictus* eggs, thus, selection for large, desiccation-resistant eggs may counteract thermal selection, especially in desiccation-prone areas. This selective force would differ greatly from what would be expected under a latitudinal cline, where egg size would increase with latitude. *Wyeomyia smithii* exhibits an egg size cline that is likely caused by thermal selection (Armbruster et al. 2001), indicating that the absence of a cline is not specific to container-inhabiting species. However, while *Ae. albopictus* oviposits its eggs above the water level of containers (Amerasinghe and Alagoda 1984), *W. smithii* oviposits eggs on the surface of the water (Bradshaw 1983), which may lead to reduced desiccation stress. As a result, *W. smithii* may not be subjected to clinal selection for desiccation resistance.

Because gene flow is expected to homogenize the genetic composition of geographically distinct populations (Slatkin 1987), it may also inhibit the evolution of latitudinal clines in some cases. However, *D. melanogaster* populations appear to be relatively panmictic (Singh and Rhomberg 1987), so the presence of clines in this species on multiple continents implies that the force of selection must be strong relative to the homogenizing effect of gene flow (Singh and Rhomberg 1987b, Gockel et al. 2001). In contrast, *Ae. albopictus* is characterized by high levels of population structure.
throughout its native and invasive ranges (Black et al. 1988a, b, Kambhampati and Rai 1991, Birungi and Munstermann 2002, Lourenço-de-Oliveira et al. 2003). Consequently, the homogenizing force of genetic drift is unlikely to explain the absence of clines for *Ae. albopictus* populations across their native and invasive range.

A number of additional evolutionary forces may explain why populations have evolved non-clinal differences in egg size. For example, one explanation for the absence of an expected cline is the lack of sufficient genetic variation. However, the populations examined in this study have evolved geographic differences in egg size (Fig. 1, Table 2). Populations from the US and Japan varied significantly for egg size within country (Table 1) but population differentiation did not occur in the pattern of a latitudinal cline. These results indicate that a lack of genetic variation can not be invoked to explain the lack of a cline in egg size. It is possible that egg size variation among populations may be evolving neutrally due to random genetic drift (Whitlock 2003). However, this is unlikely because egg size is important trait closely related to offspring fitness in arthropods (Fox and Czesak 2000). Population differentiation may instead be due to local adaptation of egg size. Additional studies are needed to investigate the strength of selection for egg size in local populations.

In summary, increasing egg size with increasing latitude may be an adaptive response to differences in temperature along latitudinal range for other insect species (Harvey 1983, Azevedo et al. 1996, Armbruster et al. 2001). However, I find no evidence to suggest that evolution of egg size is affected by climate in *Ae. albopictus*. 

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Alternatively, other ecologically relevant factors, such as the absence of a larval density selection or selection for desiccation resistance, may drive egg size evolution in this mosquito. Future studies should investigate the potential selective affects of abiotic and biotic factors that exhibit local variation in order to further elucidate this issue.
Table 1. Geographic origin of 22 populations of *Ae. albopictus* collected for egg size comparisons (in bold) and weather stations used to calculate the temperature index ($T_I$; in parentheses below). Unless otherwise listed, all weather stations were located in the same state/prefecture and country as the populations collected.

<table>
<thead>
<tr>
<th>Location</th>
<th>Country</th>
<th>Latitude and Longitude</th>
<th>Gen&lt;sup&gt;a&lt;/sup&gt;</th>
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<td>Newark, New Jersey</td>
<td>USA</td>
<td>40° 44’ N, 74° 4’ W</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>(Newark INTL Airport, ID: 286026)</td>
<td>40° 43’ N, 74° 10’ W</td>
</tr>
<tr>
<td>Berlin, New Jersey</td>
<td>USA</td>
<td>39° 46’ N, 74° 59’ W</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Glassboro 2 NE, ID: 283291)</td>
<td>39° 44’ N, 75° 06’ W</td>
</tr>
<tr>
<td>Manassas, Virginia</td>
<td>USA</td>
<td>38° 38’ N, 77° 25’ W</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Reagan NTL Airport, ID: 448906)</td>
<td>38° 52’ N, 77° 02’ W</td>
</tr>
<tr>
<td>Waverly, Virginia</td>
<td>USA</td>
<td>37° 3’ N, 77° 7’ W</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Stony Creek 1 E, ID: 448129)</td>
<td>36° 57’N, 77° 24’ W</td>
</tr>
<tr>
<td>Henderson, North Carolina</td>
<td>USA</td>
<td>36° 21’ N, 78° 22’ W</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Henderson 2 NNW, ID: 313969)</td>
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</tr>
<tr>
<td>Fayetteville, North Carolina</td>
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<td>35° 2’ N, 78° 51’ W</td>
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<td></td>
<td></td>
<td>(Fayetteville PWC, ID: 313017)</td>
<td>35° 4’ N, 78° 52’ W</td>
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<td>New Zion, South Carolina</td>
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<td>Jacksonville, Florida</td>
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<table>
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<th>Location</th>
<th>Country</th>
<th>Latitude, Longitude</th>
<th>Distance</th>
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<td>Jacksonville Beach, ID: 084366</td>
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<td>30° 17’ N, 81° 24’ W</td>
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<td>Deland 1 SSE, ID: 082229</td>
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<td>29° 01’ N, 81° 19’ W</td>
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<tr>
<td>Oakhill, Florida</td>
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<tr>
<td>Vero Beach, Florida</td>
<td>USA</td>
<td>27° 35’ N, 80° 22’ W</td>
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</tr>
<tr>
<td>Vero Beach MUNI ARPT, ID: 089214</td>
<td>USA</td>
<td>27° 39’ N, 80° 25’ W</td>
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<tr>
<td>West Palm Beach, Florida</td>
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<td>26° 40’ N, 80° 9’ W</td>
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<tr>
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<td>Sakata, Yamagata</td>
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<td>(Sakata, Station No: 47587)</td>
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<td>Sendai, Kagoshima</td>
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<td>38° 16’ N, 140° 52’ E</td>
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<td>(Sendai, Station No: 47590)</td>
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<td>38° 15.7’ N, 140° 53.8’ E</td>
<td></td>
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<tr>
<td>Aizuwakamatsu, Fukushima</td>
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<td>37° 29’ N, 139° 33’ E</td>
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<tr>
<td>(Wakamatsu, Fukuoka, Station No: 47570)</td>
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<td>37° 29.3’ N, 139° 54.6’ E</td>
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<td>Khoriyama, Fukushima</td>
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<tr>
<td>(Utsunomiya, Station No: 47615)</td>
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<td></td>
</tr>
<tr>
<td>Tokyo Metropolis</td>
<td>Japan</td>
<td>35° 41’ N, 139° 46’ E</td>
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</tr>
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<td>(Tokyo, Station No: 47662)</td>
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<td>35° 41.4’ N, 139° 45.6’ E</td>
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</tr>
<tr>
<td>Hiroshima, Hiroshima</td>
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<tr>
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<tr>
<td>Location</td>
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<td>Number of Generations</td>
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<td>Shimonoseki, Yamaguchi</td>
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<tr>
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<td>33° 56.9' N, 130° 55.5' E</td>
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<td>Kagoshima, Kagoshima</td>
<td>31° 33' N, 130° 33' E</td>
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<td>Okinawa, Okinawa</td>
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<td>26° 20.2' N, 126° 48.2' E</td>
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*a = number of generations in the lab*
**Table 2.** ANOVA results for effects of country and population nested within country on *Ae. albopictus* egg volume.

<table>
<thead>
<tr>
<th>Source</th>
<th>df&lt;sub&gt;num&lt;/sub&gt;</th>
<th>df&lt;sub&gt;den&lt;/sub&gt;</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>Approx. F</th>
<th>P value</th>
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<tr>
<td>Country</td>
<td>1</td>
<td>20</td>
<td>0.89</td>
<td>0.89</td>
<td>0.18</td>
<td>0.68</td>
</tr>
<tr>
<td>Population (Country)</td>
<td>20</td>
<td>688</td>
<td>100.04</td>
<td>5.00</td>
<td>10.28</td>
<td>&lt; 0.001</td>
</tr>
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</table>
Figure 1. Effects of latitude and country of origin (● = Japan, ○ = US) on mean egg volume (±1 SE) for *Ae. albopictus*. Results of ANCOVA are in upper right corner, ns = $P > 0.05$. 
Figure 2. Effects of latitude and country of origin (● = Japan, ○ = US) on temperature index ($T_i$). Results of ANCOVA in upper right corner, *** indicates $P < 0.001$, ns = $P > 0.05$. 
Appendix A. Population-specific pupal mass-fecundity regression equations

<table>
<thead>
<tr>
<th>Population</th>
<th>$n_{wl-pm}$</th>
<th>$n_{fol-wl}$</th>
<th>$r^2$</th>
<th>Regression equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>FL1</td>
<td>27</td>
<td>37</td>
<td>0.81</td>
<td>$\text{fec} = 26.97 x \text{pm} - 10.73$</td>
</tr>
<tr>
<td>FL2</td>
<td>43</td>
<td>43</td>
<td>0.73</td>
<td>$\text{fec} = 25.96 x \text{pm} - 17.00$</td>
</tr>
<tr>
<td>FL3</td>
<td>30</td>
<td>46</td>
<td>0.71</td>
<td>$\text{fec} = 16.53 x \text{pm} + 2.15$</td>
</tr>
<tr>
<td>FL1 X FL3</td>
<td>21</td>
<td>27</td>
<td>0.41</td>
<td>$\text{fec} = 11.06 x \text{pm} + 28.43$</td>
</tr>
<tr>
<td>FL3 X FL1</td>
<td>24</td>
<td>28</td>
<td>0.56</td>
<td>$\text{fec} = 13.73 x \text{pm} + 16.04$</td>
</tr>
<tr>
<td>FL2 X FL1</td>
<td>23</td>
<td>54</td>
<td>0.14</td>
<td>$\text{fec} = 9.28 x \text{pm} + 20.44$</td>
</tr>
<tr>
<td>FL1 X FL2</td>
<td>22</td>
<td>41</td>
<td>0.47</td>
<td>$\text{fec} = 13.91 x \text{pm} + 25.17$</td>
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<tr>
<td>FL2 X FL3</td>
<td>24</td>
<td>30</td>
<td>0.53</td>
<td>$\text{fec} = 13.71 x \text{pm} + 6.00$</td>
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<tr>
<td>FL3 X FL2</td>
<td>21</td>
<td>46</td>
<td>0.52</td>
<td>$\text{fec} = 14.12 x \text{pm} + 6.91$</td>
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<tr>
<td>NJ1</td>
<td>41</td>
<td>36</td>
<td>0.62</td>
<td>$\text{fec} = 20.27 x \text{pm} - 8.60$</td>
</tr>
<tr>
<td>NJ2</td>
<td>37</td>
<td>46</td>
<td>0.55</td>
<td>$\text{fec} = 16.86 x \text{pm} + 6.65$</td>
</tr>
<tr>
<td>NJ3</td>
<td>38</td>
<td>42</td>
<td>0.72</td>
<td>$\text{fec} = 19.33 x \text{pm} + 3.97$</td>
</tr>
<tr>
<td>FL3 X NJ1</td>
<td>49</td>
<td>40</td>
<td>0.71</td>
<td>$\text{fec} = 16.96 x \text{pm} + 1.01$</td>
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<tr>
<td>NJ1 X FL3</td>
<td>22</td>
<td>41</td>
<td>0.23</td>
<td>$\text{fec} = 10.39 x \text{pm} + 15.70$</td>
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<tr>
<td>FL1 X NJ2</td>
<td>21</td>
<td>16</td>
<td>0.49</td>
<td>$\text{fec} = 17.68 x \text{pm} - 12.74$</td>
</tr>
<tr>
<td>NJ2 X FL1</td>
<td>22</td>
<td>35</td>
<td>0.50</td>
<td>$\text{fec} = 23.70 x \text{pm} - 27.66$</td>
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<tr>
<td>FL2 X NJ3</td>
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<td>58</td>
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<td>$\text{fec} = 17.00 x \text{pm} + 12.00$</td>
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<tr>
<td>NJ3 X FL2</td>
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<td>43</td>
<td>0.33</td>
<td>$\text{fec} = 11.80 x \text{pm} + 16.00$</td>
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<tr>
<td>HAW</td>
<td>38</td>
<td>29</td>
<td>0.62</td>
<td>$\text{fec} = 15.15 x \text{pm} + 11.50$</td>
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<tr>
<td>MAL</td>
<td>35</td>
<td>47</td>
<td>0.65</td>
<td>$\text{fec} = 21.24 x \text{pm} - 1.76$</td>
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<td>JPN</td>
<td>31</td>
<td>45</td>
<td>0.62</td>
<td>$\text{fec} = 11.74 x \text{pm} + 10.52$</td>
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<tr>
<td>FL3 X HAW</td>
<td>35</td>
<td>28</td>
<td>0.68</td>
<td>$\text{fec} = 34.41 x \text{pm} - 40.90$</td>
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<tr>
<td>HAW X FL3</td>
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<td>54</td>
<td>0.55</td>
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<tr>
<td>FL1 X MAL</td>
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<td>0.51</td>
<td>$\text{fec} = 10.81 x \text{pm} + 29.02$</td>
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<td>36</td>
<td>0.67</td>
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<tr>
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<td>JPN X FL2</td>
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<td>38</td>
<td>0.67</td>
<td>$\text{fec} = 19.62 x \text{pm} - 5.36$</td>
</tr>
</tbody>
</table>
November 16, 2009

Deborah Ladner O'Donnell
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Email: debsterl@aol.com

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immunosuppression by a malaria parasite in its mosquito vector. P. Roy. Soc.

competence of geographic strains of *Aedes albopictus* for dengue1 virus. J. Am.
Mosq. Control Assoc. 3: 378-386.


Bradshaw, W. E. 1983. Interaction between the mosquito *Wyeomyia smithii*, the
plants as hosts for aquatic insect communities. Plexus Publishing, Medford, NJ.

smithii*: seasonal and geographic adaptations, pp. 167-185. In V. K. Brown and
I. Hodek [eds.], Diapause and life cycle strategies in insects. Dr W. Junk
Publishers, The Hauge.


de Oliveira, R. L., M. Vazeille, A. M. B. de Filippis, and A. B. Failloux. 2003. Large genetic differentiation and low variation in vector competence for dengue and yellow fever viruses of *Aedes albopictus* from Brazil, the United States, and the


Lourenço-de-Oliveira, R., M. Vazeille, A. M. B. de Filippis, and A. B. Failloux. 2003. Large genetic differentiation and low variation in vector competence for


Mathsoft. 1999. S-Plus user's guide. Mathsoft, Seattle, WA.


egg size and number in anadromous masu salmon *Oncorhynchus masou*. J. Fish Biol. 74: 699-705.


**NOAA. 2004.** Climatography of the United States, No. 20, 1971-2000, National Climatic Data Center, Asheville, NC.


infections of Aedes aegypti are modulated by the mosquito's RNA interference pathway. PLoS Pathog. 5.


